Leading Historical Regulatory Assessments of Glyphosate Safety

A Comprehensive Overview

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European Food Safety Authority (EFSA)

Glyphosate: Response to Comments on the Human Health Draft Risk Assessment

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CONCLUSION ON PESTICIDES PEER REVIEW

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Peer review of the pesticide risk assessment of the active substance glyphosate

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Abstract

The conclusions of EFSA following the peer review of the initial risk assessments carried out by the Assessment Group on Glyphosate (AGG), consisting of the competent authorities of France, the Netherlands, Sweden and Hungary, acting jointly as rapporteur Member State for the pesticide active substance glyphosate are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012. The conclusions were reached on the basis of the evaluation of the representative uses of glyphosate as a herbicide as proposed by the applicants, covering uses pre-sowing, pre-planting and pre-emergence plus post-harvest in vegetables and sugar beet; post-emergence of weeds in orchards, vineyards, row vegetables, railway tracks against emerged annual, biennial and perennial weeds. Moreover, uses as spot treatment against invasive species in agricultural and non-agricultural areas, and in vegetables and sugar beet against couch grass are also included. The reliable endpoints, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are reported where identified.

Keywords: glyphosate, peer review, risk assessment, pesticide, herbicide

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Summary

Commission Implementing Regulation (EU) No 844/2012 lays down the procedure applicable for the renewal of the approval of glyphosate submitted under Article 14 of Regulation (EC) No 1107/2009. Glyphosate is covered under the fifth stage of the renewal work programme (AIR V). By Commission Implementing Regulation (EU) 2019/724 amending Commission Implementing Regulation (EU) No 686/2012, on 10 May 2019, four Member States (France, Hungary, the Netherlands and Sweden) were appointed to act jointly as rapporteurs for the assessment of the application for renewal of the approval for glyphosate. The four Member States formed the Assessment Group on Glyphosate (AGG) and jointly assumed the role of the rapporteur Member State (RMS).

In accordance with Article 1 of Regulation (EU) No 844/2012, an application for the renewal of the approval for glyphosate was submitted by the deadline of 15 December 2019 by a consortium of 8 companies[1] – the Glyphosate Renewal Group (GRG).

An initial evaluation of the dossier on glyphosate was provided by the four RMSs of the AGG in the renewal assessment report (RAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012.

For glyphosate, the formal assessment of the proposal for harmonised classification and labelling in accordance with Regulation (EC) No 1272/2008 has been conducted by the European Chemicals Agency (ECHA) in parallel to the EFSA peer review. When carrying out the risk assessment in the framework of the peer review, EFSA adopted ECHA's hazard assessment and the conclusions of the ECHA Committee for Risk Assessment (RAC) on harmonised classification and labelling delivered in their Opinion on 30 May 2022 (ECHA, 2022).

The following overall conclusions were derived by the peer review.

The representative uses of glyphosate proposed at EU level were a herbicide applied as a foliar spray to target weeds when growing vegetables, sugar beet, in orchards, in vineyards, on railway tracks, on fallow agricultural and non-agricultural land. These uses result in a sufficient **herbicidal efficacy** against the target emerged annual weeds, emerged perennial and biennial weeds, giant hogweed and Japanese knotweed, and couch grass.

The assessment of the data package revealed no issues that could not be finalised or that needed to be included as critical areas of concern with respect to **identity, physical–chemical and technical properties** of the active substance and the formulation for representative uses, and **analytical methods**.

In the area of **mammalian toxicology** and non-dietary exposure, no critical areas of concern were identified. The assessment of the reference specification could not be finalised since one of the impurities showed a potential for clastogenicity in an *in vitro* chromosome aberration test that was not appropriately followed up *in vivo*. This impurity was present in some of the batches used in toxicity studies at levels representative of the proposed reference specification, however a maximum level for this impurity cannot be established while this issue is not clarified. There were no indications of acute toxicity or genotoxicity in studies performed with the formulation for representative uses 'MON 52276'. Toxicological studies were available for all co-formulants but one (present in significant amount in the final formulation), for which repeated-dose toxicity information over short- and long term was not available. In order to reach a final conclusion on the risk assessment of 'MON 52276', repeated-dose toxicity data for this component should be assessed.

In the area of **residues**, the consumer risk assessment could not be finalised. Although preliminary results indicated residues in rotational crops above the limit of quantification, the number of rotational crop field trials was insufficient to address all relevant scenarios. Therefore, a higher consumer exposure to residues of glyphosate than the one considered in the current risk assessment cannot be excluded. However, it is not expected that this might lead to an exceedance of the toxicological reference values. Therefore, no critical concern was identified.

The data available on **environmental fate and behaviour** were sufficient to carry out the required environmental exposure assessments at EU level for the representative uses. In some small hydrological catchments and some larger river systems, the route of groundwater exposure via bank infiltration and the connectivity of surface water bodies to groundwater aquifers may be relevant. Therefore, further information would be useful for assessors in national regulatory competent

[1] It is noted that at the time of application (December 2019) there were 9 companies in the consortium of GRG. By the time of dossier submission (June 2020) and thereafter, 8 companies remained in GRG supporting the renewal of approval of glyphosate.

authorities to assess groundwater concentrations that may result from this exposure pathway. However, the groundwater exposure assessment was finalised for most typical small hydrological catchments and most typical larger river systems, where the connectivity of surface water bodies to groundwater aquifers is limited.

The assessment of the data package revealed no issues that could not be finalised or that needed to be included as critical areas of concern with respect to **ecotoxicology** for the representative uses assessed. A high long-term risk to mammals was concluded for 12 of the 23 representative uses based on tier 1 assumptions. Suitable data to refine the risk assessment were not available. The assessment for aquatic macrophytes, when contact exposure via spray drift occurs, could not be finalised. Insufficient information was provided to draw a firm conclusion on the impact to **biodiversity** via indirect effects and trophic interactions for the representative uses. In addition, the experts acknowledged the lack of harmonised methodologies and agreed specific protection goals, and that the risks for biodiversity are complex and depend on multiple factors.

Studies reporting effects on **microbiome** were considered and taken into account for the risk assessment in the areas of mammalian toxicology and ecotoxicology. Currently, no internationally agreed guidelines for the risk assessment of microbiome are in place in the pesticide area. Further research in the field of microbiome is needed to understand its relevance for risk assessment and to develop dedicated strategies and methodologies accordingly.

Following the assessment based on the available evidence, glyphosate does not meet the criteria for **endocrine disruption** as laid down in points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) No 2018/605.

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Background

Commission Implementing Regulation (EU) No 844/2012[2] (hereinafter referred to as 'the Regulation'), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009[3]. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States (MSs), the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of an additional 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3).

Glyphosate is covered under the fifth stage of the renewal work programme (AIR V). By Commission Implementing Regulation (EU) 2019/724[4] amending Commission Implementing Regulation (EU) No 686/2012[5], on 10 May 2019, four MSs (France, Hungary, the Netherlands and Sweden) were appointed to act jointly as rapporteurs for the assessment of the application for renewal of the approval for glyphosate. The four MSs formed the Assessment Group on Glyphosate (AGG) and jointly assumed the role of the RMS.

In accordance with Article 1 of the Regulation, an application for the renewal of the approval for glyphosate has been submitted by the deadline of 15 December 2019 by a consortium of 8 companies[1] – the Glyphosate Renewal Group (GRG).

On 8 June 2020, a supplementary dossier for renewal of the approval for glyphosate had been submitted by the GRG to the four RMSs of the AGG. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and on 18 August 2020 informed the applicants (GRG), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on glyphosate in the RAR, which was received by EFSA on 15 June 2021 (AGG, 2021).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the MSs and the applicants, the GRG, for consultation and comments on 23 September 2021. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 24 November 2021. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of reporting tables. In addition, the applicants were invited to respond to the comments received. The comments and the applicants' response were evaluated by the RMS in column 3 of the reporting tables.

The need for expert consultation and the necessity for additional information to be submitted by the applicants in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA, the RMS and the European Commission on 9 February 2022. On the basis of the comments received, the applicants' response to the comments and the RMS' evaluation thereof, it was concluded that additional information should be requested from the applicants, and that EFSA should conduct an expert consultation in the areas of mammalian toxicology, residues, environmental fate and behaviour and ecotoxicology.

[2] Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, p. 26–32.

[3] Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

[4] Commission Implementing Regulation (EU) 2019/724 of 10 May 2019 amending Implementing Regulation (EU) No 686/2012 as regards the nomination of rapporteur Member States and co-rapporteur Member States for the active substances glyphosate, lambda-cyhalothrin, imazamox and pendimethalin and amending Implementing Regulation (EU) No 844/2012 as regards the possibility that a group of Member States assumes jointly the role of the rapporteur Member State. OJ L 124, 13.5.2019, p. 32–35.

[5] Commission Implementing Regulation (EU) No 686/2012 of 26 July 2012 allocating to Member States, for the purposes of the renewal procedure, the evaluation of the active substances whose approval expires by 31 December 2018 at the latest. OJ L 200, 27.7.2012, p. 5–10.

The outcome of the telephone conference, together with EFSA's further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting tables. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an experts' consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation tables, together with the outcome of the experts' consultation and the written consultation on the assessment of additional information, were reported in the final column of the evaluation tables.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with MSs via a written procedure in May 2023.

This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the formulation for representative uses, evaluated on the basis of the representative uses of glyphosate as a herbicide as proposed by the applicants, covering uses as pre-sowing, pre-planting and pre-emergence plus post-harvest in vegetables and sugar beet; post-emergence of weeds in orchards, vineyards, row vegetables, railway tracks against emerged annual, biennial and perennial weeds. Moreover, uses as spot treatment against invasive species in agricultural and non-agricultural areas, and in vegetables and sugar beet against couch grass are also included in the EU peer review.

In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review, if any, are presented in the conclusion.

A list of the relevant end points for the active substance and the formulation for representative uses is provided in Appendix B. In addition, the considerations as regards the cut-off criteria for glyphosate according to Annex II of Regulation (EC) No 1107/2009 are summarised in Appendix A.

A key supporting document to this conclusion is the Peer Review Report (EFSA, 2023a), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

- the comments received on the RAR;
- the reporting tables (17 February 2022);
- the evaluation tables (July 2023);
- the reports of the scientific consultation with MS experts, including their Annexes where relevant;
- the comments received on the assessment of the additional information;
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (AGG, 2023), and the Peer Review Report, both documents are considered as background documents to this conclusion and thus are made publicly available. In addition, the list of newly available publications on glyphosate brought to EFSA's attention after the public consultation phase until the time point of drafting the EFSA conclusion, and screened for potential impact on the risk assessment, is also made publicly available as part of the background documentation to the conclusion (EFSA, 2023b).

It is recommended that this conclusion and its background documents would not be accepted to support any registration outside the EU for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulation for representative uses

Glyphosate is the ISO common name for *N*-(phosphonomethyl)glycine (IUPAC).

The formulation for representative uses for the evaluation was 'MON 52276', a soluble concentrate (SL) containing 360 g/L of glyphosate as isopropylammonium salt (IUPAC name isopropylammonium *N*-(phosphonomethyl)glycinate) (486 g/L), plus co-formulants.

The representative uses evaluated are:

- pre-sowing, pre-planting and pre-emergence applications by tractor-mounted broadcast spraying in vegetables (root, tuberous, bulb, fruit-vegetable, *Brassica*, leaf and stem) and sugar beet against emerged annual, biennial and perennial weeds;
- post-harvest, pre-sowing and pre-planting applications by tractor-mounted broadcast spraying in vegetables (root, tuberous, bulb, fruit-vegetable, *Brassica*, leaf and stem) and sugar beet against emerged annual, biennial and perennial weeds and cereal volunteers;
- post-emergence of weeds inter-row application by ground-directed, fully shielded (hooded) spraying in vegetables (root, tuberous, bulb, fruit-vegetable, legume and leaf vegetables) against emerged annual, biennial and perennial weeds;
- post-emergence of weeds in-row band application by ground-directed, fully shielded (hooded) spraying in orchards (citrus, stone and pome fruits, kiwi, nut, banana and table olives) and vines (table and wine grape, leaves not intended for human consumption) against emerged annual, biennial and perennial weeds;
- train spray applications directed on railway tracks against emerged annual, biennial and perennial weeds;
- post-emergence-shielded spot treatment spray applications against invasive species (giant hogweed and Japanese knotweed) in agricultural and non-agricultural areas, and against couch grass in vegetables (root, tuberous, bulb, fruit-vegetable, *Brassica*, leaf and stem vegetable) and sugar beet for post-harvest, pre-sowing and pre-planting applications.

Full details of the Good Agricultural Practices (GAPs) can be found in the list of end points in Appendix B.

Data were submitted to conclude that the representative uses of glyphosate proposed at EU level result in a sufficient herbicidal effect following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014b).

The information on the active substance, co-formulants and isopropylammonium counter ion declared in the formulation for representative uses has all been considered for the assessments during the peer review.

As regards the literature search carried out by the applicants, there is evidence that the exclusion criteria for relevance of literature used by the applicants at the rapid screening were not properly applied, as also noted by the RMS. Reasons for having excluded several of the ecotoxicology-related publications identified by the literature search at the rapid screening step seemed not pertinent after reading the title and/or abstract. However, where subsequently identified as potentially relevant, these publications were added to the RAR and further assessed. Overall, considering that the public consultation also resulted in available scientific literature being assessed also from a broader time frame than that required by the regulatory framework, EFSA concludes that it is unlikely that relevant evidence from the peer-reviewed scientific literature has been missed by the peer review.

Conclusions of the evaluation

1. Identity, physical/chemical/technical properties and methods of analysis

The following guidance documents were followed in the production of this conclusion: European Commission (2000b, 2010, 2012).

An updated common EU **reference specification** was proposed by the RMS and the GRG comprising of eight applicants. The proposed common reference specification was based on batch data from industrial plant productions. The proposed minimum purity of the active substance as manufactured is 950 g/kg (the minimum purity for individual sources ranged from 950 to 990 g/kg). The technical grade active ingredient was manufactured in the majority of cases as a technical material (TC), but also as a technical concentrate (TK). Based on the data submitted in support of the renewal of approval process, an update of the common EU reference specification is proposed (i.e. two

additional relevant impurities were identified: triethylamine and formic acid, and some of the significant impurities were deleted from the specification). *N*-nitroso-glyphosate (NNG), formaldehyde, triethylamine and formic acid were considered relevant impurities at levels of < 1 mg/kg, < 1 g/kg, ≤ 2 g/kg and ≤ 4 g/kg, respectively (see Section 2). It is noted that the toxicological relevance of one impurity is inconclusive (see Section 2); hence additional data consisting of spectral data, content of the impurity before and after storage of the formulation and method for its analysis in the formulation might be required. The current and the proposed common reference specifications cannot be concluded as sufficiently supported by the toxicological information available, whilst the genotoxicity profile of one impurity needs clarification (see Section 2). The proposed reference specification is supported by the batches used in the ecotoxicological studies (see Section 5).

The proposed minimum purity of 950 g/kg met the requirements of the FAO specification 284/TC (2016), covering glyphosate technical materials of Monsanto, Cheminova, Syngenta and Helm. It should be noted that the FAO specification contains only NNG and formaldehyde as relevant impurities, with a higher specification level of 1.3 g/kg for formaldehyde.

For each source, an individual technical specification was derived based on the batch data submitted for the renewal. The RMS compared each individual source specification to the newly proposed EU reference specification according to the criteria given in the guidance document SANCO/10597/2003 rev. 10.1 (European Commission, 2012) and concluded that they were equivalent except from some sources, however EFSA notes that this equivalence check should be considered as provisional for all sources due to the inconclusive toxicological relevance of an impurity (see Section 2). Batch data were not submitted by applicant Ciech Sarzyna, therefore no further consideration could be made.

Some **data gaps** relevant to the specifications and batch analysis were set (see Section 10).

The main data regarding the identity of glyphosate and its physical and chemical properties are given in Appendix B. A **data gap** for *n*-octanol/water partition coefficient for the metabolite *N-acetyl* AMPA was identified. A **data gap** was also set for determination of the content of the relevant impurities: formic acid and triethylamine before and after 2-year storage at ambient temperature of the formulation for representative uses (see Section 10).

In general, adequate methods are available for the generation of data required for the risk assessment, except for specific plant residue studies for which EFSA considers that the efficiency for the extraction procedure used was not addressed according to SANTE/2017/10632 (European Commission, 2022).[6] The RMS disagrees. In addition, a **data gap** for validation data for the method used in a toxicological study was identified (see Sections 2 and 10).

Appropriate methods of analysis are available for the determination of the active substance and impurities in the technical material, and for the determination of the active substance and the relevant impurities formaldehyde, NNG, triethylamine and formic acid in the formulation for representative uses. Pending on the outcome of the data gap on toxicological data on a component of a co-formulant (see Sections 2 and 10), a method for its determination in the formulation might be required at MS level.

Appropriate liquid chromatography with tandem mass spectrometry (LC–MS/MS) methods are available for monitoring the components of the residue definition for food and feed of plant origin, with limits of quantification (LOQs) of 0.025 mg/kg for glyphosate, (aminomethyl)phosphonic acid (AMPA) and *N*-acetyl glyphosate in all representative commodity groups. It should be noted that different options for the residue definition for enforcement for plant matrices are proposed to risk managers for consideration (see Section 3).

Residues of glyphosate and *N*-acetyl glyphosate can be monitored in food of animal origin by the LC–MS/MS method with LOQs of 0.025 mg/kg in meat, milk, egg, liver, kidney and fat, respectively. Residues of glyphosate and AMPA in honey can be determined by the LC–MS/MS method with a LOQ of 0.025 mg/kg for each analyte. However, *N*-acetyl glyphosate was also included in the residue definition for monitoring in honey; therefore, a validated monitoring method for *N*-acetyl glyphosate residues in honey is needed (**data gap**, see Section 10). It is noted that different options for the residue definition for enforcement in honey are proposed to risk managers for consideration (see Section 3).

The residue definition for monitoring in soil was defined as glyphosate and AMPA. The compounds of the residue definition in soil can be monitored by LC–MS/MS, with LOQs of 0.05 mg/kg for both compounds. An appropriate LC–MS/MS method is available for monitoring residues of glyphosate and AMPA in groundwater, drinking water and surface water with LOQs of 0.03 μg/L for both substances. Residues of glyphosate in air can be monitored by gas chromatography–mass spectrometry (GC–MS) with a LOQ of 5 $\mu g/m^3$.

[6] See Evaluation Table, section 1, open point 1.31 (EFSA, 2023a).

Residues of glyphosate and AMPA in body fluids can be monitored by LC–MS/MS with LOQs of 0.01 mg/L, while residues of glyphosate and AMPA in tissues can be determined by the LC–MS/MS method with LOQs of 0.025 mg/kg for each analyte.

2. Mammalian toxicity

The toxicological profile of glyphosate and its metabolites was discussed at the Pesticides Peer Review Experts' Teleconference (TC) 80 in November–December 2022. The following guidance documents were followed in the production of this conclusion: European Commission (2003, 2012), EFSA (2014b), EFSA PPR Panel (2017), EFSA (2022) and ECHA (2017).

The assessment relies on studies submitted by the applicants and carried out according to internationally agreed guidelines and quality standards, as well as on relevant studies from peer reviewed scientific literature. Studies using formulated products other than the one for the representative uses as test material were considered for their reliability and relevance, and discussed as part of the weight of evidence (WoE) in the risk assessment for the active substance and the formulation for representative uses.

Regarding the proposed **reference specification**, the impurities *N*-nitroso-glyphosate (NNG), formaldehyde, triethylamine and formic acid are identified as relevant (see Section 1) based on their hazard properties, as classified according to Annex VI of Regulation (EC) No 1272/2008[7] (CLP Regulation). Regarding the other impurities occurring in batches from the different manufacturing sources, none were found to be relevant, except for one impurity, which showed a potential for clastogenicity in an *in vitro* chromosome aberration test that was not appropriately followed up *in vivo*. Therefore, the toxicological relevance for this impurity is inconclusive (**data gap**, see Section 9.1). This impurity was present in some of the batches used in toxicity studies at levels representative of the proposed reference specification, however its maximum level in any of the specifications cannot be established while its genotoxicity profile has not been clarified. Accordingly, the assessment of any reference specification cannot be finalised (see Section 9.1).[8] The RMS disagrees with this conclusion and considers the genotoxic potential not to be of toxicological concern at the level of the proposed reference specification, since the impurity was present at a 7-fold higher level than that proposed for the reference specification in one *in vivo* micronucleus test performed with glyphosate. It is noted that the relevance assessment of the impurities was based on toxicological studies and quantitative structure–activity relationship (QSAR) analysis; a detailed summary of the QSAR assessment has not been provided by the applicants and was identified as a **data gap** (see Section 10). Another **data gap** was identified for clarification on the composition of some of the batches used in the toxicological studies (see Section 10).

The analytical methods used in feed, body fluids and tissues, air and any additional matrices in support of the critical toxicity studies used to set reference values are overall considered fit-for-purpose (see Section 1). A **data gap** was identified due to the lack of the analytical report including information on the analytical method validation in a toxicological study (see Sections 1 and 10).

The **oral absorption** of glyphosate is estimated to account for 20% of the administered doses (in the range between 1 and 10 mg/kg body weight (bw)). **Excretion** occurs predominantly through faeces and to a lesser extent in urine and it is almost completed within 48 h; biliary and pulmonary routes of elimination are negligible. In rats, glyphosate is rapidly **distributed**, with the highest levels being reached in bones, kidneys and liver; the evidence does not suggest bioaccumulation in mammals.[9] The **metabolism** of glyphosate is limited; less than 1% of the parent compound is eliminated as AMPA and major rat metabolites were not detected in the available studies. Based on comparative *in vitro* metabolism, major metabolic interspecies (mouse, rat, rabbit, dog) differences were not observed and unique human metabolites were not identified.

The **residue definition** for body fluids and tissues consists of glyphosate and AMPA.

Glyphosate has low **acute toxicity** by the oral, dermal and inhalation exposure routes. Clinical signs including diarrhoea, reduced activity, ataxia, piloerection, convulsions, and hunched posture were observed in rats and mice only following acute oral exposure to > 2,000 mg/kg bw. Glyphosate does not have skin irritating or sensitising properties. It is a severe eye irritant (ECHA, 2022). Testing for

[7] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1–1355.
[8] See experts' consultation point 2.36 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[9] See experts' consultation point 2.1 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).

phototoxicity is not required for glyphosate in accordance with data requirement provisions stipulated in Commission Regulation (EU) No 283/2013. The currently available data do not give rise to any concern between glyphosate exposure and respiratory health effects (i.e. irritation and sensitisation).[10] The ECHA Committee for Risk Assessment (RAC) (ECHA, 2022) concluded that there were no clear human data to support classification for respiratory tract irritation and no specific data which clearly indicated respiratory tract irritation in studies with animals. For respiratory sensitisation, RAC considered that no classification is warranted based on insufficient data.

Many **short-term oral** toxicity studies were provided for rats, mice and dogs. The dog and the rat were the most sensitive species, followed by the mouse. Common target organs/critical effects for toxicity included the gastrointestinal tract, decreased body weight gain and reduced food consumption, and changes in clinical chemistry including increased alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in plasma, possibly indicative of altered liver metabolism. Effects in salivary glands, consisting of cellular alterations in the parotid gland (basophilic staining of the cytoplasm and hypertrophy not associated with degeneration/necrosis inflammatory conditions and not progressing to preneoplastic lesions in long-term studies), were observed in rodents. They were considered as a local effect of unclear adversity based on the nature of the histopathological characteristics with lack of clinical correlates in rodents and of unclear human relevance.[11] The relevant short-term no observed adverse effect level (NOAEL) in dog is 53 mg/kg bw per day based on decreased food consumption, increased gamma-glutamyl transferase (GGT), increased ALP and bilirubin at the lowest observable adverse effect level (LOAEL) of 252 mg/kg bw per day in a 90-day repeated dose toxicity study. In rats, the relevant short-term oral NOAEL is 79 mg/kg bw per day, based on effects on the caecum (i.e., mucosal atrophy) and increased ALP reported at the LOAEL of 730 mg/kg bw per day in a 90-day repeated dose toxicity study. The relevant short-term oral NOAEL in mice is 1,221 mg/kg bw per day derived from a 90-day repeat dose toxicity study, based on decreased food consumption, liver effects (increased ALP), caecum (distension not accompanied by histopathological changes) and increased incidence of cystitis in the urinary bladder, reported at the LOAEL of 6,295 mg/kg bw per day.

Glyphosate is unlikely to be **genotoxic** based on a WoE approach[12]; this is in line with ECHA RAC assessment (ECHA, 2022).

After **long-term exposure**, target organs/critical effects regarding toxicity included the gastro-intestinal tract, salivary glands (local effects), eyes, liver and lungs in rats; and reduced body weight gain and urinary bladder in mice, with higher dose levels producing liver and kidney lesions, stomach cysts and increased mortality in mice.[13] The relevant long-term NOAEL is 59.4 mg/kg bw per day based on increased incidences of liver (small livers, focal haemorrhage small cyst, and pale and mottled appearance) and lung (emphysema, collapse, petechiae and ecchymoses) lesions, increased ALP and cataracts observed at the LOAEL of 595.2 mg/kg bw per day in a 2-year study in rats.[14] Lower LOAELs were identified ranging from 300 to 362 mg/kg bw per day in other long-term studies for stomach mucosal irritation,[15] increased caecum weight, clinical chemistry (increase ALP) and decreased adrenal weight. Glyphosate may induce oxidative stress as shown in some *in vitro* and *in vivo* studies, but increased oxidative stress was not consistently demonstrated in the available studies. Regarding epidemiological studies investigating oxidative stress endpoints, a conclusion could not be drawn on the possible relationship between glyphosate exposure and changes in oxidative stress parameters based on the limited database and outcome from available human observational studies.[16] Based on all the available evidence, it was agreed that glyphosate is not carcinogenic in rats up to the highest dose level tested of 1,214 mg/kg bw per day in males and 1,498 mg/kg bw per day in females. In the mouse studies, no carcinogenic effects were seen up to 988 mg/kg bw per day in males and 1,081 mg/kg bw per day in females.[17] The currently available human epidemiological studies do not provide conclusive evidence that glyphosate exposure is associated with any cancer-related health effect.[18] ECHA RAC concluded that glyphosate is unlikely to be carcinogenic for humans (ECHA, 2022).

[10] See experts' consultation point 2.32 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[11] See experts' consultation points 2.2 and 2.15 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[12] See experts' consultation points 2.1, 2.2, 2.3, 2.4 (identified following comments by public) and experts' consultation 2.17 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[13] See experts' consultation point 2.16 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[14] See experts' consultation point 2.14 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[15] See experts' consultation point 2.18 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[16] See experts' consultation point 2.17 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[17] See experts' consultation point 2.5 (identified following comments by the public) at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[18] See experts' consultation point 2.19 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).

With regard to **reproductive toxicity** studies, the relevant reproductive toxicity NOAEL is 351 mg/kg bw per day, based on decrease in homogenised resistant spermatid count in F0 males observed at the limit dose of 1,063 mg/kg bw per day in a two-generation reproductive toxicity study in rats. For offspring toxicity, the relevant NOAEL is 293 mg/kg bw per day, based on reduced body weight observed at the LOAEL of 985 mg/kg bw per day in another two-generation toxicity study in rats. For parental toxicity, the relevant NOAEL is 417 mg/kg bw per day, based on increased liver and kidney weights observed at the LOAEL of 2,151 mg/kg bw per day in a further two-generation toxicity study in rats.[19,20] From the assessment of currently available human epidemiological studies, no conclusions could be drawn on a causal association between glyphosate exposure and effects on reproductive endpoints.[21]

With regard to **developmental toxicity**, the relevant maternal toxicity NOAEL is 300 mg/kg bw per day, based on findings observed at 1,000 mg/kg bw per day in two rat developmental toxicity studies, including clinical signs (in both studies); the relevant developmental toxicity NOAEL is 300 mg/kg bw per day, based on reduced ossification and skeletal variations in foetuses observed in a rat developmental toxicity study at 1,000 mg/kg bw per day.

With regard to fetal development in rabbits, no teratogenic effect was observed. The relevant NOAELs for developmental and maternal toxicity were identified in a rabbit developmental toxicity study. For developmental toxicity, a NOAEL of 150 mg/kg per day was identified, based on increased incidence of post-implantation loss at 450 mg/kg bw per day and reduced fetal weight at 300 mg/kg bw per day; the relevant maternal toxicity NOAEL is 50 mg/kg bw per day based on reduced body weight gain between gestation days 11 to 29.[22]

In 2022, the ECHA RAC Committee (ECHA, 2022) concluded that no classification is warranted for adverse effects on reproduction and development.

There is no indication of **neurotoxicity** potential of glyphosate from one acute and two subchronic toxicity studies in rats and one delayed neurotoxicity study in domestic hens. The overall NOAEL is 1,000 mg/kg bw for acute systemic toxicity and 2,000 mg/kg bw (highest tested dose) for acute neurotoxicity; the NOAEL for subchronic systemic toxicity is 395 mg/kg bw per day based on reduced body weight gain and food consumption, while in the absence of neurotoxicity findings in the 90-day neurotoxicity study in rats, the NOAEL for subchronic neurotoxicity is 1,499 mg/kg bw per day (highest tested dose).

There is insufficient evidence of an effect of glyphosate active substance and glyphosate-based herbicides (GBHs) on neurotransmitters.[23] The integration of human observational studies with the limited experimental evidence from *in vitro* and *in vivo* studies does not trigger a concern for parkinsonism.[23] From the epidemiological studies, insufficient evidence on the possible association between glyphosate exposure and autism spectrum disorder (ASD) or amyotrophic lateral sclerosis (ALS) was concluded.[23] A **developmental neurotoxicity study** (DNT) with glyphosate is not present in the dossier and considered not needed based on the lack of neurotoxicity effects in the regulatory dataset on glyphosate active substance. New evidence on glyphosate was highlighted during the experts' meeting discussion[24]: an *in vivo* study in rats where DNT-related endpoints were assessed and considered as not affected by the high doses administered to dams (2.16 and 4.65 g/kg bw per day during gestation and lactation period, respectively), and ToxCast/Tox 21 data, where glyphosate was not showing any activity in all tested *in vitro* assays, except for one parameter at high concentrations (i.e. AC_{50} 31.7 μM). Additional data, including public literature studies on GBHs and studies on other glyphosate salts (including glyphosate-trimesium), showing some DNT effects, were also assessed by the peer review.[23] Considering the overall body of evidence, a pattern of effects suggesting DNT liabilities was not clearly identified for glyphosate and the current toxicological reference values were considered protective. However, a **data gap** is identified for the applicants to clarify the cause of the DNT effects seen in the public literature studies with GBHs and in the study with glyphosate-trimesium (see Section 10).

[19] See experts' consultation point 2.20 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[20] See experts' consultation point 2.22 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[21] See experts' consultation point 2.7 (identified following comments by the public) at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[22] See experts' consultation point 2.21 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[23] See experts' consultation point 2.27 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[24] See experts' consultation point 2.27 and Annex 8 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).

There are no indications of **immunotoxicity** potential for glyphosate in the available 28-day toxicity study in female mice; a NOAEL of 1,448 mg/kg bw per day (highest tested dose) has been derived.[25]

Several studies from the published literature investigated the potential effects of glyphosate on the **human and animal gut microbiome**, and possible consequent effects on health. Based on the current state of knowledge, considering that standardised regulatory guidance and/or established harmonised criteria are currently not available for the assessment of microbiome, no definitive conclusions can be drawn from these studies. However, the available mammalian toxicity dataset supports a sufficiently protective assessment for any health impact possibly mediated by the microbiome on humans, livestock and pet animals. Consistently, the previous conclusions on the lack of impact of glyphosate on animal gut microbiome and health (EFSA, 2018a) remain valid. Further developments are needed to understand the importance of the microbiome in risk assessment and identify dedicated strategies and methodologies accordingly (Merten et al., 2020).[26]

The impact of glyphosate on the microbiome was also discussed at the Pesticides Peer Review Experts' TC 82 on ecotoxicology and similar conclusions were reached.

Toxicological reference values (TRVs) have been derived for glyphosate[27] as follows. The **acceptable daily intake** (**ADI**) is 0.5 mg/kg bw per day, based on a NOAEL of 53 mg/kg bw per day from a 90-day study in dogs. The ADI is supported by the NOAEL of 59.4 mg/kg bw per day from a 2-year rat study and covering the NOAEL of 50 mg/kg bw per day for maternal toxicity identified in a rabbit developmental toxicity study. The standard uncertainty factor (UF) of 100 was applied. Glyphosate-induced effects on the salivary glands in rodents are likely to be a local effect of unclear adversity and human relevance, that were considered as not relevant for the derivation of TRVs.[28] The **acute reference dose** (**ARfD**) is 1.5 mg/kg bw, based on a NOAEL for developmental effects of 150 mg/kg bw per day identified in a rabbit developmental toxicity study. The standard UF of 100 was applied. During the previous peer review of glyphosate (EFSA, 2015), maternal and developmental NOAELs from a rabbit developmental toxicity study were selected for the derivation of the previous ADI (0.5 mg/kg bw per day) and ARfD (0.5 mg/kg bw), respectively. In the current peer review process, the reliability of this rabbit developmental toxicity study was re-considered; another study, as reported above, was deemed as more appropriate to derive TRVs.[22]

The **acceptable operator exposure level** (**AOEL**) is 0.1 mg/kg bw per day, based on the same considerations as for the ADI, applying a correction factor for limited oral absorption of 20%. This value is the same as previously established by the peer review (EFSA, 2015).

The **acute AOEL** (**AAOEL**) is 0.3 mg/kg bw, based on the same point of departure as for setting the ARfD, applying a correction factor for limited oral absorption of 20%.

Regarding glyphosate **metabolites**, an overview of their toxicological profile can be found in Table 1 and in Table 3 in Section 7.

The metabolites AMPA, *N*-methyl AMPA and *N*-acetyl AMPA were concluded as unlikely to be genotoxic, based on the available data. For the minor metabolites in genetically modified (GM)-tolerant crops *N*-glyceryl AMPA and *N*-malonyl AMPA, the submitted QSAR analysis did not suggest any specific concern for genotoxicity. Nonetheless, the analysis itself was not sufficiently reliable to cover clastogenicity and aneugenicity potential (**data gap**, see Section 10).

As regards general toxicity, AMPA and *N*-acetyl AMPA displayed a similar qualitative and quantitative toxicological profile to glyphosate and the TRVs of glyphosate were concluded as applicable. For *N*-acetyl glyphosate, general toxicity was sufficiently investigated, while the aneugenic potential was not addressed (**data gap**, see Section 10). Since aneugenicity has a threshold-based mechanism and this metabolite is of no greater toxicity than glyphosate (similar toxicological profile), the same TRVs were concluded as applicable.

[25] See experts' consultation point 2.28 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[26] See experts' consultation point 2.30 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a). See also Annex 9 to experts' consultation point 2.30.
[27] See experts' consultation point 2.34 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[28] See experts' consultation point 2.15 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).

Table 1: Overview table of the toxicological profile of metabolites found as residues in livestock and/or crops

Metabolite	Genotoxicity	General toxicity Toxicological reference values (TRVs)	Additional source of human exposure[(a)] (e.g. groundwater)
AMPA	Unlikely to be genotoxic	TRVs of glyphosate apply	No
N-acetyl AMPA	Unlikely to be genotoxic	TRVs of glyphosate apply	No
N-acetyl glyphosate	Negative for both mutagenicity and clastogenicity; aneugenicity not sufficiently investigated (data gap)	TRVs of glyphosate apply	No
N-methyl AMPA	Unlikely to be genotoxic	No data, not needed for consumer risk assessment	No
N-glyceryl AMPA	Negative for mutagenicity. Clastogenicity and aneugenicity not sufficiently investigated(data gap)	No data, not needed for consumer risk assessment	No
N-malonyl AMPA	Negative for mutagenicity. Clastogenicity and aneugenicity not sufficiently investigated(data gap)	No data, not needed for consumer risk assessment	No

(a): As a groundwater metabolite please refer to the assessment summarised under Section 7.

Based on an *in vitro* study with human skin conducted with the formulation for representative uses, 'MON 52276', the **dermal absorption** values are 0.096% for the concentrate (360 g/L) and 0.23% and 0.68% for the two in-use dilutions (28.8 g/L and 2.4 g/L, respectively). Appropriate pro-rata corrections were applied when necessary for the representative uses under consideration.[29]

Based on the EFSA model predictions for tractor-mounted and hand-held application techniques, the operator **exposure estimates** are below the (A)AOEL for all representative uses, for an operator wearing workwear and no further personal protective equipment (PPE). Similarly, the predicted exposure levels for residents and bystanders (both adults and children) are lower than the (A)AOEL, without specific risk mitigation measure (considering the default buffer zone of 2–3 m), and the estimates for recreational exposure (in non-agricultural areas) are also below the AOEL. Different scenarios were considered for workers,[29] for which no re-entry is expected shortly after application for applications on bare-soil (pre-planting) or on railway tracks. For the uses on vegetables, the predicted worker exposure is below the AOEL for both tasks of inspection (2 or 8 h) and reaching/picking. For the uses in orchards crops and vines, considering the downward application of the herbicide, only re-entry for inspection (8 h) is considering relevant, also triggering exposure estimates below the AOEL (without the need of gloves). The same outcome applies to the uses on invasive species.

Based on the available biomonitoring studies, the estimated systemic exposure levels to glyphosate are all below the AOEL/AAOEL or ADI/ARfD for the EU population.[30] It is noted that existing uncertainties due to limited relevance and reliability of some data were addressed by using the P_{95}/max concentrations when available.[31]

With regard to the toxicological information available for the formulation for representative uses 'MON 52276', studies were performed on acute toxicity and genotoxicity endpoints. With regard to the co-formulants contained in 'MON 52276', toxicological studies were available for all components but one (present in significant amount in the final formulation). This component is exempted from REACH requirements because of its chemical nature. MS experts and the RMS considered that the available toxicological information is sufficient to conclude on the safety of 'MON 52276'. However, EFSA concludes that repeated-dose toxicity data for this component should be assessed to reach a final conclusion on the risk assessment of 'MON 52276' (a **data gap** has been identified by EFSA post-experts' meeting, see Section 10).

3. Residues

The assessment in the residue section is based on the following guidance documents: OECD (2009, 2011), European Commission (2011) and JMPR (2004, 2007). All data assessed as reliable that inform

[29] See experts' consultation point 2.35 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[30] See Annex 10 to experts' consultation point 2.33 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[31] See experts' consultation point 2.33 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).

on the defined data requirements, approval criteria[32] or criteria for product authorisation,[33] whether unpublished regulatory studies provided by the applicants or published peer reviewed scientific literature, have been used for the assessment of residues in plant and animal commodities. Where the test material used in an investigation was a formulated product, this information was assessed if relevant for the assessment of the active substance or the formulation for representative uses.

Glyphosate was discussed at the Pesticides Peer Review Experts' TC 83 on residues in November–December 2022.

The metabolism of glyphosate in primary crops was investigated in several crops, including genetically modified plants containing the CP4-EPSPS,[34] GOX[35] or GAT[36] modifications.

In conventional crops (non-tolerant),[37] acceptable metabolism studies were available for the categories fruit (lemon and grapes), cereal/grass crops (wheat) and pulses and oil seeds (soya bean, coffee). In addition, several other metabolism studies in fruit, root crops, cereals, pulses and sugar cane were considered supportive. The acceptable studies investigated the metabolism of ^{14}C-glyphosate when applied as soil (citrus, grapes, soya bean and coffee) and foliar treatment (grapes, wheat and coffee). Soil applied coffee experiments also investigated the metabolite ^{14}C-AMPA. Most studies were conducted with ^{14}C-glyphosate-trimesium. Evidence provided from the peer reviewed scientific literature (Jianmei et al., 2005; Satchivi et al., 2000) showed that no differences - neither in the rate nor the amount of glyphosate absorbed – were observed when compared with diammonium and isopropylammonium salt formulations. Therefore, all studies, regardless of the salt formulation, can be used to assess the metabolism of glyphosate in plants. Following soil application, the uptake of glyphosate was very low in comparison to when application was to foliage. Limited translocation was also observed after local foliar application. Unchanged glyphosate was observed as the major component with low amounts of AMPA (up to 6.4% TRR in soya bean straw). *N*-methyl AMPA, *N*-methyl glyphosate and methylphosphonic acid were only found in hydroponic experiments classified as supportive and were considered not needing further consideration with respect to the residues assessment.

Several studies with glyphosate-tolerant crops with CP4-EPSPS, with GOX and with GAT modifications were available. It is noted that the representative uses evaluated in the current renewal process do not include tolerant crops. Therefore, the studies were considered solely to complete the scientific assessment. Especially the studies with EPSPS and with GOX modifications confirm the metabolic picture found in the conventional crops. Some minor metabolites found in these modified crops (*N*-glyceryl AMPA, *N*-malonyl AMPA and *N*-methyl AMPA) were not considered relevant to conventional crops, but require screening for genotoxic potential to address the safety of glyphosate residues in tolerant crops (outstanding **data gaps** for *N*-glyceryl AMPA and *N*-malonyl AMPA; for *N*-methyl AMPA it was concluded that it is unlikely to be genotoxic; see Sections 2 and 10). In GAT modified crops, the specific metabolites *N*-acetyl glyphosate and *N*-acetyl AMPA were found.[38] It is noted that the aneugenic potential of *N*-acetyl glyphosate has not been addressed (**data gap**, see Sections 2 and 10).

Acceptable confined rotational crop studies dosed with radiolabelled glyphosate or glyphosate-trimesium in conventional crops are available for leafy crops (lettuce), root crops (radish and carrot) and cereals (wheat and barley). Several non-fully guideline compliant studies were supporting these results. The main residue component found in food and feed parts of the investigated conventional crops is the metabolite AMPA.

[32] Provided for in Article 4 of Regulation (EC) No 1107/2009.

[33] Set out in Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

[34] CP4-EPSPS: Tolerance to glyphosate is obtained by the introduction of a gene that codes for the expression of a modified EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) enzyme, making the plant insensitive towards glyphosate EPSPS inhibition. This modification is considered not to have an effect on the nature of the residues of glyphosate upon metabolisation by the plant.

[35] GOX: Glyphosate oxidoreductase, protein obtained by the introduction of a gene from *Ochrobactrum anthrop* acting by breaking down glyphosate to AMPA and glyoxylate, which have no herbicidal activity. This modification is considered not to have an effect on the nature of the residues of glyphosate upon metabolisation by the plant.

[36] GAT: Glyphosate *N*-acetyltransferase, protein obtained by the introduction of a gene from *Bacillus licheniformis*, giving rise to *N*-acetyl glyphosate which denotes no herbicidal activity. This modification is considered to affect the nature of the residues of glyphosate upon metabolisation by the plant by forming *N*-acetyl metabolites.

[37] Traditionally bred variety that does not exhibit resistance to glyphosate.

[38] Further information is given in the report of the Pesticides Peer Review Experts' TC 83 under experts' consultation point 3.4 (EFSA, 2023a) and in the EFSA Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for glyphosate according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2019).

The data selected as reliable are considered sufficient to elucidate the metabolic pathway and the nature of residues in plants (including those derived from soil residue uptake in crops planted in rotation) to cover all crop categories. Based on this evidence, separate **plant residue definitions** for **risk assessment** can be proposed for conventional crops: Sum of glyphosate and AMPA, expressed as glyphosate; and for glyphosate-tolerant crops: Sum of glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA, expressed as glyphosate. For **enforcement** purposes, two options are proposed for risk managers to consider. Both options address crops with glyphosate-tolerant modifications that were identified as being on the market in 2019 in the context of the Article 12 MRL review (EFSA, 2019) and consider specific metabolites that prevail in the crops. **Option 1** is according to Codex (FAO-WHO, 2019)[39] and relevant for soya bean, oilseed rape (OSR), maize (including sweet corn)[40]: Sum of glyphosate and *N*-acetyl glyphosate, expressed as glyphosate; and for all other crops: Glyphosate only. **Option 2** is according to the proposal in the EFSA MRL Art.12 Reasoned Opinion (EFSA, 2019) and relevant for soya bean, OSR, cotton, maize (including sweet corn), sugar beet[40]: sum of glyphosate, AMPA and *N*-acetyl glyphosate, expressed as glyphosate; for all other crops: glyphosate only.

Public peer reviewed studies[41] did not confirm the transfer of AMPA in relevant amounts to crops from sources other than the use of glyphosate based herbicide products, suggesting AMPA as a specific marker for glyphosate use. AMPA was found in rotational field trials (see below). This is in line with the assessment that AMPA is a good environmental marker for glyphosate (see Section 4). As further information on additional residue trials for the representative uses and on the magnitude of residues in rotational crops is required (data gap, see below), and depending on the outcome of these trials, AMPA might be a better marker compound **than glyphosate** and risk managers may further consider the need to include AMPA in the enforcement residue definition for plants.

A large number of residue trials in conventionally grown crops were submitted, in most of them samples were analysed for glyphosate and AMPA. Many of these residue trials deviated from guidance and/or the critical GAPs (cGAPs). Those residue trials that can be considered reliable, i.e. cGAP compliant and analysing for glyphosate and AMPA with a valid analytical method and supported by sufficient storage stability data, are given in the list of endpoints in Appendix B (for further information on the validity assessment see Appendix D of this conclusion) and the **data gaps** identified in line with the current guideline SANTE/2019/12752 (European Commission, 2019) are detailed in Section 10.

It is noted that the RMS and the MS experts present at the experts' meeting do not agree with the data gaps set by EFSA to provide a sufficient number of GAP compliant residue field trials that are supported by storage stability data and a validated analytical method for some individual crop groups, except for residue trials for table olives in Northern EU (NEU). Instead, they suggested a wider extrapolation from the existing data to all crop groups (except table olives) and also to address pre-sowing, pre-planting, pre-emergence and inter-row uses based on the argument that residues for glyphosate and AMPA were below the LOQ in all cases except that of table olives.[42] In EFSA's view, the data need to be provided for completeness even if, taking into account the large amount of data available, their current absence does not raise an area of concern. In addition, the different views between EFSA and RMS on the validity of several trials with respect to the interpretation of the application of the agreed demonstrated storage stability and the analytical method for AMPA have been reflected and transparently reported in Appendix D.

Processing studies were submitted demonstrating the stability of glyphosate and AMPA under standard conditions simulating food processing operations, and processing factors were proposed for several crop commodities (see Appendix B).

Confined rotational crop studies for glyphosate-tolerant rotational crops are not available and would be needed in case glyphosate-tolerant crops were ever authorised in the EU. For the uses in conventional crops, an interim report of a study on the magnitude of residues in rotational crops in lettuce, carrot and wheat (results for only maximum two plant back intervals) indicated that residues of AMPA were present in rotational crops at levels above the LOQ (LOQ = 0.025 mg/kg), and therefore the study should be completed to enable the full assessment of rotational crop residues (**data gap,** see Section 9.1). In addition, a **data gap** has been identified for sufficient studies

[39] FAO and WHO (2019)). Pesticide residues in food 2019 – Extra Joint FAO/WHO Meeting on Pesticide Residues Evaluation Part I: Residues. Rome. https://www.fao.org/publications/card/en/c/CA6010EN/

[40] Due to the potential presence of glyphosate tolerant sources in the market (e.g. imported products), risk managers should consider to apply the proposed residue definition to all the monitored samples from these crops.

[41] Eaton et al. (2022) and the therein referenced article Grandcoin et al. (2017).

[42] Further information is given in the report of the Pesticides Peer Review Experts' TC 83 under experts' consultation point 3.6 (EFSA, 2023a).

investigating the magnitude of residues in rotational crops (i.e. carrot, lettuce, wheat), as well as in additional crops, as appropriate. Given the limited data available, these data are considered necessary to finalise the consumer risk assessment (see Section 9.1).

Taking into account the residues from primary crops and the limited results from rotational crops, animal studies for all groups of livestock are triggered. Metabolism in ruminants and poultry was addressed in several studies administering radiolabelled forms of glyphosate alone (as such or as trimesium salt), as a mixture of glyphosate with AMPA (9:1) or as *N*-acetyl glyphosate. Despite some shortcomings, all studies were considered acceptable except those dosed with glyphosate (acid form) that deviated from the guideline. Overall, glyphosate is the main component of the residue and only one metabolite (AMPA), major in several matrices, has been identified in these studies. On this basis and considering only the representative non- GM plant uses, the **residue definition for risk assessment** in **animal** commodities is proposed as sum of glyphosate and AMPA, expressed as glyphosate. In view of future MRL-setting procedures and assuming that conventional and glyphosate-tolerant crops could be included in the animal diet, the residue definition should be extended as follows: sum of glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA, expressed as glyphosate. It is noted, that the aneugenic potential of *N*-acetyl glyphosate has not been addressed (**data gap**, see Sections 2 and 10). Given that the main compounds are good markers and considering that it cannot be excluded that livestock are fed with genetically GAT-modified crops imported from third countries, the **residue definition for enforcement** purposes in **animal** commodities is confirmed as sum of glyphosate and *N*-acetyl glyphosate, expressed as glyphosate, with the view of future MRL-setting procedures. Several feeding studies conducted on dairy cows and laying hens fed with the same substances as in the metabolism studies were submitted. A feeding study on pig using the glyphosate/AMPA mixture was also provided. The studies employing glyphosate-trimesium were not considered acceptable due to a non-valid analytical method and lack of scientific evidence addressing its comparable absorption with respect to glyphosate. The studies with the mixture of glyphosate and AMPA are valid and sufficient to exclude residues above the LOQ in animal commodities with regard to the representative uses. Based on the latter studies and the preliminary estimated residue intakes by livestock, MRLs were proposed for animal commodities. However, these proposals are based on the representative uses limited to conventional crops only and MRL proposals might be significantly changed if the nature and level of residues present in feed commodities from glyphosate-tolerant GM crops are taken into account.

According to the SANCO Technical guidelines for MRL setting in **honey** (European Commission, 2016), the same **residue definitions** as for plant commodities should be applicable. It is noted that a validated analytical method for monitoring of residues of *N*-acetyl glyphosate in honey (not originating from the representative use but that has the potential to be present in imported honey) is not available (**data gap**, see Sections 1 and 10). Recent valid field studies analysing glyphosate and AMPA in honey were presented and indicate the need to increase the current MRL of 0.05–15 mg/kg.

The consumer risk assessment limited to the representative uses was performed using the EFSA PRIMo version 3.1 and using the supervised trials median residue (STMR) and highest residue (HR) values derived for plants grown as primary and rotational crops and animal commodities. The maximum chronic intake was calculated to be 3% of the ADI (NL toddler) and the highest acute intake is 2% of the ARfD for honey and other apicultural products. These assessment results are provisional, and a finalisation is still pending the **data gaps** identified on rotational crops and consequently the update of the animal dietary burden calculation.

4. Environmental fate and behaviour

Glyphosate was discussed at the Pesticides Peer Review Experts' TC 81 in November 2022. All data assessed as reliable that inform on the defined data requirements, approval criteria or criteria for product authorisation, whether unpublished regulatory studies provided by the applicants or published peer reviewed scientific literature, have been used for the assessment of environmental fate and behaviour. Where the test material used in an investigation was a formulated product, this information coming from different formulations was assessed equally, independently of whether the material was 'MON 52276' or another formulation.

The rates of dissipation and degradation in the environmental matrices investigated were estimated using the FOCUS (2006) kinetics guidance. In **soil** laboratory incubations under aerobic conditions in the dark, glyphosate exhibited low to high persistence, forming the major (> 10% applied radioactivity

(AR)) metabolite AMPA (max. 42% AR), which exhibited moderate to very high persistence. Mineralisation of the phosphonomethyl ^{14}C radiolabel to carbon dioxide accounted for 17–71% AR after 70–364 days. The formation of unextractable residues (not extracted by aqueous ammonium hydroxide) for this radiolabel accounted for 2.5–22% AR after 14–364 days. In anaerobic soil incubations glyphosate was stable compared to aerobic incubation conditions. Under the conditions of a laboratory soil photolysis study the only metabolite reaching levels triggering assessment was AMPA. Glyphosate and AMPA both exhibited characteristics between having low mobility and being immobile in soil. It was concluded that the adsorption of both glyphosate and AMPA was not pH dependent. In satisfactory field dissipation studies carried out at two sites in Germany, one in Switzerland, one in Ontario (Canada) and two in California (USA) (spray applications to the soil surface on bare soil plots) glyphosate exhibited low to moderate persistence. Sample analyses were carried out for AMPA in addition to glyphosate. This confirmed that AMPA was a major soil metabolite also under field conditions (max. 49% as parent equivalents). However, reliable AMPA dissipation rates could not be estimated from the available field studies leading to the identification of a **data gap** (see Section 10). Consequently, the exposure assessment for the representative uses being assessed was completed with the available laboratory AMPA kinetic endpoints. Field study DegT50 values for glyphosate were derived following normalisation to FOCUS reference conditions (20°C and pF2 soil moisture) in line with the EFSA (2014a) DegT50 guidance for one of the German and both California USA trial sites. The glyphosate field data endpoints were combined with laboratory values to derive modelling endpoints in line with the DegT50 guidance. The peer review confirmed the RMS assessment that soil degradation of glyphosate was best described by biphasic kinetics (except for an incubation in one soil) and that both glyphosate and AMPA degradation was pH dependent, with both compounds degrading more slowly under acidic soil conditions than when soil pH was in the neutral to alkaline range. The experts at the Pesticides Peer Review Experts' TC 81 agreed the use of the kinetic endpoints from the experiments that represented the slowest degradation (and fastest degradation for glyphosate when AMPA is kinetically generated from its glyphosate precursor), be used for exposure modelling for assessing the representative uses at EU level. This approach ensures that assessments covered use situations in acidic soils where degradation was slower, but also neutral/ alkaline conditions where the formation of AMPA might be greater. However, they agreed that if refinement would be needed for other uses in future exposure assessments, geomean soil DegT50 values should be used, splitting the dataset of reliable kinetic endpoints using the geomean value below $pH_{(water)}$ 6.5 to cover fields/areas with acidic soil conditions, and those above this value for alkaline fields/areas. The geomean endpoints that result from this approach have been included in Appendix B. It was agreed to use the arithmetic mean kinetic formation fraction for AMPA from glyphosate from all reliable soils in exposure modelling, independent of the pH of the soil incubation.

In laboratory incubations in dark aerobic natural sediment **water** systems, glyphosate exhibited moderate to high persistence, forming the major metabolites AMPA (max. 16% AR in water and 19% AR in sediment) and HMPA (max. 10% AR in water). Like glyphosate, these two metabolites also exhibited moderate to high persistence. The unextractable sediment fraction (not extracted by aqueous monopotassium phosphate or aqueous sodium hydroxide) was a sink for the phosphonomethyl ^{14}C radiolabel, accounting for 14–22% AR at study end (100 days). Mineralisation of this radiolabel accounted for 6–48% AR at the end of the studies. In incubations where AMPA was applied as test substance, two further unidentified sediment metabolites were elucidated and ascribed the identifiers P1a and M3.3; they were estimated (estimates agreed in the Pesticides Peer Review Experts' TC 81) to have the potential to be formed at levels triggering exposure assessment at 14% and 6% of glyphosate respectively (as glyphosate molecular weight equivalents). The rate of decline of glyphosate in laboratory sterile aqueous photolysis experiments was enhanced compared to that in dark controls, with AMPA and methanediol being formed at up to 20% and 52% respectively. According to EFSA PPR Panel (2013) guidance on aquatic risk assessment and to European Commission (2003) guidance on the relevance of groundwater metabolites, the simple chemical structure of methanediol means it is considered to be not (eco)toxicologically relevant, and therefore of low risk or non-relevant. The necessary surface water and sediment exposure assessments (predicted environmental concentration (PEC) calculations) were carried out for the metabolites AMPA, HMPA, P1a and M3.3 as well as for glyphosate, using the FOCUS (2001) step 1 and step 2 approach (version 3.2 of the Steps 1–2 in FOCUS calculator). In addition for

glyphosate, appropriate step 3 (FOCUS, 2001) results were available.[43] For the representative use on railways, PEC were also available using the model and scenario parameterised in HardSpec[44] that represents UK civil engineering and climatic conditions.

The necessary groundwater exposure assessments were appropriately carried out using FOCUS (European Commission, 2014a) scenarios and the models PEARL 4.4.4, PELMO 5.5.3 and MACRO 5.5.4.[43] For the representative use on railways, PEC were also available using the model and scenario parameterised in HardSpec that represents UK civil engineering and climatic conditions. The potential for groundwater exposure from the representative uses by glyphosate and AMPA above the parametric drinking water limit of 0.1 μg/L was concluded to be low in geoclimatic situations that are represented by all 9 FOCUS groundwater scenarios and the HardSpec groundwater scenario. In a targeted monitoring study conducted in Sweden and peer reviewed in a scientific literature article (Cederlund, 2022), groundwater sampling wells (3 to 6 per site) were installed at 12 sites associated with railways; a total of 603 groundwater samples were collected in two different periods (2007–2010 and 2015–2019) and analysed for glyphosate and AMPA. Useful results were derived for wells adjacent to the railway down gradient regarding groundwater flow direction (i.e. those that have not been over sprayed so not below the rail track which were potentially influenced by preferential flow pathways). It was concluded that this information supported the exposure assessment for the single use pattern set out in the good agricultural practice table (1 × 1.8 kg a.s./ha) regarding the representative use on railways and the Swedish conditions in these periods. The results provide reassurance that groundwater exposure to glyphosate and AMPA above the parametric drinking water value of 0.1 μg/L generally did not occur in the monitored situations.

The applicants provided appropriate information to address the effect of water treatment processes on the nature of the residues that are present in surface water, when surface water is abstracted for the production of drinking water. The conclusion of this consideration was that consequent to oxidation at the disinfection stage of usual water treatment processes, glyphosate and its degradation products that trigger assessment (AMPA and HMPA) produce low molecular weight compounds with simple structures common to the degradation of naturally occurring substances in raw water, such as amino acids. The compounds identified were concluded as not being of toxicological concern.

A comprehensive review of environmental **monitoring** data, including collection of public monitoring data (raw data and aggregated data from national authorities and any regional/national agencies or research institutes) as well as open literature data was available. The monitoring reports and the published peer-reviewed papers covered the monitoring of glyphosate and its main metabolite AMPA in soil, groundwater, surface water, transitional/tidal water, sediment, drinking water and air across several European countries and different temporal scales, ranging from a single sampling occasion to multi-monthly and annual sampling schemes. The data from public monitoring have been collated and analysed by the applicants with regard to compliance with regulatory triggers (i.e. Regulatory Acceptable Concentrations (RAC) or the Drinking Water Directive[45] thresholds or Acceptable Daily Intake), considering that the whole EU data set was large enough to capture a range of agronomic, geographical, pedoclimatic and hydrogeological situations, as well as providing a good temporal coverage allowing assessment of the state of a compartment in different seasons and hydrological regimes. The applicants' approach to assess the environmental monitoring data and the reported conclusions were discussed at the Pesticides Peer Review Experts' TC 81.[46] Overall, the experts agreed that the monitoring datasets available for all the environmental compartments for glyphosate and AMPA were insufficient to use for exposure assessments in the EU regulatory framework and be assessed against a regulatory exposure assessment goal without additional information being provided (e.g. aspects such as agricultural context, including farmer usage of plant protection products, or site characterisation such as hydrogeological information). Because they are not aimed at fulfilling any higher tier assessment requirements, the results need to be taken with caution. In particular, the peer review agreed that the available groundwater monitoring data for glyphosate and AMPA cannot be used to overrule the available FOCUS PEC_{gw} values in the regulatory

[43] Simulations utilised the agreed Q10 of 2.58 (following EFSA, 2008) and Walker equation coefficient of 0.7.

[44] Hollis, Ramwell, Holman and Whelan, HardSPEC A First-tier Model for Estimating Surface- and Ground-Water Exposure resulting from Herbicides applied to Hard Surfaces. Updated Technical Guidance on Model Principles and Application for version 1.4.3.2. Version 2.1 April 2017. https://www.hse.gov.uk/pesticides/pesticides-registration/data-requirements-handbook/fate/hardspec/HardSPEC_Guidance.pdf

[45] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32–54.

[46] See experts' consultation points 4.3, 4.4, 4.5, 4.6 and 4.7 at the Pesticides Peer Review Experts' TC 81 (EFSA, 2023a).

risk assessment of pesticides. Likewise, the measured concentrations of glyphosate and AMPA from public monitoring programmes or literature articles for the soil compartment are only valid for the time and place they represent and are not equivalent to the PEC_{soil} calculated for risk assessment purposes. The experts at the Pesticides Peer Review Experts' TC 81 also acknowledged that the large proportion of land treated with glyphosate may make the route of groundwater exposure via bank infiltration and the connectivity of surface water bodies to groundwater aquifers more important issues than for other active substances. As information to address this exposure route was not available, a **data gap** was identified. However, as there are small hydrological catchments and river catchments where hydrology means groundwater would not be significantly connected with ponds, ditches, streams and rivers, this is not always a consideration (see Section 10). Consequently, an assessment not finalised has not been identified. The monitoring data for surface waters indicated concentrations below the RAC values for glyphosate and AMPA (those described in Section 5) in a very high proportion of the samples in the dataset (about 99%). In the few cases where glyphosate concentrations were above the RAC, the sites had mostly been sampled only once. Only two sites had exceedances in consecutive samples. Overall, the peer review concluded that, for regulatory purposes, the available surface water monitoring data can only be considered as supportive. For the sediment monitoring, the limited dataset provided is not representative of the EU and a comparison of sediment concentrations with the RAC value is of limited use. Since transitional/tidal water is usually not accounted for in the regulatory assessment for active substance approval, the monitoring data related to this environmental compartment were considered as supportive only. The available data from individual drinking water samples were of limited value for assessment for the whole EU as unaggregated values only originated from a few countries. For the air compartment, a limited monitoring dataset for glyphosate and AMPA was available. Despite the limited monitoring information available, also considering the intrinsic properties of glyphosate defined according to FOCUS Guidance Air (FOCUS, 2008), particulate-bound concentration as a result of wind-eroded particle transport at the short and medium range, and medium range transport during periods of spraying due to the formation of aerosols are expected to occur. Long range atmospheric transport of glyphosate in the upper atmosphere is not expected to occur due to the atmospheric half-life estimated being below 2 days (regarding photochemical oxidative degradation in air, resulting from reaction with hydroxyl radicals present in the upper atmosphere). As for the other monitoring results, the monitored results from air samplers were considered difficult to equate directly to the representative uses being assessed.

The PEC in soil, surface water, sediment and groundwater covering the representative uses assessed can be found in Appendix B of this conclusion. A key to the wording used to describe the persistence and mobility of the compounds assessed can be found in Appendix C of this conclusion.

5. Ecotoxicology

The risk assessment was based on the following guidance documents: European Commission (2002)), SETAC (2001), EFSA (2009, 2013) and EFSA PPR Panel (2013).

Several aspects were discussed at the Pesticides Peer Review Experts' TC 82 in November – December 2022. The batches used in the regulatory dossier ecotoxicity studies were demonstrated to be in compliance with the proposed technical specification.

All data assessed as reliable and relevant for informing on the defined data requirements, approval criteria or criteria for product authorisation, whether unpublished regulatory dossier studies submitted by the applicants or published peer-reviewed scientific literature, have been used for the assessment of ecotoxicology and environmental risk. Where a formulated product was used in literature studies, it was necessary to understand the relevance of the tested formulation relative to the formulation for representative uses, 'MON 52276'. Therefore, the applicants were requested to provide the composition of formulations used in the literature studies together with a consideration of whether the tested formulation is comparable to the formulation for representative uses, 'MON 52276'. This was addressed for only a number of the tested formulations and an explanation was not provided to justify why it was not possible for other formulations. The lack of this information may represent a source of uncertainty regarding the selection of the endpoints for risk assessment. A **data gap** was identified (see Section 10).

The criteria followed by the RMS for the assessment of the relevance of the tested material[47] and for the relevance and reliability of the endpoints[48] were discussed in detail during the Pesticides Peer Review Experts' TC 82. As a result of the discussions, the RMS was requested to update their evaluations following the agreed criteria.

Pending on the outcome on the data gap identified in Section 2 for one of the components in the formulation for representative uses, further consideration to non-target organisms may be necessary.

For the risk assessment for **birds**, suitable acute and reproductive toxicity data were available with glyphosate. The reliability of the endpoints from the reproduction studies was discussed and agreed at the Pesticides Peer Review Experts' TC 82.[49] In addition, three scientific peer reviewed open literature studies providing sublethal endpoints were available and evaluated in the RAR. These studies were also discussed at the Pesticides Peer Review Experts' TC 82[50] but were not considered to provide endpoints for the risk assessment.

For the risk assessment for **wild mammals**, multiple acute toxicity studies with mammals were available and the appropriate acute endpoint for the risk assessment was discussed at the experts' meeting[51] where the experts agreed with the acute endpoint selected by the RMS. The experts also discussed and agreed on the appropriate endpoint to be used in the long-term risk assessment for wild mammals.[52] Acute toxicity data for mammals were available for the formulation for representative uses, 'MON 52276'.

The risk assessment for birds and mammals was conducted in line with EFSA (2009), however, several representative uses of 'MON 52276' are not explicitly covered by the guidance. Consequently, for some uses, the exposure assessment for birds and mammals was performed using surrogate scenarios. The available risk assessment demonstrated a low acute (screening-level) and long-term (screening or tier 1) risk to birds from dietary exposure to glyphosate for all representative uses. The acute risk to mammals, from dietary exposure, was also demonstrated to be low for all representative uses. The screening-level long-term risk assessment for mammals indicated a low risk for uses at 1×0.54 kg a.s./ha[53] and at 1×0.72 kg a.s./ha.[54] For all other representative uses the screening-level long-term assessment did not exclude a risk to wild mammals. The tier 1 risk assessment resulted in a high long-term risk only to small herbivorous mammals for all uses assessed.

The refined long-term risk assessment for small herbivorous mammals considered several options.

For the representative uses to railway tracks and for the spot applications to invasive species, various exposure refinements were agreed during the Pesticides Peer Review Experts' TC 82,[55] which resulted in a low long-term risk to mammals.

For the remaining representative uses, the applicants proposed two types of refinement (i.e. degradation of glyphosate on plant material and population modelling). These refinements were discussed at the Pesticides Peer Review Experts' TC 82.[56] Regarding the degradation value (DT_{50}) used in the exposure assessment, the experts agreed that there were insufficient reliable data to use the applicants' proposed value in a refined assessment. Nevertheless, the experts acknowledged that the data suggested that the degradation of glyphosate may be faster than assumed for a tier 1 exposure assessment. Regarding the population modelling, it was performed for the common vole (representing small herbivorous mammals) in orchards. The RMS provided an in-depth assessment of the modelling according to EFSA PPR Panel (2014). The experts agreed with the RMS that, while the model showed potential for being useful, the landscape assumptions and parametrisation of the modelling were not considered appropriate. As a result, the modelling was not used for the refined assessment. Overall, there were no reliable higher tier data deemed suitable for refining the long-term risk assessment to small herbivorous mammals. Considering the diversity and complexity of the list of representative uses

[47] See experts' consultation point 5.10 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[48] See experts' consultation points 5.12, 5.4 and 5.23 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[49] See experts' consultation point 5.2 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[50] See experts' consultation point 5.4 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[51] See experts' consultation point 5.3 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[52] See experts' consultation point 5.1 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[53] Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, sugar beet.
[54] Citrus orchards, stone fruit orchards and pome fruit orchards, kiwi, nut crops, banana, table olives, vines, root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, sugar beet, legume vegetables.
[55] See experts' consultation point 5.7 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[56] See experts' consultation point 5.6 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).

for glyphosate, the experts reconsidered the problem formulation by discussing which scenarios within the representative uses lead to exposure, hence risk, of small herbivorous mammals. The experts agreed that a small herbivorous mammal is likely to be exposed for the majority of the representative uses. The exceptions were for field crops[57] (i) where the product is applied pre-emergent of the crop (but post sowing/planting) and (ii) post-emergent but when the application is made before growth stage BBCH 20. For these two scenarios, a low long-term risk to mammals was concluded. For the remaining representative uses, a high long-term risk to mammals was concluded. For a complete overview of the outcome of the risk assessment for mammals, please see Section 9.3.

The experts at the meeting agreed with the RMS that the risk to birds and mammals from the formulation for representative uses ('MON 52267') was sufficiently addressed by the risk assessment carried out for the active substance given that the available acute toxicity data for mammals did not indicate increased toxicity.[58]

From plant metabolism studies, only metabolite AMPA was identified to require further risk assessment for birds and mammals (i.e. occurring at > 10% total radioactive residues (TRR)). The available risk assessment indicated a low risk for birds and mammals for all representative uses.[59] A low risk to birds and mammals via secondary poisoning was concluded since glyphosate and metabolites AMPA and HMPA have a log K_{ow} < 3, meaning that a quantitative risk assessment was not required. A low risk to birds and wild mammals from ingestion of contaminated water was concluded for all representative uses.

According to Commission Regulation (EU) No 283/2013[60], available and relevant data for terrestrial vertebrates, including amphibians and reptiles, if any, should be provided and taken into account in the risk assessment. Several scientific peer-reviewed open literature studies were available which investigated the effects of glyphosate formulations on **reptiles** and **terrestrial phases of amphibians**. As mentioned above, the criteria for assessing the relevance and reliability of the studies were discussed and agreed at the experts' meeting.[61] Few studies were considered to provide endpoints which are potentially relevant to populations. However, when considering the available information, adverse and biologically relevant endpoints were not obtained.

The available data package for assessing the effects of glyphosate as active substance or in formulations on **aquatic organisms** was notably large in size and diversity. Overall, more than 600 endpoints were available for about a hundred species.

Unpublished regulatory dossier studies provided by the applicants were available to address the effects of exposure via surface water to glyphosate and the formulation for representative uses 'MON 52276' to **fish**, **aquatic invertebrates**, **algae** and **macrophytes**. The formulation for representative uses 'MON 52276' was shown to be less toxic than glyphosate.[62] Therefore, the current risk assessment covers the formulation for representative uses. Chronically exposed fish were the aquatic organisms showing adverse effects at the lowest glyphosate concentration. The chronic fish endpoint to be used for risk assessment was discussed at the Pesticides Peer Review Experts' TC 82.[63] Despite many studies were retrieved from the scientific peer-reviewed open literature, none of those considered sufficiently reliable and relevant provided an endpoint lower than the one selected from unpublished regulatory dossier studies provided by the applicants.[64] Thus, the surface water Ecological Threshold Option Regulatory Acceptable Concentration (ETO-RAC = 0.1 mg a.e./L) was derived from the selected chronic fish endpoint (NOEC = 1 mg a.e./L). Based on this RAC and on the estimated predicted environmental concentrations (PEC_{sw}), a low risk due to exposure via surface water to fish, aquatic invertebrates, algae and macrophytes could be concluded for all the representative uses of glyphosate.

The effects of glyphosate (either as active substance or formulated) to the **aquatic stage of amphibians** were investigated in several studies retrieved from the open literature. A comparison of

[57] Field crops: Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, sugar beet.
[58] See experts' consultation points 5.3 and 5.4 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[59] See experts' consultation point 5.5 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[60] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1–84.
[61] See experts' consultation point 5.23 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[62] The only exception to this pattern was for macrophytes: in that case 'MON 52276' presented a slightly lower endpoint, which was retained for the risk assessment of glyphosate as well.
[63] See experts' consultation point 5.15 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[64] See experts' consultation point 5.13 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).

the hazard data with fish was carried out and discussed at the Pesticides Peer Review Experts' TC 82.[65] For acute, lethal effects, due to exposure to glyphosate, the lowest fish endpoint was agreed to be protective for amphibians. For chronic exposure to glyphosate, a proper comparison between fish and amphibians could not be carried out, since relevant and reliable chronic endpoints for amphibians were not available. A full comparability between fish and aquatic stages of amphibians would anyway be hampered by the different response types being measured for the two groups.

Unpublished regulatory dossier studies provided by the applicants were also available to address the toxicity of glyphosate due to exposure via contaminated sediment to **sediment-dwelling organisms**. Based on this information and on the estimated PEC_{sed}, a low risk due to sediment-borne exposure was concluded for all the representative uses of glyphosate.

One study from the public literature (Sesin et al., 2021)[66] investigated how a single glyphosate formulation would result in different levels of effect to aquatic macrophytes, depending on the route of exposure. In particular, the study highlighted that overspraying the emerged parts of the plants resulted in larger effects when compared to other routes of exposure, including the standard exposure via contaminated surface water normally considered in the risk assessment. The study was considered reliable and its general findings plausible, also in light of the mode of action (MoA) of glyphosate; however, a regulatory endpoint was not derived since the study is not relevant due to the test material (experiment was carried out with a formulation containing the surfactant polyethoxylated tallow amine); in addition, two non-standard species, whose general level of sensitivity is not known, were tested. Nonetheless, direct contact of the emerged parts of macrophytes via spray drift is likely to occur in the field for the representative uses, and the standard hazard assessment is not considered suitable to address this route of exposure for glyphosate. Considering the lack of further data, a **data gap** for addressing the risk to aquatic macrophytes due to contact exposure via spray drift of glyphosate was identified and this resulted in an assessment not finalised (see Section 9.1).

The available information was sufficient to conclude a low risk for **metabolites** AMPA and HMPA for all the representative uses of glyphosate. Data were not available for metabolites P1a and M3.3 which are expected to form in the sediment (see Section 4). Nonetheless, assuming as a worst-case M3.3/P1a as 10 times more toxic than the parent compound AMPA, and thus using the AMPA endpoint for sediment-dwelling organisms divided by a factor of 10 in a screening risk assessment, a low risk for all the representative uses of glyphosate was concluded.

A number of regulatory dossier toxicity studies provided by the applicants were available to address the effects of glyphosate and of the formulation for representative uses 'MON 52276' to honey **bees** (*Apis mellifera*). The available data package included all the required study types (i.e. acute oral and contact, chronic and larval toxicity studies). Since the necessary acute tests were available using the formulation 'MON 52276', the current risk assessment covers the formulation for representative uses. In addition, acute studies (oral and contact) were available to *Bombus terrestris* and an acute contact test was available to *Osmia bicornis* for glyphosate (test material glyphosate-isopropylammonium). Reliable and relevant information for lethal effects from scientific peer-reviewed open literature evaluated in the revised RAR did not indicate higher toxicity when compared to the regulatory studies.

The acute risk to honey bees in accordance with European Commission (2002) was concluded to be low for all the representative uses. Similarly, the risk from acute exposure was predicted to be low for all the representative uses when assessed in accordance with EFSA (2013) for honey bees and considering the available endpoints for the non-*Apis* bees. By using EFSA (2013), low chronic risk to adult and larvae honey bees was concluded for all the representative uses (at screening level risk assessment or Tier 1). Risk assessments for chronic exposure (adult and larvae) for non-*Apis* bees were not available.

A number of laboratory studies from scientific peer-reviewed open literature investigating different types of sublethal effects were available.[67] Furthermore, a colony feeder study, which included an assessment of sublethal effects, was available. However, with a lack of a quantified link between the observed effects and the consequences for the colony, the endpoints derived from these studies can be used to inform the overall assessment, but they could not be used for a quantitative risk assessment.

[65] See experts' consultation point 5.11 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[66] The reliability of this study and its impact on the risk assessment were discussed at the experts' meeting; see experts' consultation point 5.14 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[67] See experts' consultation point 5.16 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).

An assessment of the accumulative effects was not available. For the relevant plant metabolite AMPA, data gaps were identified in Section 3. Therefore, the potential occurrence of AMPA in pollen and nectar could not be estimated for a risk assessment to bees (**data gap**, see Section 10).

To address the risk for **non-target arthropods other than bees**, extended laboratory studies with the formulation for representative uses 'MON 52276' were available with the standard species, *Aphidius rhopalosiphi* and *Typhlodromus pyri*, as well as with the ground beetle *Poecilus cupreus* and the spider *Pardosa* sp.[68] Therefore, the current risk assessment covers the formulation for representative uses. Tier 1 (glass plate) studies with the standard species were only considered supporting; however, all experts agreed that the data set available was sufficient to perform the risk assessment according to European Commission (2002).[69] Relevant scientific peer-reviewed publications evaluating direct effects of glyphosate on non-target arthropods were not identified in the open literature in accordance with the criteria agreed at the Pesticides Peer Review Experts' TC 82. Based on the available data and risk assessment, low in- and off-field risk to non-target arthropods other than bees was concluded for all the representative uses of glyphosate.

Chronic toxicity studies were conducted with **earthworms** (*Eisenia fetida*), and **soil meso- and macrofauna** (the collembola *Folsomia candida* and the predatory mite *Hypoaspis aculeifer*) for the active substance and the formulation for representative uses 'MON 52276'. Furthermore, a chronic toxicity study was provided with the only pertinent soil metabolite of glyphosate (i.e. AMPA). The formulation for representative uses 'MON 52276' was not shown to be of higher toxicity than glyphosate. Therefore, the current risk assessment covers the formulation for representative uses. The endpoints used for risk assessment for soil organisms were agreed by the experts.[70] Relevant and reliable peer-reviewed publications evaluating direct effects of glyphosate on soil organisms were not identified in the open literature in accordance with the criteria agreed at the Pesticides Peer Review Experts' TC 82. Low chronic risk to earthworms and soil meso- and macrofauna (other than earthworms) was concluded for glyphosate and the metabolite AMPA for all representative uses. Studies on the effects of glyphosate, the formulation for representative uses and the metabolite AMPA on **soil microorganisms** were available. Based on the available data, which included a relevant peer-reviewed publication identified from the open literature, the risk to soil microorganisms from exposure to glyphosate, the formulation for representative uses and the metabolite AMPA was considered low for all representative uses.

Appropriate data for risk assessments for **non-target terrestrial plants** were available (i.e. vegetative vigour and seedling emergence tests with the formulation for representative uses 'MON 52276'). In accordance with the criteria as agreed at the Pesticides Peer Review Experts' TC 82,[71] relevant and reliable data from the scientific peer-reviewed open literature studies were not identified for non-target terrestrial plants.

The deterministic risk assessment considering the lowest available endpoint as agreed by the experts[71] indicated a high risk for all representative uses. However, a low risk for non-target terrestrial plants was identified for all representative uses by implementing appropriate risk mitigation measures. The required risk mitigation measures (5 to 10 m in-field non-sprayed buffer strip without or with combination of other drift reducing technology) vary with the different representative uses (see Section 8.1).

An assessment of risk to **biodiversity via indirect effects and trophic interactions** was submitted for the representative uses of glyphosate due to the specific condition related to effects on biodiversity laid down in Commission Implementing Regulation (EU) 2017/2324[72]. Such assessment considered the different environmental compartments and taxa (i.e. terrestrial vertebrates, aquatic organisms, bees, non-target arthropods, soil organisms and non-target terrestrial plants). It considered also risk mitigation and biodiversity conservation measures.

[68] The reliability of the available toxicity studies as well as the endpoints derived from some of the studies were discussed at the Pesticides Peer Review Experts' TC 82. See experts' consultation points 5.18, 5.19 and 5.20 for further details (EFSA, 2023a).
[69] See experts' consultation point 5.17 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[70] See experts' consultation point 5.21 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[71] See experts' consultation point 5.22 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[72] Commission Implementing Regulation (EU) 2017/2324 of 12 December 2017 renewing the approval of the active substance glyphosate in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJ L 333, 15.12.2017, p. 10–16.

The assessment was extensively discussed at the Pesticides Peer Review Experts' TC 82.[73] In general, the current lack of a harmonised approach to assess biodiversity within the prospective risk assessment was recognised by the experts. EFSA suggested that this is a general issue that could be addressed during the development and agreement on specific protection goals for non-target organisms.[74] The MS experts agreed with this suggestion, and they also agreed that the standard risk assessments address direct effects only, with the partial exception of aquatic organisms (see below for more details). In relation to the specific peer review of the submitted assessment, the discussion considered (i) the adequacy of the data collection, (ii) the criteria for the assessment of relevance and reliability of the available data, and (iii) the proposed mitigation measures.

Regarding the data collection, it was noted that a specific systematic literature search was not available, although requested to the applicants during the peer review process following the public consultation. Therefore, the assessment provided is lacking an appropriate problem formulation, search strategy and methodology. Overall, the experts considered the data presented of questionable scientific quality and of limited use to address the topic.

Regarding the assessment of relevance of the studies in relation to the representative uses, the experts agreed that the criteria are determined by the test material and the test conditions; therefore, it was agreed to re-evaluate the studies according to those criteria and consider them appropriately in the overall WoE.[75] It is noted that the relevance of the test material was not considered applicable for studies reporting indirect effects due to plant removal.

Regarding the risk mitigation measures, the applicants proposed the implementation of a multi-functional field margin (MFFM) in areas where more than 15 hectares are treated (for GAPs where 100% of the area is treated). Although this mitigation was considered as potentially useful, the experts noted that its effectiveness will be context and landscape dependent. The adequacy of the size limitation of the field (> 15 ha) and threshold for 100% of the treated area was not scientifically supported. The quality of the MFFM, in terms of structure and composition, was not specified and the extent to which the MFFM, and its quality, could mitigate effects on biodiversity was not quantified.

Based on the evaluation presented in the revised RAR by the RMS for the different groups of non-target organisms, most of the studies were considered to be of low relevance for the representative uses.

For terrestrial vertebrates, the studies reported evidence of negative indirect effects of glyphosate, but generally reversible (i.e. recovery occurred within a few years post-application). However, the dataset was considered too limited to reach a firm conclusion.

For aquatic organisms, the experts agreed that in principle the ETO-RAC is suitable to cover both direct and indirect effects including trophic interaction among the aquatic food chain, as indicated in the EFSA PPR Panel (2013). However, the experts noted that some specific issues (e.g. disruption of the biofilm, community shifts in microbes, effects via contact on emergent macrophytes via spray drift, indirect effects driven by direct effects occurring outside of the water system) are not currently covered in the EFSA PPR Panel (2013). Overall, for aquatic organisms, the dataset was also considered too limited to reach a conclusion for indirect effects not covered by the direct effects.

For bees, relevant studies investigating impact on indirect effects due to removal of weeds and the reduction of floral resources were not provided.

For terrestrial non-target plants, it was highlighted during the Pesticides Peer Review Experts' TC 82 that it would be necessary to ensure that the function of any MFFM would be effective. This would require measures to effectively limit the spray-drift reaching the MFFM.

Overall, the experts recognised that the risks associated with the representative uses of glyphosate for biodiversity are complex and depend on multiple factors. Furthermore, it was reflected that indirect effects as a result of removal of the target weeds are likely to be similar for any broad-spectrum herbicide used in the same manner. The experts also recognised that indirect effects on non-target organisms may not be addressed by the assessment of direct toxicity effects, i.e. a low risk based on standard toxicity effects cannot be used to exclude indirect effects. However, a quantification of direct toxic effects could be useful to understand the resulting effects on higher trophic levels. Overall, on the basis of the information provided, the experts agreed that a conclusion cannot be reached to

[73] See experts' consultation point 5.25 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[74] See experts' Annex '*Glyphosate: biodiversity assessment within the context of Regulation (EC) No 1107/2009: A discussion paper from the EFSA WG on Glyphosate (sub-group biodiversity)*' to the report of the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[75] See expert consultation point 5.10 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a) for the general discussion on the relevance of the formulations.

exclude possible negative impacts on non-target species, habitats and ecosystems due to indirect effects via trophic interactions for all the representative uses of glyphosate, including uses where less than 50% of the surface is treated (i.e. band and spot applications) and railway uses. The experts also recognised that risk mitigation measures for the off-field (i.e. the use of the proposed 75% drift reduction nozzles) as well as the implementation of a MFFM could be beneficial. A general **data gap** was identified to address several aspects (see Section 10). When addressing this general data gap, the issue should be considered more specifically for the different groups of non-target organisms, including a consideration of the effectiveness of possible risk mitigation measures at landscape level, for all the uses being assessed.

For the current assessment, studies were identified (both via literature search and submitted during the consultation phase) on the potential effects of glyphosate and formulations on the **microbiome of non-target organisms**. The information was assessed for relevance and reliability using criteria agreed during the Pesticides Peer Review Experts' TC 82.[76] The impact of glyphosate on the microbiome was discussed at the Pesticides Peer Review Experts' TC 82[77] and also at the Pesticides Peer Review Experts' TC 80[78] on mammalian toxicology. Only for bees, the studies identified were evaluated as relevant and reliable and responses due to glyphosate exposure on bees' gut microbiota identified, such as changes in the abundance of core microbial species. In particular, a decrease in abundance and growth of bee gut bacterium *Snodgrasella alvi* was observed. Generally, it was acknowledged that the relevance of these effects at the population level is unknown.

6. Endocrine disruption properties

The assessment of the endocrine disruption (ED) potential of glyphosate was discussed at the Pesticides Peer Review Experts' TC 84 on Mammalian Toxicology and Ecotoxicology joint session on endocrine disruption (December 2022) for both humans and non-target organisms.

In the context of the peer review, considering the extensive amount of data available in the RAR, both regulatory studies and studies retrieved through a systematic literature search, EFSA with the support of the EFSA Working Group on Endocrine Disruptors (EFSA ED WG) conducted an ED assessment in line with the ECHA/EFSA (2018) guidance using a structured approach. A number of studies were also available with formulated products other than the one for the representative uses. However, the criteria for the identification of endocrine disruptors, as laid down in Commission Regulation (EU) No 2018/605[79], do not apply to formulations. Therefore, studies with formulated products were only considered by the EFSA ED WG to understand their possible impact on the ED assessment of glyphosate active substance.

The approach used by EFSA and the RMS was not fully congruent in terms of data included in the ED assessment,[80] partially owing to slight difference in the assessment of relevance and reliability of the available data. Nevertheless, the overall outcomes reached individually by EFSA and the RMS were aligned and agreed by the experts at the Pesticides Peer Review Experts' TC 84.[81]

With regard to the assessment of the ED potential of glyphosate for both **humans and non-target organisms** according to the ECHA/EFSA (2018) guidance, in determining whether glyphosate interacts with the oestrogen, androgen, steroidogenesis (EAS) and thyroid (T) mediated pathways, the number and type of effects induced, and the magnitude and pattern of responses observed across the available information were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in overt toxicity. The assessment is therefore providing a WoE analysis of the potential interaction of glyphosate with the EAS and T signalling pathways, using the available evidence in the dataset.

For **humans**, with regard to the T-modality, the data set was considered complete and a pattern of T-mediated adversity was not identified. With regard to the EAS-modalities, the dataset was also considered complete and a pattern of EAS-mediated adversity was not observed.

[76] See experts' consultation points 5.10 and 5.12 at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[77] See experts' consultation point 5.1 (identified following comments by public) at the Pesticides Peer Review Experts' TC 82 (EFSA, 2023a).
[78] See experts' consultation point 2.30 at the Pesticides Peer Review Experts' TC 80 (EFSA, 2023a).
[79] Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties. OJ L 101, 20.4.2018, p. 33–36.
[80] The RMS did not consider in their assessment studies conducted with formulated products.
[81] See experts' consultation point 5.24 at the Pesticides Peer Review Experts' TC 84 (EFSA, 2023a) including its Annexes with the EFSA ED WG report.

In conclusion, based on the available information[82] and according to the ECHA/EFSA (2018) guidance, the ED criteria according to point 3.6.5 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605, are not met for the EAS- and T-modalities for the active substance glyphosate.[83]

For **mammals as non-target organisms**, the same conclusion drawn for humans was reached.

Regarding the **non-mammalian species**, several species and taxa were tested with glyphosate active substance including fish, birds, amphibians, reptiles, aquatic and terrestrial invertebrates, and many *in vivo* mechanistic, EATS-mediated and 'sensitive to but not diagnostic' parameters were investigated. Therefore, the dataset was considered, overall, complete for the investigation of both EATS-adversity and endocrine activity.

The overall WoE did not show any convincing pattern of **EATS-mediated adversity and/or endocrine activity.**

Although invertebrate non-target organisms are currently not fully addressed by the ECHA/EFSA (2018) guidance due to the lack of knowledge and test guidelines, especially at the mechanistic level, the guidance recommends evaluating the data with invertebrates when available by applying the general principles of the guidance. Although a clear pattern of adversity attributable to an ED MoA was not observed, in general, a clear conclusion on the ED potential of glyphosate on invertebrates could not be drawn as, in the vast majority of the studies, there was no clear dose- response and several drawbacks were noted in the studies impacting their reliability.

Overall, based on the available evidence and assessment, glyphosate does not meet the criteria for the EATS-modalities as laid down in point 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.

[82] See experts' consultation point 2.29 at the Pesticides Peer Review Experts' TC 84 (EFSA, 2023a).
[83] See experts' consultation point 2.23 at the Pesticides Peer Review Experts' TC 84 (EFSA, 2023a).

7. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 2–5)

Table 2: Soil

Compound (name and/or code)	Ecotoxicology
Glyphosate	Low risk to soil organisms
AMPA	Low risk to soil organisms

Table 3: Groundwater[(a)]

Compound (name and/or code)	> 0.1 µg/L at 1 m depth for the representative uses[(b)] Step 2	Biological (pesticidal) activity/relevance Step 3a.	Hazard identified Steps 3b. and 3c.	Consumer RA triggered Steps 4 and 5	Human health relevance
Glyphosate	No	Yes	–	–	Yes
AMPA	No	No, though assessment not triggered	No Unlikely to be genotoxic; Same TRVs as glyphosate apply	No	Assessment not triggered

(a): Assessment according to European Commission guidance of the relevance of groundwater metabolites (2003).
(b): FOCUS scenarios or a relevant lysimeter.

Table 4: Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Glyphosate	Low risk to aquatic and sediment-dwelling organisms via surface water and sediment **Data gap** for the risk to aquatic macrophytes due to contact exposure via spray drift
AMPA	Low risk to aquatic and sediment-dwelling organisms
HMPA	Low risk to aquatic and sediment-dwelling organisms
P1a (sediment only)	Low risk to sediment-dwelling organisms
M3.3 (sediment only)	Low risk to sediment-dwelling organisms

Table 5: Air

Compound (name and/or code)	Toxicology
Glyphosate	> 5 mg/L air/4 h (nose-only)

8. Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of MS and/or applicant's proposal(s) during the peer review, if any, are presented in this section (see Table 6). These measures applicable for human health and/or the environment leading to a reduction of exposure levels of operators, workers, bystanders/residents, environmental compartments and/or non-target organisms for the representative uses are listed below. The list may also cover any RMMs as appropriate, leading to an acceptable level of risks for the respective non-target organisms.

It is noted that final decisions on the need of RMMs to ensure the safe use of the plant protection product containing the concerned active substance will be taken by risk managers during the decision-making phase. Consideration of the validity and appropriateness of the RMMs remains the responsibility of MSs at product authorisation, taking into account their specific agricultural, plant health and environmental conditions at national level.

8.1. Particular conditions proposed for the representative uses evaluated

Table 6: Risk mitigation measures (RMMs) proposed for the representative uses assessed in addition to those already specified in the GAP table as part of the representative uses applied for[(a)]

Representative use	PRE-SOWING, PRE-PLANTING, PRE-EMERGENCE			POST-HARVEST, PRE-SOWING, PRE-PLANTING				
	Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, Sugar beet			Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, Sugar beet				
	Tractor-mounted broadcast spray			Tractor-mounted broadcast spray				
	1× 1.44 kg a.s./ha	1× 1.08 kg a.s./ha	1× 0.72 kg a.s./ha	1–2 × 1.08/1.44 kg a.s./ha	1–3 × 0.72/ 1.08 kg a.s./ha	1–3 × 0.72 kg a.s./ha	Cereal volunteers 1 × 0.54 kg a.s./ha	Cereal volunteers 1 × 0.54 kg a.s./ha once every 3 years
	Max appl. rate of 1.44 kg a.s./ha in any 12 months period	Max appl. rate of 1.08 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 12 months period	Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 2.16 kg a.s./ ha in any 12 months period	Max appl. rate of 0.54 kg a.s./ha in any 12 months period	Max appl. rate of 0.54 kg a.s./ha in any 36 months period
	Use No 1a	Use No 1b	Use No 1c	Use No 2a	Use No 2b	Use No 2c	Use No 3a	Use No 3b
Risk to non-target terrestrial plants	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]	No additional measures needed.	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]	No additional measures needed.		

(a): Use of at least 75% drift reducing nozzles is specified in the GAP table.
(b): RMMs such as 10-m no-spray buffer zone or 5 m no-spray buffer zone in combination with an application of 50% drift-reducing nozzles or application of 90% drift-reducing nozzle.

Representative use	POST-EMERGENCE OF WEEDS														
	Orchard crops: citrus, stone and pome fruits, kiwi, nut crops, banana, and table olives			Vines (table and wine grape, leaves not intended for human consumption)			Vegetables (Root vegetable plants and tuberous plants, bulb plants, fruit-vegetable plants, Legume vegetables, Leafy vegetables		Railway tracks		Invasive species in agricultural and non-agricultural areas		Root vegetable plants and tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, Sugar beet		
	Ground directed, fully-shielded (hooded) spray, band application (Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50% of the total orchard area)			Ground directed, fully-shielded (hooded) spray, band application (Band application in the rows below the vine stock or as spot treatments. The treated area represents not more than 50% of the total vineyard area)			Inter-row application: ground directed, fully-shielded (hooded) spray (Applications are made between the crop rows. The rate refers to the treated area only, which represents not more than 50% of the total area)		Ground directed, spray		Spot treatment (shielded)	Spot treatment (shielded), cut stem: spray appl.	Spot treatment (shielded) Post-harvest, pre-sowing, pre-planting Application to existing row cropland after harvest for removal of couch grass The treated area represents not more than 20% of the cropland		
	1–2 × 1.08/ 1.44 kg a.s./ha	1–3 × 0.72/ 1.08 kg a.s./ha	1–3 × 0.72 kg a.s./ha	1–2 × 1.08/ 1.44 kg a.s./ha	1–3 × 0.72/ 1.08 kg a.s./ha	1–3 × 0.72 kg a.s./ha	1 × 1.08 kg a.s./ha	1 × 0.72 kg a.s./ha	2 × 1.8 kg a.s./ha	1 × 1.8 kg a.s./ha	1 × 1.8 kg a.s./ha	1 × 1.8 kg a.s./ha	1 × 1.08 kg a.s./ha	1 × 0.72 kg a.s./ha	1 × 0.72 kg a.s./ha once every three years
	Max appl. rate of 2.88 kg a.s./ha treated area in any 12 months period		Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 2.88 kg a.s./ha treated area in any 12 months period		Max appl. rate of 2.16 kg a.s./ha treated area in any 12 months period	Max appl. rate of 1.08 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 12 months period	Max appl. rate of 3.6 kg a.s./ha in any 12 months period	Maximum application rate of 1.8 kg a.s./ha in any 12 months period			Max appl. rate of 1.08 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 36 months period
	Use No 4a	Use No 4b	Use No 4c	Use No 5a	Use No 5b	Use No 5c	Use No 6a	Use No 6b	Use No 7a	Use No 7b	Use No 8	Use No 9	Use No 10a	Use No 10b	Use No 10c
Risk to non-target terrestrial plants	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]		No additional measures needed.	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]		No additional measures needed.	RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed[(b)]	No additional measures needed.	RMMs already specified in the GAP table are not sufficient. More efficacious measures such as 90% drift-reducing nozzles are needed.		RMMs already specified in the GAP table are not sufficient. More efficacious measures are needed.[(b)]		No additional measures needed.		

(a): Use of at least 75% drift reducing nozzles is specified in the GAP table.
(b): RMMs such as 10 m no-spray buffer zone or 5 m no-spray buffer zone in combination with an application of 50% drift-reducing nozzles or application of 90% drift-reducing nozzles.

9. Concerns and related data gaps

9.1. Issues that could not be finalised

An issue is listed as 'could not be finalised' if there is not enough information available to perform an assessment, even at the lowest tier level, for one or more of the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011[33] and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as 'could not be finalised' if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following issues or assessments that could not be finalised have been identified, together with the reasons including the associated data gaps where relevant, which are reported directly under the specific issue to which they are related:

1) The assessment of the reference specification cannot be finalised since one of the impurities showed a potential for clastogenicity in an *in vitro* chromosome aberration test that was not appropriately followed up *in vivo*. Although some batches used in the toxicological studies contained this impurity at levels representative of the proposed reference specification, a conclusion on the maximum level of this impurity in any reference specification cannot be drawn without a clarification on its clastogenic potential (see Section 2).
    a) Clarification on the clastogenic potential of one impurity present in the reference specification, following up on the positive results obtained in an *in vitro* chromosome aberration test needs to be provided (relevant for all applicants/sources of glyphosate, see Section 2).
2) The consumer dietary risk assessment could not be finalised since the data set on the magnitude of residues in rotational crops is not complete (see Section 3).
    a) Final report of the magnitude of the residues in rotational crops study in carrot, lettuce and wheat is required (relevant for the representative uses in all crops which are grown in rotation; see Section 3).
    b) Sufficient studies investigating the magnitude of residues in rotational crops (i.e. carrot, lettuce, wheat) including additional crops (as appropriate) are required (relevant for the representative uses in all crops which are grown in rotation; see Section 3).
3) The risk assessment for aquatic macrophytes due to contact exposure via spray drift could not be finalised (see Section 5).
    a) Further information to investigate the risk for aquatic macrophytes due to contact exposure via spray drift is needed, including an assessment of the toxicity of the active substance and the formulation to standard macrophytes species via this route of exposure (relevant for all representative uses, see Section 5).

9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 regarding the hazard cut-off criteria outlined in Appendix A.

The following critical areas of concern are identified, together with any associated data gaps, where relevant, which are reported directly under the specific critical area of concern to which they are related:

Critical areas of concern were not identified.

9.3. Overview of the concerns identified for each representative use considered (Table 7)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 7.)

Table 7: Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all representative uses or risk assessment scenarios

Representative use		PRE-SOWING, PRE-PLANTING, PRE-EMERGENCE			POST-HARVEST, PRE-SOWING, PRE-PLANTING				
		Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, Sugar beet			Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica*, leaf and stem vegetable plants, Sugar beet				
		Tractor-mounted broadcast spray			Tractor-mounted broadcast spray				
		1× 1.44 kg a.s./ha	1× 1.08 kg a.s./ha	1 × 0.72 kg a.s./ha	1–2 × 1.08/1.44 kg a.s./ha	1–3 × 0.72/1.08 kg a.s./ha	1–3 × 0.72 kg a.s./ha	Cereal volunteers 1 × 0.54 kg a.s./ha	Cereal volunteers 1 × 0.54 kg a.s./ha once every 3 years
		Max appl. rate of 1.44 kg a.s./ha in any 12 months period	Max appl. rate of 1.08 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 12 months period	Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 0.54 kg a.s./ha in any 12 months period	Max appl. rate of 0.54 kg a.s./ha in any 36 months period
		Use No 1a	Use No 1b	Use No 1c	Use No 2a	Use No 2b	Use No 2c	Use No 3a	Use No 3b
Operator risk	Risk identified								
	Assessment not finalised								
Worker risk	Risk identified								
	Assessment not finalised								
Resident/ bystander risk	Risk identified								
	Assessment not finalised								
Consumer risk	Risk identified								
	Assessment not finalised	X[2(f)]	X[2(f)]	X[2(f)]	X[2(f)]	X[2(f)]	X[2(f)]	X[2(f)]	X[2(f)]
Risk to wild non-target terrestrial vertebrates	Risk identified	X[(c)]	X[(c)]		X[(e)]	X[(d)]	X[(d)]		
	Assessment not finalised								
Risk to wild non-target terrestrial organisms	Risk identified								
	Assessment not finalised								

Representative use		PRE-SOWING, PRE-PLANTING, PRE-EMERGENCE			POST-HARVEST, PRE-SOWING, PRE-PLANTING				
other than vertebrates									
Risk to aquatic organisms	Risk identified								
	Assessment not finalised	X[3(g)]	X[3(g)]	X[3(g)]	X[3(g)]	X[3(g)]	X[3(g)]	X[3(g)]	X[3(g)]
Groundwater exposure to active substance	Legal parametric value breached								
	Assessment not finalised								
Groundwater exposure to metabolites	Legal parametric value breached[(a)]								
	Parametric value of 10 µg/L[(b)] breached								
	Assessment not finalised								

The superscript numbers relate to the numbered points indicated in Section 9.1. Where there is no superscript number, see Section 5 for further information.

(a): It should be noted that the classification proposed in the context of this evaluation procedure under Regulation (EC) No 1107/2009 concurs with the harmonised classification and labelling in accordance with Regulation (EC) No 1272/2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission (2003).

(c): High long-term risk to mammals (identified at tier 1) for pre-sowing and pre-planting uses. Low risk to mammals for the pre-emergent uses (but after the sowing/planting).

(d): High long-term risk to mammals (identified at tier 1) for 2 or 3 applications of 0.72 kg a.s/ha and for 1, 2 or 3 applications of 1.08 kg a.s/ha. Low risk to mammals for a single application of 0.72 kg a.s./ha.

(e): High long-term risk to mammals (identified at tier 1) for all scenarios within this representative use.

(f): Rotational crop field trials are required regarding uses on agricultural/cropped land for all crop groups that can be grown in rotation (i.e. not for orchard crops such as kiwi, olives, grapes, citrus, stone, pome and tree fruit), to finalise the livestock dietary burden calculation and the consumer risk assessment.

(g): Assessment not finalised for aquatic macrophytes, only for contact exposure via spray drift.

POST-EMERGENCE OF WEEDS

Representative use		POST-EMERGENCE OF WEEDS														
		Orchard crops: citrus, stone and pome fruits, kiwi, nut crops, banana, and table olives			Vines (table and wine grape, leaves not intended for human consumption)			Vegetables (Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, Legume vegetables, Leafy vegetables		Railway tracks		Invasive species in agricultural and non-agricultural areas		Root vegetable plants & tuberous plants, bulb plants, fruit-vegetable plants, *Brassica,* leaf and stem vegetable plants, Sugar beet		
		Ground-directed, fullyshielded (hooded) spray, band application (Band application in the rows below the trees or as spot treatments. The treated area represents not more than 50% of the total orchard area)			Ground-directed, fully shielded (hooded) spray, band application (Band application in the rows below the vine stock or as spot treatments. The treated area represents not more than 50% of the total vineyard area)			Inter-row application: ground-directed, fully shielded (hooded) spray (Applications are made between the crop rows. The rate refers to the treated area only, which represents not more than 50% of the total area) Crop BBCH < 20		Ground-directed, spray		Spot treatment (shielded)	Spot treatment (shielded), cut stem: spray appl.	Spot treatment (shielded) Post-harvest, pre-sowing, pre-planting Application to existing row cropland after harvest for removal of couch grass The treated area represents not more than 20% of the cropland		
		1–2 × 1.08/1.44 kg a.s./ha	1–3 × 0.72/1.08 kg a.s./ha	1–3 × 0.72 kg a.s./ha	1–2 × 1.08/1.44 kg a.s./ha	1–3 × 0.72/1.08 kg a.s./ha	1–3 × 0.72 kg a.s./ha	1 × 1.08 kg a.s./ha	1 × 0.72 kg a.s./ha	2 × 1.8 kg a.s./ha	1 × 1.8 kg a.s./ha	1 × 1.8 kg a.s./ha	1 × 1.8 kg a.s./ha	1 × 1.08 kg a.s./ha	1 × 0.72 kg a.s./ha	1 × 0.72 kg a.s./ha once every three years
		Max appl. rate of 2.88 kg a.s./ha treated area in any 12 months period		Max appl. rate of 2.16 kg a.s./ha in any 12 months period	Max appl. rate of 2.88 kg a.s./ha treated area in any 12 months period		Max appl. rate of 2.16 kg a.s./ha treated area in any 12 months period	Max appl. rate of 1.08 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 12 months period	Max appl. rate of 3.6 kg a.s./ha in any 12 months period	Maximum application rate of 1.8 kg a.s./ha in any 12 months period				Max appl. rate of 1.08 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 12 months period	Max appl. rate of 0.72 kg a.s./ha in any 36 months period
		Use No 4a	Use No 4b	Use No 4c	Use No 5a	Use No 5b	Use No 5c	Use No 6a	Use No 6b	Use No 7a	Use No 7b	Use No 8	Use No 9	Use No 10a	Use No 10b	Use No 10c
Operator risk	Risk identified															
	Assessment not finalised															
Worker risk	Risk identified															
	Assessment not finalised															
Resident/ bystander risk	Risk identified															
	Assessment not finalised															
Consumer risk	Risk identified															
	Assessment not finalised							X[2(e)]	X[2(e)]			X[2(e)]	X[2(e)]	X[2(e)]	X[2(e)]	X[2(e)]

Representative use		POST-EMERGENCE OF WEEDS														
Risk to wild non-target terrestrial vertebrates	Risk identified	X[(d)]	X[(c)]	X[(c)]	X[(d)]	X[(c)]	X[(c)]							X[(d)]		
	Assessment not finalised															
Risk to wild non-target terrestrial organisms other than vertebrates	Risk identified															
	Assessment not finalised															
Risk to aquatic organisms	Risk identified															
	Assessment not finalised	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]	X[3(f)]
Groundwater exposure to active substance	Legal parametric value breached															
	Assessment not finalised															
Groundwater exposure to metabolites	Legal parametric value breached[(a)]															
	Parametric value of 10 µg/L[(b)] breached															
	Assessment not finalised															

The superscript numbers, if any, relate to the numbered points indicated in Section 9.1. Where there is no superscript number, see Section 5 for further information.

(a): It should be noted that the classification proposed in the context of this evaluation procedure under Regulation (EC) No 1107/2009 concurs with the harmonised classification and labelling in accordance with Regulation (EC) No 1272/2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission (2003).

(c): High long-term risk to mammals (identified at tier 1) for 2 or 3 applications of 0.72 kg a.s/ha and for 1, 2 or 3 applications of 1.08 kg a.s/ha. Low risk to mammals for a single application of 0.72 kg a.s./ha.

(d): High long-term risk to mammals (identified at tier 1) for all scenarios within this representative use.

(e): Rotational crop field trials are required regarding uses on agricultural/cropped land for all crop groups that can be grown in rotation (i.e. not for orchard crops such as kiwi, olives, grapes, citrus, stone, pome and tree fruit), to finalise the livestock dietary burden calculation and the consumer risk assessment.

(f): Assessment not finalised for aquatic macrophytes, only for contact exposure via spray drift.

10. List of other outstanding issues

Remaining data gaps not leading to critical areas of concern or issues not finalised but considered necessary to comply with the data requirements, and which are relevant for some or all of the representative uses assessed at EU level (unless stated otherwise). Although not critical, these data gaps may lead to uncertainties in the assessment and are considered relevant.

These data gaps refer to the representative uses assessed (unless stated otherwise) and are listed in the order of the sections:

- n-octanol/water partition coefficient for metabolite *N*-acetyl AMPA (relevant for all representative uses evaluated except that on railway tracks; see Section 1).
- Determination of the content of the relevant impurities: formic acid and triethylamine before and after storage of the formulation for representative uses, for 2 years at ambient temperature (relevant for all representative uses evaluated; see Section 1).
- Additional validation data for the determination of repeatability and recovery for formaldehyde, and for repeatability for NNG of the method for the determination of impurities in the technical active substance of one source (relevant for Industrias Afrasa and all representative uses evaluated; see Section 1).
- Determination of the precision of the method used for the analysis of two non-relevant impurities in a batch (relevant for Industrias Afrasa and all representative uses evaluated; see Section 1).
- Validation data to demonstrate a sufficiently validated LOQ of at least 0.8 g/kg for formaldehyde and 0.8 mg/kg for NNG of the methods for the determination of the impurity in the technical material (relevant for Industrias Afrasa and all representative uses evaluated; see Section 1).
- Validation data of the method used for determination of triethylamine in the submitted quality control data for one source (relevant for Industrias Afrasa and all representative uses evaluated; see Section 1).
- Validation data to demonstrate a sufficiently validated LOQ of at least 3.2 g/kg for formic acid of the method for the determination of the impurity in the technical active substance (relevant for Albaugh and all representative uses evaluated; see Section 1).
- Analysis of formic acid, with a validated method, in 5 representative and recent (within the last 5 years of manufacture) batches according to Good Laboratory Practice (GLP) (relevant for Industrias Afrasa and all representative uses evaluated; see Section 1).
- Analysis of formic acid in five representative and recent (within the last 5 years of manufacture) batches according to GLP (relevant to Sinon, and all representative uses evaluated; see Section 1).
- Validated analytical method for monitoring of *N*-acetyl glyphosate residues in honey (not relevant for the representative uses assessed, but relevant when there is a need to enforce MRLs in imported honey; see Sections 1 and 3).
- Analytical report including information on the validation of the analytical method used in a toxicological study (study CA 5.4.2/0.15, report no. 14613.402.078.14) (relevant for all representative uses evaluated; see Sections 1 and 2 and open point in the evaluation table set under experts' consultation point 2.3 (EFSA, 2023a)).
- Detailed summary of the QSAR analysis provided to assess the toxicological relevance of the impurities present in the reference specification according to the recommendations given in the 'Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology' (EFSA, 2018b) (relevant for all representative uses evaluated; see Section 2).
- Applicant to clarify the identity (CAS, name, structure) of some of the impurities listed in the composition of the batches used in toxicological studies and to clarify whether these impurities are the ones in the reference specification (relevant for all representative uses evaluated; see Section 2).
- A data gap is set to identify whether the DNT findings reported in the studies with glyphosate-trimesium and with GBHs are due to glyphosate (relevant for all representative uses evaluated; see Section 2).
- The aneugenic potential of the metabolite *N*-acetyl glyphosate has not been addressed (not relevant for the representative uses assessed, but relevant for future uses on modified crops or

consumer risk assessment for animal products as a result of the import of feed that is modified crops or imported honey; see Sections 2 and 3).
- The aneugenicity and clastogenicity of metabolites *N*-glyceryl AMPA and *N*-malonyl AMPA have not been sufficiently investigated. An *in vitro* micronucleus test will be needed to address the metabolites' clastogenic/aneugenic potential (not relevant for the representative uses assessed, but relevant for future uses on modified crops to ensure safety with respect to minor metabolites; see Sections 2 and 3).
- For one of the components of the formulation for representative uses 'MON 52276', repeated-dose toxicity information over short- and long term was not available (see Section 2); therefore, in order to allow a final conclusion on the risk assessment of 'MON 52276', repeated dose toxicity data for this component (short- and long term) should be assessed (relevant for all representative uses evaluated; see Section 2).
- Additional field residue trials are needed to complete the data package for the proposed representative uses assessed, as detailed below. For detailed explanation regarding the possibility for extrapolation between crop groups, please refer to the list of endpoints in Appendix B. It should be noted that the number of trials reflect the minimum requirements and more trials than indicated below may be needed if residues > LOQ are obtained either for glyphosate or AMPA (for relevant uses see below and Section 3):

For post-emergence uses, supervised residue field trials with an acceptable method of analysis validated for glyphosate and AMPA, and analysed within the demonstrated storage stability of residues period for glyphosate and AMPA, are requested for:

- Banana: At least three Southern EU (SEU) additional residue trials with AMPA/glyphosate analysed within the demonstrated storage stability period or studies demonstrating a longer storage stability of AMPA in banana are needed (relevant for the representative uses in banana; see Section 3).
- Grapes: Six Northern EU (NEU) and seven SEU additional residue trials with AMPA/glyphosate analysed within the demonstrated storage stability period or studies demonstrating a longer storage stability of AMPA are needed. The results from these trials can cover the uses in kiwi, pome fruit, citrus fruit, stone fruit and tree nuts (relevant for the representative uses in grapes, kiwi, pome fruit, citrus fruit, stone fruit and tree nuts; see Section 3).
- Table olives: At least four NEU residue trials with analysis of glyphosate and AMPA and at least four SEU residue trials with AMPA/glyphosate analysed within the demonstrated storage stability period are needed (relevant for the representative uses in table olives; see Section 3).

For post-harvest, pre-sowing, pre-planting, pre-emergence uses, supervised residue field trials with an acceptable method of analysis validated for glyphosate and AMPA, and analysed within the demonstrated storage stability of residues period for glyphosate and AMPA, are requested for:

- Root and tuber vegetables:
  - carrot: at least two NEU and two SEU additional trials;
  - potato: at least two NEU and two SEU additional trials;
  - sugar beet: at least four NEU and two SEU additional trials.
- Bulb vegetables:
  - bulb onion: at least two NEU and two SEU additional trials;
  - spring onion (possible extrapolation from leek, see data gap for leek);
  - bulb vegetables (other than onion and spring onion): at least three NEU and three SEU additional trials for each.
- Fruiting vegetables:
  - cucumber or courgette: at least three NEU and two SEU additional trials;
  - tomato: at least two NEU and four SEU additional trials.
- *Brassica* vegetables:
  - cauliflower or broccoli: at least two NEU and three SEU additional trials;
  - head cabbage: at least two NEU and two SEU additional trials;

  - kale (leafy *Brassica*): at least three NEU and three SEU additional trials or extrapolation from pre-emergence trials in lettuce may be considered;
  - Kohlrabies: at least three NEU and three SEU additional trials.
- Leaf vegetables:
  - lettuce (pre-emergence): at least two NEU and two SEU additional trials;
  - open leaf lettuce: at least one NEU and three SEU additional trials;
  - vine leaves: at least three NEU and three SEU additional trials;
  - witloof: at least three NEU and three SEU additional trials.
- Stem vegetables:
  - leek: at least two NEU and one SEU additional trials (extrapolation possible to spring onion);
  - stem vegetables (other than leek): at least three NEU and three SEU additional trials for each.

For inter-row uses, supervised residue field trials with an acceptable method of analysis validated for glyphosate and AMPA, and analysed within the demonstrated storage stability of residues period for glyphosate and AMPA, are requested for:

- Root and tuber vegetables:
  - carrot: at least four NEU additional trials;
  - potato: at least four NEU and four SEU additional trials.
- Bulb vegetables:
  - bulb onion: at least two NEU and one SEU additional trials;
  - bulb vegetables (other than onion): at least three NEU and three SEU additional trials for each.
- Fruiting vegetables:
  - tomato: at least four NEU additional trials;
  - cucumber or courgette: at least one NEU additional trials.
- Leaf vegetables:
  - open leaf lettuce: at least four NEU and four SEU additional trials;
  - vine leaves: at least three NEU and three SEU additional trials;
  - witloof: at least three NEU and three SEU additional trials.
- Legume vegetables (fresh):
  - beans (with pods): at least one SEU additional trial.
- Reliable AMPA soil DegT50 endpoints from at least three field trial sites were not available (relevant for all representative uses evaluated; see Section 4).
- Further information to address the route of groundwater exposure via bank infiltration and the connectivity of surface water bodies to groundwater aquifers, which can be relevant in some small hydrological catchments and some larger river systems (relevant for all representative uses evaluated; see Section 4).
- Although available for some tested formulations, a complete consideration of the composition of formulations used in the literature studies used for the ecotoxicological assessment together with a consideration of whether the tested formulation is comparable to the formulation for representative uses, 'MON 52276', was missing (see Section 5).
- The potential occurrence of metabolite AMPA in pollen and nectar needs to be further investigated (relevant for all representative uses, see Section 5).
- Although the studies were fully considered in the assessments described in Section 5, sufficiently detailed summaries were not provided for several of the literature studies[84] (relevant for all representative uses, see Section 5).

[84] Refer to Evaluation table section 5, data requirements 5.66, 5.71, 5.72, 5.81 and 5.83 (EFSA, 2023a).

- For further addressing the risk to biodiversity via indirect effects and trophic interactions it was considered needed (1) to perform a systematic literature search for data collection; (2) to quantify, in a spatial and temporal context, the direct effects on the weeds (including the impact on the seed bank), non-target plants, non-target arthropods and bees in order to inform the extent of potential indirect effects via trophic interactions; (3) to demonstrate how both specific and general mitigation measures may address the impact due to indirect effects (see Section 5).

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Abbreviations

AAOEL	acute acceptable operator exposure level
AMPA	(aminomethyl)phosphonic acid
a.s.	active substance
ADI	acceptable daily intake
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ALS	amyotrophic lateral sclerosis
AOEL	acceptable operator exposure level
AR	applied radioactivity
ARfD	acute reference dose
ASD	autism spectrum disorder
bw	body weight
CAS	Chemical Abstracts Service
DNT	developmental neurotoxicity study
DT_{50}	period required for 50% dissipation (define method of estimation)
DT_{90}	period required for 90% dissipation (define method of estimation)
EAS	oestrogen, androgen and steroidogenesis modalities
ECHA	European Chemicals Agency
EEC	European Economic Community
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ETO	ecological threshold option
FAO	Food and Agriculture Organization of the United Nations
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP	Good Agricultural Practice
GBH	glyphosate-based herbicides
GAT	glyphosate *N*-acetyltransferase
GC–MS	gas chromatography–mass spectrometry
GGT	gamma glutamyl transferase
GLP	Good Laboratory Practice
GOX	glyphosate oxidoreductase
HR	hazard rate
InChiKey	International Chemical Identifier Key.
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K_{doc}	organic carbon linear adsorption coefficient
K_{Foc}	Freundlich organic carbon adsorption coefficient
K_{oc}	normalised organic carbon to water partition coefficient
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LOAEL	lowest observable adverse effect level
LOQ	limit of quantification
MFFM	multi-functional field margin
MOA	mode of action
MRL	maximum residue level
MS	Member State
NNG	*N*-Nitroso-glyphosate
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OSR	oilseed rape
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in groundwater
PEC_{sed}	predicted environmental concentration in sediment

PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
PPE	personal protective equipment
QSAR	quantitative structure–activity relationship
RAC	regulatory acceptable concentration
RAR	Renewal Assessment Report
REACH	Registration, Evaluation, Authorisation of Chemicals Regulation
SFO	single first-order
SL	soluble concentrate
SMILES	simplified molecular-input line-entry system
STMR	supervised trials median residue
TK	technical concentrate
TRR	total radioactive residue
UF	uncertainty factor
WHO	World Health Organization

Appendix A – Consideration of cut-off criteria for glyphosate according to Annex II of Regulation (EC) No 1107/2009 of the European Parliament and of the Council

Properties		Conclusion[(a)]
CMR	**Carcinogenicity (C)**	Glyphosate is not classified as carcinogen category 1A or 1B from: Harmonised classification according to Regulation (EC) No 1272/2008 and its Adaptations to Technical Process (Table 3.1 of Annex VI of Regulation (EC) No 1272/2008 as amended): CLP00, and proposed classification according to ECHA RAC opinion (May 2022).
	Mutagenicity (M)	Glyphosate is not classified as mutagen category 1A or 1B from: Harmonised classification according to Regulation (EC) No 1272/2008 and its Adaptations to Technical Process [Table 3.1 of Annex VI of Regulation (EC) No 1272/2008 as amended]: CLP00, and proposed classification according to ECHA RAC opinion (May 2022).
	Toxic for Reproduction (R)	Glyphosate is not classified as toxic for reproduction category 1A or 1B from: Harmonised classification according to Regulation (EC) No 1272/2008 and its Adaptations to Technical Process (Table 3.1 of Annex VI of Regulation (EC) No 1272/2008 as amended): CLP00, and proposed classification according to ECHA RAC opinion (May 2022).
Endocrine disrupting properties		Glyphosate is not considered to meet the criteria for endocrine disruption for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II of Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.
POP	**Persistence**	Glyphosate is not considered to be a persistent organic pollutant (POP) according to point 3.7.1 of Annex II of Regulation (EC) No 1107/2009.
	Bioaccumulation	
	Long-range transport	
PBT	**Persistence**	Glyphosate is not considered to be a persistent, bioaccumulative and toxic (PBT) substance according to point 3.7.2 of Annex II of Regulation (EC) No 1107/2009.
	Bioaccumulation	
	Toxicity	
vPvB	**Persistence**	Glyphosate is not considered to be a very persistent, very bioaccumulative substance according to point 3.7.3 of Annex II of Regulation (EC) No 1107/2009.
	Bioaccumulation	

(a): Origin of data to be included where applicable (e.g. EFSA, ECHA RAC, Regulation).

Appendix B – List of end points for the active substance and the formulation for representative uses

Appendix B can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2023.8164

Appendix C – Wording EFSA used in Section 4 of this conclusion, in relation to DT and K_{oc} 'classes' exhibited by each compound assessed

Wording	DT_{50} normalised to 20°C for laboratory incubations[85] or not normalised DT_{50} for field studies (SFO equivalent, when biphasic, the DT_{90} was divided by 3.32 to estimate the DT_{50} when deciding on the wording to use)
Very low persistence	< 1 day
Low persistence	1 to < 10 days
Moderate persistence	10 to < 60 days
Medium persistence	60 to < 100 days
High persistence	100 days to < 1 year
Very high persistence	A year or more

Note these classes and descriptions are unrelated to any persistence class associated with the active substance cut-off criteria in Annex II of Regulation (EC) No 1107/2009. For consideration made in relation to Annex II, see Appendix A.

Wording	K_{oc} (either K_{Foc} or K_{doc}) mL/g
Very high mobility	0–50
High mobility	51–150
Medium mobility	151–500
Low mobility	501–2,000
Slight mobility	2,001–5,000
Immobile	> 5,000

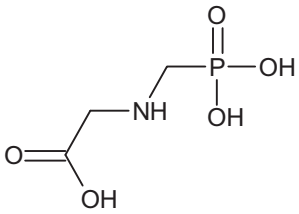
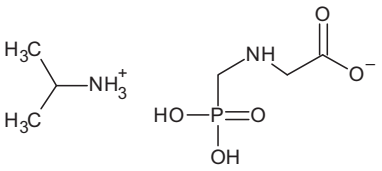
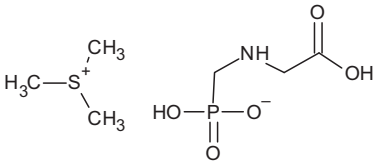
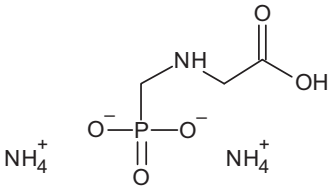
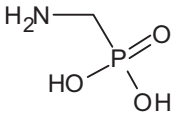
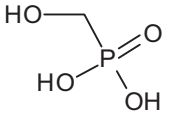
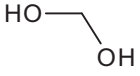
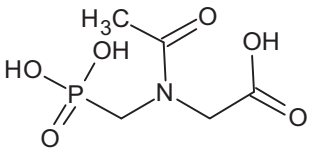
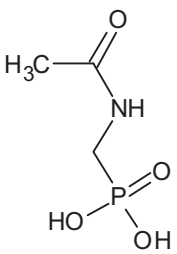
Based on McCall et al. (1980).

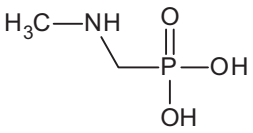
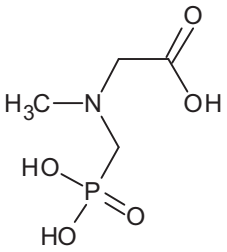
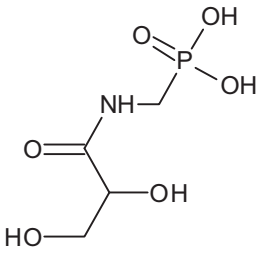
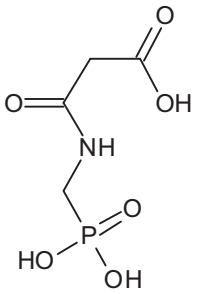
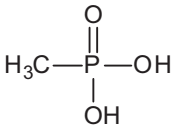
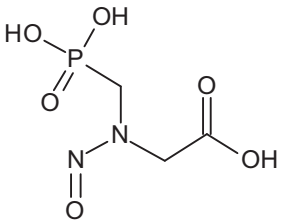
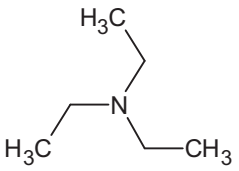
[85] For laboratory soil incubations normalisation was also to field capacity soil moisture (pF2/10 kPa). For laboratory sediment water system incubations, the whole system DT values were used.

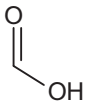
Appendix D – EFSA assessment of residue field trials – primary crops

Appendix D can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2023.8164

Appendix E – Used compound codes

Code/trivial name[a]	IUPAC name/SMILES notation/ InChiKey[b]	Structural formula[c]
glyphosate	*N*-(phosphonomethyl)glycine C(C(=O)O)NCP(=O)(O)O XDDAORKBJWWYJS-UHFFFAOYSA-N	
glyphosate-isopropylammonium	*N*-(phosphonomethyl)glycine - isopropylamine (1:1) or isopropylammonium *N*-(phosphonomethyl)glycinate O=C([O-])CNCP(=O)(O)O.[NH3+]C(C)C ZEKANFGSDXODPD-UHFFFAOYSA-N	
glyphosate-trimesium	trimethylsulfonium *N*-[(hydroxyphosphinato)methyl]glycine [O-]P(=O)(O)CNCC(O)=O.C[S+](C)C RUCAXVJJQQJZGU-UHFFFAOYSA-M	
glyphosate-diammonium	diammonium *N*-(phosphonatomethyl) glycine [NH4+].[NH4+].[O-]P([O-])(=O)CNCC(O)=O CPHCYTUHSKEDOI-UHFFFAOYSA-N	
AMPA ((aminomethyl) phosphonic acid) (M02)	(aminomethyl)phosphonic acid NCP(=O)(O)O MGRVRXRGTBOSHW-UHFFFAOYSA-N	
HMPA	(hydroxymethyl)phosphonic acid OCP(=O)(O)O GTTBQSNGUYHPNK-UHFFFAOYSA-N	
methanediol	Methanediol OCO CKFGINPQOCXMAZ-UHFFFAOYSA-N	
***N*-acetyl glyphosate (M04)**	*N*-acetyl-*N*-(phosphonomethyl)glycine OC(=O)CN(CP(=O)(O)O)C(C) = O BFECXRMSKVFCNB-UHFFFAOYSA-N	
***N*-acetyl AMPA (M05)**	(acetamidomethyl)phosphonic acid CC(=O)NCP(=O)(O)O FDNUAHPLMXZWLS-UHFFFAOYSA-N	

Code/trivial name[a]	IUPAC name/SMILES notation/ InChiKey[b]	Structural formula[c]
***N*-methyl AMPA (M03)**	[(methylamino)methyl]phosphonic acid CNCP(=O)(O)O HSMRCPIZVMDSHN-UHFFFAOYSA-N	
***N*-methyl glyphosate (M09)**	*N*-methyl-*N*-(phosphonomethyl)glycine CN(CC(=O)O)CP(=O)(O)O SGVDYFNFBJGOHB-UHFFFAOYSA-N	
***N*-glyceryl AMPA (M06)**	[(2*RS*)-(2,3-dihydroxypropanamido) methyl]phosphonic acid O=C(NCP(=O)(O)O)C(O)CO LFMJDSWPGBSPFL-UHFFFAOYSA-N	
***N*-malonyl AMPA (M07)**	3-oxo-3-[(phosphonomethyl)amino] propanoic acid O=C(CC(=O)O)NCP(=O)(O)O XVCISTXLOJFXDA-UHFFFAOYSA-N	
methylphosphonic acid (M08)	methylphosphonic acid CP(=O)(O)O YACKEPLHDIMKIO-UHFFFAOYSA-N	
***N*-nitroso-glyphosate (NNG)**	[nitroso(phosphonomethyl)amino]acetic acid O=NN(CC(=O)O)CP(=O)(O)O BJYYBQPCMQGLLZ-UHFFFAOYSA-N	
triethylamine	*N*,*N*-diethylethanamine CCN(CC)CC ZMANZCXQSJIPKH-UHFFFAOYSA-N	

Code/trivial name[a]	IUPAC name/SMILES notation/ InChiKey[b]	Structural formula[c]
formic acid	formic acid O=CO BDAGIHXWWSANSR-UHFFFAOYSA-N	
formaldehyde	Formaldehyde C=O WSFSSNUMVMOOMR-UHFFFAOYSA-N	

(a): The name in bold is the name used in the conclusion.
(b): ACD/Name 2021.1.3 ACD/Labs 2021.1.3 (File Version N15E41, Build 123232, 7 July 2021).
(c): ACD/ChemSketch 2021.1.3 ACD/Labs 2021.1.3 (File Version C25H41, Build 123835, 28 August 2021).

European Chemicals Agency (ECHA)

Glyphosate: questions and answers

May 2022

30 May 2022

Glyphosate: questions and answers

1. Why didn't the current classification change?

ECHA's Risk Assessment Committee formed its independent scientific opinion based on their evaluation of a new proposal prepared by the Assessment Group on Glyphosate - Sweden, France, The Netherlands and Hungary: the current classification of glyphosate does not change.

The committee's independent experts assessed a large number of scientific studies and information received from our consultation against criteria in the EU's classification, labelling and packaging regulation.

They found that the available scientific evidence did not meet the criteria to classify glyphosate for specific target organ toxicity, or as a carcinogenic, mutagenic or reprotoxic substance under the EU's CLP regulation.

This is in line with the previous RAC opinion from 2017.

Assessment Group on glyphosate

2. On what data is RAC's opinion based? Did the committee take into account all available new studies?

The harmonised classification and labelling proposal takes into account a broad range of scientific studies: all data that was included in the previous assessment (for the opinion adopted in 2017) as well as both published and unpublished data since then addressing all the required CLP hazard classes, including specific target organ toxicity following repeated exposure, carcinogenicity, mutagenicity and toxicity to reproduction.

The Assessment Group on Glyphosate - Sweden, France, The Netherlands and Hungary - carefully assessed the available data and included all relevant and appropriate information in the preparation of the dossier.

RAC's independent experts also assessed a large number of studies and comments received in the consultation.

Assessment Group on Glyphosate

3. What is ECHA's role in the glyphosate assessment?

ECHA implements the harmonised classification and labelling (CLH) process for hazardous chemical substances. The aim is to protect human health and the environment from those hazards that matter the most.

Active substances, the main chemicals in plant protection products (PPP), such as glyphosate, are classified for their hazards as part of their approval process in the EU. This is done through the CLH process managed by ECHA, whereby substances are proposed for harmonised classification by Member States and evaluated by RAC. This avoids double work, because the harmonised classification is used under many other regulatory frameworks. It also avoids divergences between the hazard assessments done by other European agencies, such as the European Food Safety Authority (EFSA). EFSA manages the overall evaluation of active substances in PPP.

In 2019, a group of companies (the Glyphosate Renewal Group GRG) applied under the Plant Protection Products (PPP) Regulation[1] to renew the approval of glyphosate for use after the current approval expires at the end of 2022. The application was assessed by a group of four EU Member States (France, Hungary, the Netherlands and Sweden – called the Assessment Group on Glyphosate, AGG) and it will be peer reviewed by the European Food Safety Authority, EFSA.

In parallel with the EFSA peer review risk assessment, ECHA's Committee for Risk Assessment (RAC) adopted an opinion on the proposal for harmonised classification of glyphosate. This opinion is based on a proposal prepared by the same group of four Member States that assess the industry renewal application.

The harmonised classification and labelling focuses solely on the hazardous properties of the active substance: its potential to cause harm. It does not assess risk via exposure of humans or the environment to glyphosate. This will be part of the peer review of the risk assessment by EFSA.

EFSA's assessment

4. What happens next?

The adopted opinion will be published on ECHA's website and sent to EFSA by mid-August. EFSA will carry out the risk assessment of glyphosate which is foreseen to be ready in July 2023.

The European Commission will analyse EFSA's conclusions and the renewal assessment report that was prepared by Sweden, France, Hungary and The Netherlands. The Commission will then put forward a renewal report and a draft regulation to Member States on whether the approval of glyphosate can be renewed or not.

EFSA's assessment

European Commission: status of glyphosate in the EU

5. What information will ECHA publish?

RAC's opinion will be finalised and published by mid-August and sent to EFSA. This opinion will detail RAC's scientific reasoning in coming to their conclusion.

The CLH report from the Assessment Group on Glyphosate has been available on ECHA's website since September 2021.

This report includes summaries of studies, a comparison of the data with the criteria for classification which are described in the CLP Regulation, and an assessment of the evidence and arguments leading to the proposals for classification. ECHA does not publish the full study reports which are the intellectual property of the companies who own them.

The Glyphosate Renewal Group has listed the studies on their website and their website indicates that it is possible to "request a copy of all the reports of the additional glyphosate studies that were commissioned by the Glyphosate Renewal Group or its member companies

[1] Regulation (EC) No 1107/2009 EUR-Lex - 32009R1107 - EN - EUR-Lex (europa.eu)

for the 2020 Scientific Dossier". RAC has access to relevant full study reports.

The CLH report addresses the following hazard classes: acute toxicity, STOT RE (specific target organ toxicity, repeated exposure), eye damage/irritation, respiratory and skin sensitisation, STOT SE (specific target organ toxicity, single exposure), skin corrosion / irritation, carcinogenicity, germ cell mutagenicity, reproductive toxicity and toxicity to the aquatic environment, as well as relevant physical hazards.

ECHA has already published the non-confidential comments received during the consultation on the CLH report. At the end of the CLH process, ECHA will also publish the "Response to Comments" (RCOM) documents from the consultation on the CLH report and from the additional targeted consultation, which will include the responses of the dossier submitter and RAC to the comments received.

Glyphosate Renewal Group: Owned Studies Archive

6. How does ECHA avoid conflicts of interest?

ECHA is an organisation that issues decisions, opinions and recommendations strictly based on science. Therefore, it is important for the Agency to guarantee the independence of its work from private interests.

To safeguard its independence, ECHA has established a comprehensive policy which obliges anyone taking up a position in ECHA to complete a detailed declaration of interests before they can start to work for the Agency.

On glyphosate, staff of the ECHA secretariat perform an accordance check of an incoming proposal from the Member States and provide administrative support throughout the process. The ECHA secretariat does not provide any opinion on the classification and labelling proposal itself. Staff members assigned to the dossier have filled out an annual declaration of interest (like all ECHA staff members) and have also been checked for any potential personal interest in the file.

The scientific opinion on glyphosate will be prepared by ECHA's Committee for Risk Assessment (RAC), which is composed of independent scientific experts nominated by the Member States and appointed by the Management Board (or co-opted by the Committee); most of them are public officials, or academics from universities.

Before being appointed by ECHA's Management Board, all Committee members are screened against five exclusion criteria. Once appointed they also submit updated declarations of interest annually, which are reviewed by the Chair of the Committee and published on ECHA's website for transparency reasons and peer review. Furthermore, each meeting of RAC starts with an oral declaration of specific interests with regard to the agenda items to be discussed. These oral declarations are recorded in the meeting minutes and members with conflicting interests abstain from decision making.

RAC is a collegial body (decisions built mainly on consensus), which means that no single individual could influence the outcome of the process by him or herself.

With all these checks and controls, ECHA is confident that no-one that has an apparent conflict of interest has participated in the decision-making process.

ECHA's Conflict of Interest Prevention policy

U.S. Environmental Protection Agency (EPA)

Glyphosate: Response to Comments on the Human Health Draft Risk Assessment

April 2018

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

Date: April 23, 2018

SUBJECT: Glyphosate: Response to Comments on the Human Health Draft Risk Assessment

PC Code: 417300; 103601; 103603; 103604; 103605; 103607; 103608; 103613
Decision No.: 542736
Petition No.: NA
Risk Assessment Type: NA
TXR No.: NA

MRID No.: NA

DP Barcode: D448021

Registration No.: NA
Regulatory Action: Registration Review
Case No.: 178
CAS No.: 1071-83-6; 38641-94-0; 70393-85-0; 114370-14-8; 40465-76-7; 69254-40-6; 34494-04-7; 70901-12-1
40 CFR: § 180.364

FROM: Monique M. Perron, Sc.D., Toxicologist
Tom Bloem, Chemist
Risk Assessment Branch 1 (RAB1)
Health Effects Division (HED; 7509P)

THROUGH: Christine L. Olinger, Branch Chief
RAB1/HED (7509P)

TO: Khue Nguyen, Chemical Review Manager
Ricardo Jones, Team Leader
Amy Blankinship, Acting Branch Chief
Pesticide Re-evaluation Division (PRD; 7508P)

The Office of Pesticide Programs received thousands of public comments related to the human health draft risk assessment (DRA) for glyphosate in support of glyphosate's registration review. Comments addressing the human health risk assessment came from a wide array of stakeholders, including environmental non-governmental organizations (e.g., Natural Resource Defense Council, Center for Biological Diversity, Food & Water Watch, Environmental Working Group, Pesticide Action Network), consumer groups (e.g., Moms Across America, Environmental Action), pesticide registrants (e.g., the Joint Glyphosate Task Force, the Scotts Miracle-Gro Company), and private citizens (including anonymous commenters and growers). OPP has thoroughly reviewed all of the comments received during the public comment period. The Agency appreciates the substantial pubic interest in glyphosate. Due to the large volume of comments received on the risk assessment, the Agency identified the most detailed, substantive, and/or unique comments and addressed them as part of the identified "themes" below. Overall,

the comments received do not result in substantive changes to the Agency's human health risk assessment for glyphosate. EPA continues to conclude that exposure to glyphosate when used according to the label does not result in human health risk, including infants and children.

Cancer Assessment

- *Potential genotoxicity and carcinogenicity of glyphosate*
- *Consideration of Scientific Advisory Panel (SAP) recommendations*
- *Disagreement with International Agency for Research on Cancer (IARC) classification*
- *Weight of evidence evaluation of animal carcinogenicity data*

Several commenters expressed concern regarding the Agency's cancer assessment and disagreement with the Agency's cancer classification. Many of the commenters cited the IARC cancer classification of "probably carcinogenic to humans". The Agency conducted an independent evaluation of the cancer potential of glyphosate and concluded that glyphosate is "not likely to be carcinogenic to humans." This conclusion is based on a weight-of-evidence evaluation in accordance with the Agency's 2005 Guideline for Carcinogen Risk Assessment.

In December 2016, the Agency's evaluation of the human carcinogenic potential of glyphosate was presented to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) SAP for external peer review. This evaluation included an in-depth review of all relevant animal carcinogenicity and genotoxicity studies for the active ingredient glyphosate, as well as epidemiological studies that investigated potential carcinogenic effects from using pesticide products containing glyphosate. The epidemiological data was considered in this evaluation since it represents the best available data for evaluating human exposures and potential risk of cancer in the absence of epidemiological data on the active ingredient alone.

Although the panel did not reach complete consensus on several charge questions, none of the panel members believed glyphosate should be classified as "likely to be carcinogenic to humans" or "carcinogenic to humans". The Agency utilizes SAP reports as they represent the full continuum of opinions expressed. In the specific case of glyphosate, given the variety of opinions expressed, the Agency focused on statements where consensus appeared to be reached to revise the Issue Paper. The revised Issue Paper (D444689; TXR 0057688; G. Akerman; 12-DEC-2017) and a response to the SAP report (D444688; TXR 0057689; G. Akerman; 12-DEC-2017) along with associated supporting documents were released in December 2017[1].

Several public comments to the human health DRA were also received regarding the Agency's weight of evidence evaluation of the animal carcinogenicity data, including statistical evaluation, evaluation of preneoplastic and related non-neoplastic lesions, and use of historical controls. These comments have already been addressed in the response to the SAP report. Further information on the Agency's weight of evidence evaluation of the potential carcinogenicity of glyphosate can be found in the *Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential* (D444689; TXR 0057688; G. Akerman; 12-DEC-2017).

[1] https://www.epa.gov/ingredients-used-pesticide-products/draft-human-health-and-ecological-risk-assessments-glyphosate

The Agency's conclusion that glyphosate is "not likely to be carcinogenic" is consistent with other countries and regulatory authorities/international organizations including the Canadian Pest Management Regulatory Agency, Australian Pesticide and Veterinary Medicines Authority, European Food Safety Authority, the European Chemicals Agency, German Federal Institute for Occupational Safety and Health, The Joint FAO/WHO Meeting on Pesticide Residues, the New Zealand Environmental Protection Authority, and Food Safety Commission of Japan.

The Agency's analysis is more robust compared with IARC's evaluation. IARC considered a subset of the studies included in the Agency's evaluation. For instance, IARC only considered 8 animal carcinogenicity studies, while the Agency utilized 15 acceptable animal carcinogenicity studies in its evaluation. The Agency also did not use some studies that IARC incorporated into their evaluation because the studies were not appropriate for determining the human carcinogenic potential of glyphosate. For example, genotoxicity studies conducted in non-mammalian species (i.e., worms, fish, reptiles, plants) were excluded from the Agency's evaluation because they were not considered relevant for informing the genotoxic risk in humans. Furthermore, the Agency's process for evaluating the potential carcinogenicity of glyphosate is more transparent than IARC's process. As part of the SAP process, public participation is encouraged with the Agency's draft evaluation and all supporting documents provided in advance of the meeting, several opportunities available for oral and written public comments to be submitted, and the meeting was open to the public and available by webcast. The SAP meeting is well-documented with publication of a full transcript of the meeting and a final report drafted by SAP panel members. Additionally, the Agency drafted a response to the SAP report, which addressed the panel recommendations and identified revisions that were made in the Agency's Issue Paper following the SAP meeting. In contrast, IARC meetings are not accessible to the public. The committee deliberations are closed and the process does not allow for public comments to be submitted for consideration. Furthermore, there are no materials available in advance of the meeting, reports are final without any external peer review, and conclusions are not well described.

Glyphosate toxicological studies

- *Use and availability of industry generated studies*
- *The Agency should conduct search of open literature studies*
- *Chronic, developmental, reproductive, dermal, inhalation, neurotoxic, immunotoxic, and ocular effects*

Several commenters assert that the Agency relies too heavily on industry-funded studies and that these studies are not accessible to the public and requested the Agency to use open literature studies for the glyphosate hazard evaluation.

In the case of glyphosate, the Agency is aware of a significant number of studies published in the open literature. In 2012, the Office of Pesticide Programs (OPP) published a guidance document to provide guidance procedures for considering and using open literature toxicity studies to support human health risk assessment[2]. This guidance assists OPP scientists in their judgement of the scientific quality of open literature publications and has been applied in the glyphosate

[2] U.S. EPA (2012). *Guidance for considering and using open literature toxicity studies to support human health risk assessment.* http://www.epa.gov/pesticides/science/lit-studies.pdf

review. More recently, the National Academy of Sciences National Research Council (NRC) has encouraged the Agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision making[3]. The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies"[4].

As part of the glyphosate registration review process, the Agency reviewed the open literature for hazard identification and characterization purposes in order to identify studies that could potentially impact the human health risk assessment. The first search was performed in late 2011/early 2012 and another search was performed in October 2015 using the concepts consistent with systematic review, such as detailed tracking of search terms and which literature have been included or excluded. The Agency also considered studies that were submitted by non-profit groups or members of the public as part of this 2015 review. These reviews are summarized in the DRA and a separate memo (D417703; TXR 0056885; M. Perron; 12-DEC-2017). Only a limited number of studies were deemed acceptable and appropriate for consideration in risk assessment. None of the studies were found to have an impact on the hazard characterization or draft human health risk assessment for glyphosate.

A fit-for-purpose systematic review was also executed to obtain relevant and appropriate open literature studies with the potential to inform the human carcinogenic potential of glyphosate and reviewed by the SAP in 2016. This additional review identified numerous epidemiological and genotoxicity studies from the literature in addition to the guideline genotoxicity and animal carcinogenicity studies submitted to the Agency. Details regarding this review can be found in the *Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential* (D444689; TXR 0057688; G. Akerman; 12-DEC-2017).

Under FIFRA, the Agency requires substantial amounts of toxicology and exposure data to be collected and submitted for pesticide registration. These studies, defined under the Title 40 Code of Federal Regulations (40 CFR) Part 158 Toxicology Data Requirements, provide information on a wide range of adverse health outcomes, routes of exposure, exposure durations, species, and lifestages. In general, many of these studies are commissioned and submitted by the pesticide producers. To ensure data quality and consistency, the Agency has standard guidelines for how testing is to be conducted. The Agency's test guidelines are largely harmonized with those established by the Organisation for Economic Co-operation and Development (OECD). Harmonization also eases comparisons across studies and chemicals. Laboratories must also conduct studies in accordance with Good Laboratory Practices (GLP) standards (40 CFR Part 160) to further ensure the quality and integrity of the data submitted to the Agency. Study reports must include a statement on whether they were conducted in accordance with the GLP procedures. The Agency's Office of Enforcement and Compliance Assurance (OECA) periodically inspects labs that conduct studies to support pesticide registrations to ensure they are in compliance with GLPs.

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[3] NRC 2011. "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde"; NRC 2014. "Review of EPA's Integrated Risk Information System (IRIS) Process"

[4] NRC (2014). Review of EPA's Integrated Risk Information System (IRIS) process. Washington, DC: The National Academies Press. http://www.nap.edu/catalog.php?record_id=18764

Review of all studies submitted to the Agency's Office of Pesticide Programs (OPP) is a multi-step process. Test reports must summarize and supply all the individual data obtained as part of the study; most toxicity study reports are well over a thousand pages long. An independent evaluation is prepared for each study and a Data Evaluation Record (DER) is generated to summarize the study methods, results, and conclusions. Draft DERs are subject to an internal peer review process, including review by multiple individual scientists and scientific review committees within OPP, to ensure accuracy and consistency with Agency guidance on interpretation of toxicity studies prior to finalization.

Studies evaluated by the Agency are available to the public through Freedom of Information Act (FOIA) requests, however, section 10(g) of FIFRA prohibits the Agency from disclosing certain information submitted by an applicant or registrant to any representative of a multinational pesticide producer or anybody who intends to deliver such information to a multinational pesticide producer. In order to receive registrant submitted data/studies, Section 10(g) requires requestors to sign an Affirmation of Non-Multinational Status form[5]. The form affirms the person requesting the pesticide data does not work for or represent a pesticide producer. Section 10(g) also requires the Agency to notify the data owner to whom we released the data to. Please keep in mind that registrant-submitted studies are proprietary and cannot be posted or released for public access. For more information on how to submit FOIA requests to access certain glyphosate studies, visit the Agency's website: https://www.epa.gov/foia/foia-request-process.

The entire toxicity database available is used to identify and characterize the potential health effects of a pesticide. This includes acceptable studies submitted by registrants and open literature studies. Although numerous comments were received regarding concerns for a suite of non-cancer effects, including chronic toxicity, ocular effects, developmental toxicity, reproductive toxicity, dermal effects, inhalation effects, neurotoxicity, and immunotoxicity, the available studies indicate that glyphosate will not elicit these effects, or these effects would only be observed at relatively high doses. Numerous studies are available that evaluated chronic exposure to glyphosate in rats, mice, and dogs. In most instances, effects were only seen at or near the limit dose (1000 mg/kg/day). Developmental effects in rats were only observed at a dose exceeding the limit dose (3500 mg/kg/day) and there were no developmental effects observed in rabbits. Route-specific studies are available that evaluated dermal and inhalation exposures. Dermal irritation effects were only seen at a dose exceeding the limit dose (5000 mg/kg/day), which is well above exposures expected from glyphosate use and not relevant for human health risk assessment. There were no effects observed in the inhalation study up to a dose approaching the limit concentration (0.36 mg/L). There was no evidence in the toxicological databases that glyphosate would cause ocular effects, reproductive effects, neurotoxicity, or immunotoxicity, including the guideline neurotoxicity battery, reproductive toxicity and immunotoxicity studies. Overall, the selected endpoints for risk assessment are protective of all adverse effects observed in the database.

Endocrine disruption

Some commenters assert that glyphosate is an endocrine disruptor based on open literature studies conducted primarily with commercial formulations containing glyphosate. For the few

[5] http://www2.epa.gov/foia/affirmation-non-multinational-status

studies that evaluated glyphosate alone, there were no clear endocrine-related effects observed. Glyphosate was screened under the Agency's Endocrine Disruptor Screening Program (EDSP). Under the program, a suite of Tier 1 *in vivo* and *in vitro* studies was required for glyphosate that were designed to provide the necessary empirical data to evaluate the potential of glyphosate to interact with the estrogen, androgen, or thyroid signaling pathways. In addition to the available Tier 1 assay data, other scientifically relevant information, including general toxicity data and open literature studies of sufficient quality, are considered in the Agency's weight of evidence assessment. Based on all available information, the Agency concluded using a weight of evidence approach that the existing data do not indicate that glyphosate has the potential to interact with the estrogen, androgen, or thyroid signaling pathways (TXR 0057175; G. Akerman; 29-JUN-2015)[6].

Protection of children

Several commenters assert that the Agency is not being protective of children. The Agency places top priority on the safety of children exposed to pesticides in food and/or water and living in or near areas treated with pesticides. The Food Quality Protection Act (FQPA) requires the Agency to give specific consideration to the potential for infants and children to be sensitive to pesticides[7]. Based on the 40 CFR Part 158 data requirements, pesticides typically have toxicology studies to evaluate effects in pregnant animals and their fetuses and young rats up through adulthood. Developmental and multi-generation reproduction studies are used to evaluate the potential effects of a pesticide on fetuses and offspring. Developmental studies are used to determine whether gestational exposure has an effect on the developing fetus. Multi-generation reproduction studies are used to evaluate parental and offspring toxicity, as well as reproductive toxicity, from long-term exposure to a pesticide. This includes exposure during gestation and lactation. The results of these studies are considered as part of the entire toxicity database to ensure doses selected for risk assessment are protective of any potential fetal and offspring effects.

Typical food-use pesticides have two developmental toxicity studies (one with rats and one with rabbits) and one study evaluating reproductive toxicity. In the case of glyphosate, there are 2 rat developmental, 2 rabbit developmental, and 3 reproductive toxicity studies available. The Agency found no indication that offspring are more sensitive to glyphosate from *in utero* or post-natal exposure in any of these studies. Additionally, any developmental or offspring effects were seen at doses much higher than those used for risk assessment. As part of the human health DRA to support registration review, the Agency evaluates all populations, including infants, children, and women of child-bearing age. There were no risks of concern identified for children from ingesting food/feed commodities or from entering/playing on residential areas treated with glyphosate using conservative assumptions to calculate high-end exposure estimates. Furthermore, in accordance with FQPA, aggregate exposures and risks from food, drinking

6 Available at https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and

7 HED's standard toxicological, exposure, and risk assessment approaches are consistent with the requirements of EPA's children's environmental health policy (https://www.epa.gov/children/epas-policy-evaluating-risk-children).

water, and residential exposures were calculated for adults and children. There were no aggregate risks of concern. The Agency's current risk assessment is protective of children.

Detection of glyphosate in human milk, tissues, and urine

Many commenters cite reports of glyphosate detections in human milk, tissues, and urine. The Agency has identified several issues with studies claiming to detect glyphosate in urine, tissues, and human milk. Among the key information missing from such studies are the information related to sampling methods, sample storage, sample shipping, quality assurance and quality control, and analytical methods used, which are critical to evaluating the reliability of the data. Additionally, the enzyme-linked immunosorbent assay (ELISA) method is often used in the tests. This method is known to work well with surface waters that have little or no suspended solids or with processed drinking water. However, many of the samples in these cited tests would have significant amounts of dissolved solids which may lead to issues when using the ELISA method with these sample types. Furthermore, the ELISA method is generally considered to be a semi-quantitative method that is typically used as a screening or initial test method to determine the potential presence of a chemical. The results from such a method, therefore, do not provide data that can be used quantitively for human health risk assessment.

Glyphosate is not expected to accumulate in human milk and tissues. The Agency is not aware of any peer-reviewed studies reporting detection of glyphosate in human breast milk and, due to its physicochemical properties, glyphosate is not expected to bioaccumulate in the human body. Additionally, as noted in the DRA, the Agency Biological and Economic Analysis Division (BEAD) analyzed human milk samples in response to concern from segments of the general public related to the presence of glyphosate in human milk. Glyphosate, *N*-acetyl-glyphosate, and aminomethyl phosphonic acid (AMPA) were not detected in any of the human milk samples. On the other hand, detection of trace amounts of glyphosate in urine would be expected given the chemical does not bioaccumulate and is primarily excreted un-metabolized as glyphosate by mammals. Such trace levels of glyphosate are not of concern to the Agency since the corresponding body burden (or approximate magnitude of exposure in mg/kg body weight) assuming complete excretion of the absorbed amount and virtually no metabolism, would still be well below current regulatory levels[8].

Formulations*:*

- *Toxicity of inert compounds*
- *Transparency of components*
- *Contaminants in pesticide products*

Several commenters expressed concern that glyphosate formulations are more toxic than glyphosate alone and question the toxicity of inert ingredients, the lack of transparency around other ingredients in product formulations, and other contaminants in pesticide products. Most pesticide products contain substances in addition to the active ingredient(s) that are often referred to as inert or other ingredients, which aid in the performance and effectiveness of the pesticidal

8 Niemann et al. 2015. A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. Journal of Consumer Protection and Food Safety. 10: 3-12.

product. Federal law does not require that these ingredients be identified by name or percentage on the label. In accordance with FIFRA, the Agency cannot disclose this information since these ingredients are considered trade secrets or confidential business information.

All active and inert pesticide ingredients must be approved for use by the Agency. The Agency carefully evaluates the active and inert components hazard potential (i.e., toxicity) of a pesticide product with a battery of appropriate toxicity data. However, there are tens of thousands of different registered pesticide products available in the marketplace and, though the Agency evaluates the product components, long term testing of individual products is not required. Any contaminants or impurities associated with formulation components need to be reported to the Agency and are evaluated on a case by case basis. The Agency looks at the amount of the impurity in the formulation, the manufacturing information, and what steps are taken to limit or remove impurities. A comment was received regarding formation of nitrosamines, which have been found to cause cancer. Technical grade glyphosate contains minor amounts of a nitrosamine impurity, N-nitrosoglyphosate (NNG). This contaminant was considered previously as part of the Reregistration Eligibility Decision (RED)[9]. Carcinogenicity testing of nitroso contaminants is normally required only in cases which the level of nitroso compounds exceeds 1.0 ppm. Analyses showed that greater than 92% of the individual technical glyphosate samples contained less than 1.0 ppm. No new data have been presented to warrant a reevaluation of the Agency's conclusion that the NNG content of glyphosate is not toxicologically significant.

Glyphosate has been studied in a multitude of studies and there are studies that have been conducted on numerous formulations that contain glyphosate; however, there are relatively few research projects that have attempted to directly compare glyphosate to the formulations in the same experimental design. Furthermore, there are even less instances of studies comparing toxicity across formulations. The majority of studies using commercial formulations identified as part of the systematic review are *in vitro* studies, which are difficult to translate into *in vivo* effects where metabolism and clearance would play a large role in potential toxicity. Consequently, *in vivo* studies are given more weight. In the systematic review (D417703; TXR 0056885; M. Perron; 12-DEC-2017), none of the *in vivo* studies with commercial formulations were found to be of adequate quality for use in human health risk assessment. Common limitations/deficiencies seen in these studies included lack of test material information, exposure conditions were not adequately described or documented, data were only presented as graphs and often measures of variability were not included, samples sizes were too small for the type of study conducted and/or not reported for all lifestages, only one dose was tested, and age and overall health prior to commencing a study was not reported. Furthermore, most of these studies focused on clinical chemistry measurements (i.e., enzymes, hormones, electrolytes) or histopathological examinations (without reporting severity) making it difficult to determine the adversity of the results. The relationship between any changes noted in these effects and possible adverse apical outcomes from commercial formulations has not been established. As described in the NRC report, "Toxicity Testing in the 21st Century"[10], to develop a mode of action/adverse outcome pathway (MOA/AOP) not only is it necessary to establish plausible relationships among the key events, but quantitative relationships also need to be established. In

[9] https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep-93.pdf

[10] National Research Council (NRC). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. Washington, D.C. The National Academies Press.

other words, how much of a change in one key event is needed to result in an adverse effect at the next level of biological organization? Thus, certain exposures to a chemical may impact normal physiological responses in a way that may not necessarily be adverse, and thus, the MOA/AOP concept requires an understanding of adaptive/homeostatic capacity of biological systems and their limits, relative to concentration and duration of exposure. Without an MOA/AOP understanding or even a potentially solid hypothesis, pertubations in physiology cannot be interpreted for risk assessment without understanding how these changes lead to adverse outcomes.

The Agency has been collaborating with the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences to develop a research plan intended to evaluate the role of glyphosate in product formulations and the differences in formulation toxicity. The results of this research will be considered when available.

Antibacterial properties and disruption of the gut microbiome

Many commenters assert that glyphosate has antibacterial properties and claim it contributes to antibiotic resistance and disruption of the gut microbiome. The metabolic pathway inhibited in plants by glyphosate (Shikimate pathway) is also found in many microorganisms. Although glyphosate may inhibit the Shikimate pathway in microorganisms, it has not been demonstrated to be an effective antimicrobial for treating humans. It is particularly difficult to achieve and maintain a sufficiently high concentration of glyphosate in the body to be an effective antimicrobial agent due to the low absorption and metabolism of glyphosate. Furthermore, although glyphosate may inhibit the production of certain amino acids in bacteria, these amino acids can be acquired from the body, when needed. Therefore, the inhibition does not necessarily lead to bacterial death.

Gut microbiomes (colonies of microbes in the gut) are unlikely to be altered from glyphosate exposure since the aromatic amino acids produced via the Shikimate pathway are also available in the human gut via the diet since humans are unable to synthesize them. Therefore, despite inhibition of this metabolic pathway, the microorganisms are still capable of growing and surviving. It has been suggested that glyphosate preferentially affects only "good" bacteria; however, this implies that microbes are defined by this metabolic pathway, which is not scientifically supported. Gut microbiomes are not evaluated directly in guideline toxicity studies; however, the stomach and gastrointestinal tract are routinely examined in several studies by gross evaluation and histopathological investigations. There are no indications in these studies that exposure to glyphosate induces adverse effects in those organs.

Metal chelation and nutritional deficiencies

Some commenters indicated that glyphosate is a metal chelator and consequently claim that it causes nutritional deficiencies. Glyphosate chelates with some metals in soil and aquatic environments. The relative proportion of the various chemical species of glyphosate (including dissociated species of glyphosate acid and glyphosate-metal complexes) is dependent on chemical characteristics (*e.g.*, pH, redox potential, etc.) of the environment. The Agency is unaware of any connection between metal chelation and toxicity of glyphosate in mammals. In

guideline studies for human health, exposure to glyphosate did not result in any changes in clinical or blood chemistry measurements, suggesting that glyphosate-metal chelation does not play a significant role in affecting human health.

Dietary Assessment

- *Residues in food and beverages*
- *Assessment of the dessicant use on wheat*

Many commenters point to reports of glyphosate residues being detected in food/beverage commodities such as honey, cereals, wine, and orange juice and expressed concerns about consumer safety. Due to its widespread use, trace amounts of glyphosate residues may be found in various food and beverage commodities. However, these trace amounts are not of concern to the consumer as the residue levels are well below tolerance levels established in/on food commodities treated with glyphosate. For example, the Agency has received information on glyphosate residues reported in orange juice at a maximum of 26 ppb. At this concentration, a 10 kg child would have to consume approximately 385 liters (1627 servings of an 8 oz glass) of orange juice every day to reach the chronic reference dose of 1 mg/kg/day.

As part of the human health risk assessment, the Agency evaluated dietary exposure to glyphosate for all populations, including infants, children, and women of child-bearing age. There were no dietary risks of concern for glyphosate using an unrefined analysis, which assumes all food commodities contain tolerance level residues (i.e., maximum legal residues allowed on a food commodity) of glyphosate, all food (with registered uses) has been treated with glyphosate, and using high-end estimates of glyphosate in drinking water.

Other commenters pointed to the use of glyphosate as a pre-harvest desiccant for wheat as a source of glyphosate residues in cereal products. Since the dietary exposure assessment was unrefined (assumed tolerance level residues and 100% crop treated) and the current tolerances reflect all registered uses, the wheat desiccant use was considered in the dietary analysis conducted as part of the human health DRA for registration review and there were no dietary risks of concern.

Non-Cancer Diseases

Several commenters expressed concern about the alleged link between exposure to glyphosate and various non-cancer diseases. In several instances, commenters noted a correlation in glyphosate use and some diseases; however, correlation does not imply causation. Increased prevalence of a disease may be due to many possible causes and verifying these causes should not be based on speculation. Determining whether an observed association represents a cause-effect relationship between glyphosate exposure and disease requires additional consideration of criteria, such as the modified Bradford-Hill criteria, that evaluate strength, consistency, dose response, temporal concordance and biological plausibility across multiple lines of evidence. Additionally, the plant MOA/AOP is not relevant for mammalian systems and there is a distinct lack of mechanistic understanding for the toxicity of glyphosate in mammals, which is used to inform the cause-effect relationship. As part of the Tier II Incident Report for glyphosate

(D417808; S. Recore; 6-FEB-2014;), an open literature search was conducted to identify epidemiological studies that evaluated the potential role of glyphosate and disease outcomes. The Agency reviewed studies related to a range of non-cancer effects, including adverse birth outcomes, respiratory effects, rheumatoid arthritis, diabetes, myocardial infarction, Parkinson's disease, and retinal degeneration. Most of the studies were not designed to develop data on non-cancer outcomes that could be used quantitatively or qualitatively in regulatory decision-making, but were more exploratory in nature. Additionally, in most instances, only one study was available for a specific outcome, which makes it challenging to assess consistency in the human population. Based on the available studies, the Agency could not conclude that glyphosate plays a role in any of the health outcomes studied across this epidemiologic database. The Agency also examined journal articles regarding non-cancer disease outcomes submitted with comments to the DRA to identify any epidemiological studies that were not considered as part of the Tier II report. At this time, the available scientific data do not support a cause-and-effect relationship between exposure to glyphosate and any non-cancer disease outcomes. The Agency will continue to follow the epidemiological literature concerning the potential role of glyphosate in certain non-cancer health outcomes.

European Chemicals Agency (ECHA)

CLH Report: Proposal for Harmonised Classification and Labelling

March 2017

All news

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Glyphosate not classified as a carcinogen by ECHA

ECHA/PR/17/06

ECHA's Committee for Risk Assessment (RAC) agrees to maintain the current harmonised classification of glyphosate as a substance causing serious eye damage and being toxic to aquatic life with long-lasting effects. RAC concluded that the available scientific evidence did not meet the criteria to classify glyphosate as a carcinogen, as a mutagen or as toxic for reproduction.

Helsinki, 15 March 2017 – RAC assessed glyphosate's hazardousness against the criteria in the Classification, Labelling and Packaging Regulation. They considered extensive scientific data in coming to their opinion.

The committee concluded that the scientific evidence available at the moment warrants the following classifications for glyphosate according to the CLP Regulation:

- Eye Damage 1; H318 (Causes serious eye damage)
- Aquatic Chronic 2; H411 (Toxic to aquatic life with long lasting effects)

RAC concluded that the available scientific evidence did not meet the criteria in the CLP Regulation to classify glyphosate for specific target organ toxicity, or as a carcinogen, as a mutagen or for reproductive toxicity.

The hazard classes for which classification was proposed by the German competent authority were specific target organ toxicity (repeated exposure) (category 2), eye damage/irritation (category 1), and toxicity to the aquatic environment (Aquatic Chronic 2). ECHA also assessed other hazard classes including carcinogenicity, germ cell mutagenicity and reproductive toxicity.

The adopted opinion will go through a normal editorial check before it is sent to the European Commission. The opinion will also be made available on ECHA's website at the same time.

The adopted opinion on the harmonised classification for glyphosate will be taken into account when the Commission and Member States consider whether to renew the approval to use glyphosate as an active substance in pesticides, later this year.

Background

Apart from the published studies on glyphosate, the committee also had full access to the original reports of studies conducted by industry. RAC has assessed all the scientific data, including any scientifically relevant information received during the public consultation in summer 2016.

RAC had a first discussion on glyphosate with stakeholders at its 39th meeting in December 2016.

RAC provides an independent scientific opinion on the hazard classification of the substance. The classification **is based solely on the hazardous properties of the substance**. It does not take into account the likelihood of exposure to the substance and therefore does not address the risks of exposure. The risks posed by exposure are considered for example when deciding whether to renew the approval of glyphosate as a pesticide in accordance with the EU's Plant Protection Product Regulation (Regulation (EC) N° 1107/2009).

Further information

- Video: Recording of RAC press briefing (15 March 2017)
- CLH Report submitted by the German competent authority and comments received during the public consultation
- RAC starts discussing the harmonised classification of glyphosate (News item - 7 December 2016)
- ECHA's role in assessing glyphosate (News item - 7 July 2016)
- Video: Explaining ECHA's role in the glyphosate classification process
- More information about glyphosate on ECHA's website
- How ECHA is assessing glyphosate (ECHA Newsletter - September 2016)

Committee for Risk Assessment
EFSA's web pages on glyphosate

Telakkakatu 6, P.O. Box 400
FI-00121 Helsinki, Finland

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: N-(phosphonomethyl)glycine; Glyphosate (ISO)

EC Number: **213-997-4**

CAS Number: **1071-83-6**

Index Number: **607-315-00-8**

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Version: **2.0 (Post Accordance Check)**

Date: **May 2016**

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Part A.

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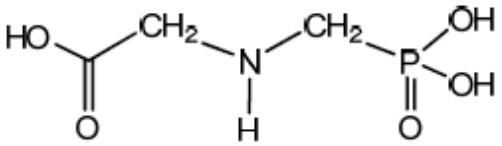
Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*N*-(phosphonomethyl)*glycine*
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	Glyphosate
EC number (if available and appropriate)	213-997-4
EC name (if available and appropriate)	Glyphosate
CAS number (if available)	1071-83-6
Other identity code (if available)	-
Molecular formula	$C_3H_8NO_5P$
Structural formula	
SMILES notation (if available)	C(CN(C[P](O)(O)=O)[H])(O)=O
Molecular weight or molecular weight range	169.1 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 95.0%

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Eye Dam. 1, H318 Aquatic Chronic 2, H411
Current proposal for consideration by RAC	STOT RE 2, H373
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Eye Dam. 1, H318 STOT RE 2, H373 Aquatic Chronic 2, H411

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification	Reason for no classification
2.1.	Explosives				Conclusive but not sufficient for classification
2.2.	Flammable gases				Conclusive but not sufficient for classification
2.3.	Flammable aerosols				Conclusive but not sufficient for classification
2.4.	Oxidising gases				Conclusive but not sufficient for classification
2.5.	Gases under pressure				Conclusive but not sufficient for classification
2.6.	Flammable liquids				Conclusive but not sufficient for classification
2.7.	Flammable solids				Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				Conclusive but not sufficient for classification
2.10.	Pyrophoric solids				Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures				Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases				Conclusive but not sufficient for classification
2.13.	Oxidising liquids				Conclusive but not sufficient for classification
2.14.	Oxidising solids				Conclusive but not sufficient for classification
2.15.	Organic peroxides				Conclusive but not sufficient for

					classification
2.16.	Substance and mixtures corrosive to metals				Conclusive but not sufficient for classification
3.1.	Acute toxicity – oral				Conclusive but not sufficient for classification
	Acute toxicity – dermal				Conclusive but not sufficient for classification
	Acute toxicity – inhalation				Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation				Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Eye Dam. 1, H318		Eye Dam. 1, H318	
3.4.	Respiratory sensitisation				Data lacking
3.4.	Skin sensitization				Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				Conclusive but not sufficient for classification
3.6.	Carcinogenicity				Conclusive but not sufficient for classification
3.7.	Reproductive toxicity				Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure				Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 2, H373		-	
3.10.	Aspiration hazard				Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2, H411		Aquatic Chronic 2, H411	
5.1.	Hazardous to the ozone layer				Data lacking

Labelling: Signal word: Danger
Pictogram: GHS05, GHS08, GHS09
Hazard statements: Causes serious eye damage, May cause damage to organs through prolonged or repeated exposure
Toxic to aquatic life with long lasting effects

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

After evaluation of the available data an additional classification as STOT RE 2 for Glyphosate is proposed based on results obtained in developmental studies in rabbits. Otherwise, the current harmonized classification is confirmed.

2.3 Current harmonised classification and labelling

Eye Dam. 1, H 318;

Aquatic Chronic 2, H 411

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Glyphosate is an active substance in plant protection products. In addition to the existing harmonised classifications for eye irritation and aquatic toxicity, a new classification (STOT RE 2) is proposed.

The re-evaluation of glyphosate as a herbicide by the European Food Safety Authority (EFSA) was required by Commission Regulation (EU) No 1141/2010 as amended by Commission Implementing Regulation (EU) No 380/2013. For this purpose, many new toxicological studies were submitted by the different applicants, especially on eye irritation, genotoxicity, carcinogenicity as well as on reproductive and developmental toxicity of glyphosate. Furthermore, a large number of scientific publications is available and should be considered for the re-evaluation of glyphosate and for the CLH proposal as well. Because of this increase of the toxicological database and also of that one on environmental effects, ECHA and its committee for risk assessment are suggested to address all relevant endpoints.

The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) published in a monograph that glyphosate is “probably carcinogenic to humans (Group 2A)” (IARC, 2015, ASB2015-8421). During the European Food Safety Authority (EFSA) peer-review process for the renewal of approval of the pesticide active substance glyphosate, the IARC evaluation regarding the potential carcinogenicity and genotoxicity of glyphosate or glyphosate -containing plant protection products was taken into consideration but EFSA and EU experts came to a different conclusion (see attached EFSA conclusion, 2015, ASB2015-11412).

The Joint Meeting on Pesticide Residues (JMPR) administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and WHO re-evaluated glyphosate in May 2016 with the following conclusion: “*The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures. Several carcinogenicity studies in mice and rats are available. The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses. In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet.*” (JMPR, 2016, ASB2016-4292).

Keeping this in mind, the CLH process administered by the European Chemicals Agency (ECHA) should result in the adoption of a harmonised classification of glyphosate for all health-related but also the environmental endpoints.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

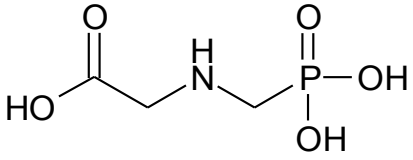
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	213-997-4
EC name:	Glyphosate
CAS number (EC inventory):	1071-83-6
CAS number:	1071-83-6
CAS name:	N-(phosphonomethyl)-glycine
IUPAC name:	N-(phosphonomethyl)-glycine
CLP Annex VI Index number:	607-315-00-8
Molecular formula:	C3H8NO5P
Molecular weight range:	169.1 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
N-(phosphonomethyl) glycine	≥ 95.0%	≥ 95.0%	

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
N-Nitroso-glyphosate	< 1 ppm	< 1 ppm	This value was decreased by the RMS based on the toxicological evaluation
Formaldehyde	< 1 g/kg	< 1 g/kg	This value was decreased by the RMS based on the toxicological evaluation

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-				

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid, crystalline powder	Hammond and Pulwer, 1986	Measured
Melting/freezing point	> 200 °C (decomposition)	Wollerton and Husband, 1997	Measured
Boiling point	> 200 °C (decomposition)	Wollerton and Husband, 1997	Measured
Relative density	d_4^{20} = 1.7018	Wollerton and Husband, 1997	Measured
Vapour pressure	< 10^{-5} Pa (20 °C)	Wollerton and Husband, 1997	Measured
Surface tension	72.7 mN/m (1 g/L in dist. H_2O, 20 °C)	Wollerton and Husband, 1997	Measured
Water solubility	10 g/L, EEC A 6 flask method	Wollerton and Husband, 1997	Measured
Partition coefficient n-octanol/water	log $P_{o/w}$ < - 1.3 EEC A 8 shake flask	Wollerton and Husband, 1997	Measured
Flash point	not required		
Flammability	not highly flammable under the conditions of the test (EEC A 10)	Wollerton and Husband, 1997	Measured
Explosive properties	not explosive	Wollerton and Husband, 1997	theoretical assessment
Self-ignition temperature	not auto-flammable (EEC A 15)	Wollerton and Husband, 1997	Measured
Oxidising properties	non-oxidising	Wollerton and Husband, 1997	Measured
Granulometry	No data	-	-
Stability in organic solvents and identity of relevant degradation products	No data	-	-
Dissociation constant	pK_{a1} = 2.25 (20 °C) pK_{a2} = 5.50 pK_{a3} = 10.34 OECD 112 titration	Wollerton and Husband, 1997	Measured
Viscosity	No data	-	-

2 MANUFACTURE AND USES

Glyphosate is a non-selective post-emergence, mono- and dicotyledonous herbicidal active substance.

3 SUBSTANCECLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not addressed in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

The main data source for the evaluation of the toxicological properties of glyphosate with regard to classification and labelling was the revised Renewal Assessment Report (RAR) dated 31 March 2015, which was written for the EU pesticides procedure. Volumes 1 and 3 are attached to the CLH dossier as background documents. This version was produced after discussion of the draft RAR of the Rapporteur Member State (RMS) Germany on an expert meeting (PRAS) hold by EFSA in February, 2015, and reflects the conclusions drawn there. The only classification that was agreed at that time was for eye irritation. Thus, it should be acknowledged that the additional German proposal for classification (STOT RE 2) has been made after that meeting and, thus, was not subject to commenting by Member States or expert meeting discussion so far. Going beyond the RAR, a number of additional long-term, reproduction and developmental studies are addressed in this CLH dossier that were found unsuitable for risk assessment purposes and, therefore, have been rejected during the EU re-evaluation process although some of them may have been used for a previous one. Even if the deficiencies in these studies do not have an impact on classification and labelling, they are at least briefly mentioned to ensure that a comprehensive picture for these endpoints is provided. With regard to genotoxicity/mutagenicity, we have included studies that do not comply with current standards only if they revealed a positive result which needed to be addressed.

Another important basis for the current evaluation is a new assessment of the International Agency for Research on Cancer (IARC) to assign glyphosate to category 2A for carcinogenicity. IARC's decision was published in July, 2015, when the IARC Monograph 112 was released. The assessment of this monograph in an addendum to the RAR by the German Federal Institute for Risk Assessment (BfR) has been completed on 31 August 2015 and was submitted in September, 2015, to EFSA as an addendum to the RAR. This addendum has been subject to thorough peer review by the competent authorities of the EU Member States. During this review process, including an expert discussion held by EFSA on 29 September 2015, all the Member States experts but one agreed that the active substance is unlikely to be genotoxic or to pose a carcinogenic threat to humans and is not proposed to be classified as such under EU regulations. The addendum and the EFSA documentation are also attached to this CLH dossier to provide background information.

All toxicological studies included in this CLH dossier were evaluated and assessed by in-house staff toxicologists of the BfR. It is emphasised that the toxicological database for glyphosate is extremely large and that the studies have come from a great number of sources. Thus, completeness of the database and identification and compilation of relevant and reliable data are crucial. In the following, the approach taken by the dossier submitter (DS) is described with particular regard to the studies and publications that are referred to in this CLH dossier.

The information that is relevant for classification and labelling of glyphosate is based on original studies of the manufacturers that were performed on a routine basis under GLP conditions and in compliance with OECD Test Guidelines for the individual toxicological endpoints. Such studies are usually confidential and are submitted to national authorities or supranational bodies to support authorisation or registration of plant protection products containing the respective active ingredient.

In case of glyphosate, these studies have been reported in detail in the RAR. Nonetheless, most of them have not been made publically available in full and they would not been found in a systematic literature review since they are proprietary to their owners.

A further source of information is published literature. For classification and labelling purposes, mainly epidemiological studies have been taken into consideration whereas there were only few published *in vivo* or *in vitro* studies with the active substance glyphosate. It must be emphasised that in most of these studies formulations of glyphosate instead of the active substance have been tested.

(1) The search for published studies was based on: The scientific literature concerning glyphosate, its salts, AMPA and also glyphosate formulations with regard to side effects on health, the environment, and non-target species as provided by the "Glyphosate Task Force" (GTF) (Carr and Bleeke, 2012, ASB2012-11583). The period from 2001 to 2011 was covered. The search was performed in five databases: Web of Science, BIOSIS Previews, CAB Abstracts, CA Plus (Chemical Abstracts Plus), and Medline.

(2) A dossier on glyphosate submitted by various non-governmental organizations (NGOs) containing further references even though a part was overlapping with the manufacturer's search.

(3) Several new publications that became available before, during and after the commenting phase of the RAR (including the "public consultation").

(4) A check of the reference lists of the submitted articles by the DS for so far unknown references.

This section contains short summaries and purpose-adapted tables frequently adopted and taken from the RAR as well as from the addendum. In case more in-depth information on the studies and effects is needed, the reader is referred to Vol. 3, chapter B.6 of the RAR where all the studies are reported in detail. Most toxicological studies were performed on behalf of various manufacturers with technical specifications from many sources. Accordingly, the purity and impurity profile were different. Impurities may have contributed to the toxic effects but there is no data to determine the extent of this contribution. In the European context this has led to the situation that a number of specifications from different applicants were not supported by the toxicological assessment (see attached EFSA conclusion, 2015, ASB2015-11412).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human data

Experimental studies in laboratory animals (mainly rats) are available in which toxicokinetics and metabolism (ADME) of glyphosate have been investigated. The understanding of toxicokinetics and metabolism of a chemical is considered as crucial for its toxicological evaluation.

Glyphosate is rapidly absorbed from the gastro-intestinal tract (GIT) following oral intake but only to a limited extent of about 20%. It is widely distributed to the various compartments, organs and tissues. Elimination is fast and virtually complete within 72-168 hours with the major part being excreted already during the first 48 hours. The absorbed part is excreted in the urine whereas the (greater) unabsorbed portion is eliminated via the faeces. Enterohepatic circulation and biliary excretion are negligible, and so is exhalation. After a period of 3 to 7 days following oral administration, total body burden accounted for ≤1% of the applied radioactivity with generally low tissue residues at study termination (Ridley and Mirly, 1988, TOX9552356; Powles & Hopkins, 1992, TOX9300343; Davies, 1996, TOX2000-1977, TOX2000-1978, TOX2000-1979; McEwen, 1995, ASB2012-11379; Knowles and Mookherjee, 1996, ASB2012-11380). Highest residues were

detected in bone, followed by kidney and liver. Due to poor oral absorption, high amounts were also found in the GIT. This pattern of distribution was confirmed by whole-body autoradiograms that showed the greatest intensity of radioactivity to be present in bone and the gastrointestinal tract not later than 24 hours after dosing. These amounts were reduced to negligible amounts within 48 hours (Powles and Hopkins, 1992, TOX9552358; Davies, 1996, TOX2000-1980). Although elimination from bone seems slower than from other tissues, the amount of radiolabel in bone tissue at 168 h after a single oral dose was relatively low accounting for not more than 0.02-0.03% of the applied dose (McEwen, 1995, ASB2012-11379).

There was no evidence of accumulation in animals based on residue analysis in organs and tissues at 72-168 h after single or repeated doses.

This pattern of absorption, distribution and elimination was not significantly changed by dose levels or by repeated administration of low doses and was independent of the sex of the test animals.

Most of the parent substance glyphosate was eliminated unchanged and only a small amount (in most studies less than 1% of the applied dose and sometimes none) was transformed to aminomethylphosphonic acid (AMPA). There is only one publication by Anadon et al. (2009, ASB2012-11542) that suggests a higher metabolism rate of up to 6.5% of the dose following oral administration of 400 mg/kg bw to rats. Formation of AMPA is assumed to be due to gastrointestinal microflora activity rather than mammalian metabolic pathways (Brewster et al., 1991, TOX9551791). AMPA was broadly investigated for many toxicological endpoints and exhibited similar or lower toxicity than glyphosate and was found to be devoid of genotoxic potential (see RAR). The same reference doses as for glyphosate are applicable.

In Table 9 the acceptable ADME studies with glyphosate and their results are compiled.

Table 9: Comparison of the distribution of radiolabelled glyphosate acid in excreta and tissues and its metabolism in valid ADME studies in the rat

Reference, Study identification, Owner	**Dosing regime and dose levels, Duration of post-observation period**	**Excretion / Distribution (mean % of applied dose)**								**Metabolism**
		Urine		**Faeces**		**Total organ / tissue / carcass residues**		**Bile**		
		♂	♀	♂	♀	♂	♀	♂	♀	
Leuschner (1995)#, TOX9650071 / Blech & Stratmann (1995) #, TOX9552251; ADAMA	0.2-0.3 mg/kg bw, single oral dose, 168 h	12.3	9.6	82.9	83.3	--	--	--	--	No metabolites found in urine following oral high dose application
	200 mg/kg bw, single oral dose, 168 h	17.1	13.2	81.8	84.4	--	--	--	--	
	0.2 mg/kg bw, single i.v. dose, 168 h	90	88.6	5.6	7.2	< 0.1*	< 0.1*	--	--	
Powles & Hopkins (1992), TOX9300343;	30 mg/kg bw, single oral dose, 168 h	29.0	30.7	58.8	56.5	0.62	0.64	--	--	No metabolites found in urine or faeces
	1000 mg/kg bw, single	30.6	22.4	53.3	60.4	0.47	0.40	--	--	

Reference, Study identification, Owner	Dosing regime and dose levels, Duration of post-observation period	Excretion / Distribution (mean % of applied dose)								Metabolism
		Urine		Faeces		Total organ / tissue / carcass residues		Bile		
		♂	♀	♂	♀	♂	♀	♂	♀	
Cheminova	oral dose, 168 h									
	30 mg/kg bw, repeated (14x) oral application followed by a single radiolabelled dose, 72 h	34.3	34.6	49.6	46.7	0.96	0.83	--	--	
	30 mg/kg bw, single i.v. dose, 168 h	86.0	84.2	3.4	1.5	1.4	1.1	--	--	
Ridley & Mirly (1988), TOX9552356 / Howe et al. (1988), TOX9552357; Monsanto	10 mg/kg bw, single oral dose, 168 h	28.6	22.5	62.4	69.4	0.48	0.36	--	--	Very limited, AMPA accounting for 0.2-0.4%
	1000 mg/kg bw, single oral dose, 168 h	17.8	14.3	68.9	69.4	<0.4	<0.4	--	--	
	10 mg/kg bw, repeated (14x) oral application followed by a single radiolabelled dose, 168 h	30.9	23.1	61.0	70.9	<0.7	<0.7	--	--	
	10 mg/kg bw, single i.v. dose, 168 h[$]	79.0	74.5	4.7	8.3	≈ 1.0	≈ 1.0	--	--	
McEwen (1995), ASB2012-11379; Arysta	Single oral gavage, 168 h; satellite groups for plasma kinetics									Very limited, traces of AMPA in urine (<0.3%) and of AMPA and another compound in faeces (<2%)
	10 mg/kg bw	22.5	19.4	74.6	84.3	0.33	0.27	--	--	
	600 mg/kg bw	30.3	29.5	74.7	74.2	0.31	0.39	--	--	
Knowles & Mook-herjee (1996), ASB2012-11380;	Single oral gavage, 168 h; satellite groups for plasma kinetics and									Very limited with <1% transformed to a compound presumed as AMPA

Reference, Study identification, Owner	Dosing regime and dose levels, Duration of post-observation period	Excretion / Distribution (mean % of applied dose)								Metabolism
		Urine		Faeces		Total organ / tissue / carcass residues		Bile		
		♂	♀	♂	♀	♂	♀	♂	♀	
Nufarm	tissue residues (up to 72 h) and 48-h biliary excretion									
	1 mg/kg bw	24.9	34.9	72.6	62.4	0.75	0.98	--	--	
	100 mg/kg bw	55.3	55.0	41.2	42.4	0.84	0.98	--	--	
	1 mg/kg bw	27.5	24.2	55.3	61.0	4.99	3.82	0.03	0.08	
Macpherson (1996), TOX2000-1981; Syngenta	Single oral gavage, 1000 mg/kg bw, 48 h	20.8	16.3	39.1	30.5	--	--	0.06	0.06	Very limited, <0.7% AMPA was found (based on examination of urinary and faecal samples obtained over 72 hours in other experiments from the same lab, i.e., Davies, 1996a-c)
Davies (1996a), TOX2000-1977; Syngenta	Single oral gavage, 10 mg/kg bw, 72 h	13.3	11.1	88.5	88.7	0.54	0.46	--	--	Not investigated
Davies (1996b), TOX2000-1978; Syngenta	Single oral gavage, 1000 mg/kg bw, 72 h	16.9	17.8	89.5	84.6	0.47	0.54	--	--	Not investigated
Davies (1996c), TOX2000-1979; Syngenta	Single oral dose (gavage) after repeated (14x) dosing, 10 mg/kg bw, 72 h (after final dose)	10.6	10.7	86.8	90.7	0.47	0.41	--	--	Not investigated

[#] Supplementary study. * Bone tissue not investigated. [$] Total recovery was rather poor.

In addition, there is a rather old (supplementary) study with dietary administration of glyphosate over 14 days to rats (Colvin and Miller, 1973, TOX9552355) where evidence of even a lower oral absorption than after gavage application was obtained. Total excretion was found to equal total intake. A supplementary study in male rabbits (Colvin and Miller, 1973, TOX9552353) demonstrated a similar pattern of toxicokinetics and metabolism as in the rat.

Following dermal exposure to rabbits, glyphosate was poorly (< 3%) absorbed (Hadfield, 2012,

ASB2012-11459) but the actual extent of dermal absorption depends very much on the product in which the active ingredient is formulated.

4.1.2 Human data

Reliable kinetic data obtained in humans are not available for glyphosate. However, based on an analysis of a total of 13 poisoning incidents with glyphosate-based herbicides in France (Zouaoui et al., 2013, ASB2014-9734), there is at least strong evidence that biotransformation of ingested glyphosate to AMPA is very limited also in man. The glyphosate:AMPA ratio in blood analyses varied between 12:1 and 6933:1 with a median value of 235:1. In urine, with data from 7 cases available, the individual ratios ranged from 243:1 to 7863:1 with a median of 422:1. These ratios were independent from the severity of symptoms or a fatal outcome.

4.2 Acute toxicity

4.2.1 Non-human information

A huge number of acute oral, dermal and inhalation studies with glyphosate is available. In the majority of experiments, the test species was the rat. A few studies have been conducted in other animal species such as the mouse suggesting that they were not more vulnerable than the rat after oral administration. The available data is compiled in Table 10, Table 11, and Table 12 and briefly summarised below for each route.

Acute oral toxicity

Table 10: Summary of acute oral toxicity studies with glyphosate acid in rats and mice

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Sharp, 1995 (Sanachem) TOX9650909	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	>2000 (limit test)	Slightly congested lungs, splenomegaly, Liver: centri-lobular congestion
Snell, 1994 (Herbex) TOX9500245	Rat, Sprague Dawley	1/sex/2000 5/sex/2000	95	Arachis oil	>2000 (limit test)	No findings
Tornai et al., 1994 (Alkaloida) TOX9650142	Rat, Wistar	5/sex/0 5/sex/5000	97.2	Water	>5000 (limit test)	♂: heart weights↓
Brown and Ogilvie, 1995 (Sinon) TOX9500377	Rat, Sprague Dawley	2/sex/250 2/sex/500 2/sex/1000 2/sex/3000 2/sex/5000 5/sex/5000	95	CMC	>5000 (limit test)	Piloerection, subdued behaviour, hunched appearance
Walker and Jones, 1992 (Barclay)	Rat, Sprague Dawley	1/sex/2000 5/sex/2000	>97	Water	>2000 (limit test)	No findings

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
TOX9551810						
Suresh, 1991 (Feinchemie, now ADAMA) TOX9551088	Rat, Wistar	5/sex/2500 5/sex/5000 5/sex/7500	96.8	Peanut oil	>7500 (estimated)	7500 mg/kg bw: mortality (2/5 ♂, 2/5 ♀); lethargy, ataxia, dyspnoea, weight loss
Brett, 1990 (Agrichem) TOX9500261	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	1% CMC	>8000	≥5000 mg/kg bw: decreased activity, abnormal gait and/or limb position
Cuthbert & Jackson, 1989 (Cheminova) TOX9552319	Rat, Sprague Dawley	5/sex/5000	98.6	0.5% CMC	>5000 (limit test)	Piloerection, reduced activity, ataxia (♂ only)
You, 2009 (Helm) ASB2012-11381	Rat, Sprague Dawley	5/females/5000	96.4	Water	>5000 (limit test)	Decreased activity, diarrhoea, piloerection, polyuria, salivation
Komura, Hitoshi, 1995 (Arysta) ASB2012-11382	Rat, Sprague Dawley	5/sex/5000	95.68	0.5% CMC	>5000 (limit test)	Decreased spontaneous motor activity and salivation
Simon, 2009 (Exxel) ASB2012-11384	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	96.66	Water	>2000	No findings
Haferkorn, 2009 (Helm) ASB2012-11385	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	98.8	0.8% hydro-xypro-pylme-thylcel-lulose	>2000 (limit test)	No findings
Haferkorn, 2010 (Helm) ASB2012-11386	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	96.4	0.8% hydro-xypro-pylme-thylcel-lulose	>2000 (limit test)	No findings
Haferkorn, 2010 (Helm) ASB2012-11387	Rat, CD	3 females/2000 (step 1) 3 females/2000 (step 2)	97.3	0.8% hydro-xypro-pylme-thylcel-lulose	>2000 (limit test)	No findings
Merkel, 2005a (Helm) ASB2012-11388	Rat, Sprague-Dawley	3 females/5000	97.23	Water	>5000 (limit test)	Diarrhea, ano-genital & facial staining, reduced faecal volume
Do Amaral	Rat, Wistar	3 females/2000	98.05	Water	>2000	No findings

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Guimaraes 2008 (Helm) ASB2012-11389		(step 1) 3 females/2000 (step 2)			(limit test)	
Taivioja, 2007 (Nufarm) ASB2012-11390	Rat, HanRcc:WIST	2 x 3 ♀/2000	95.1	PEG 300	>2000 (limit test)	Slightly ruffled fur
Reagan and Laveglia, 1988 (Monsanto) Z35389	Rat, Sprague Dawley	5/sex/5000	97.76	Water	>5000	Diarrhea, apparent urinary incontinence and hair loss on the abdomen
Heenehan et al., 1979 (Monsanto) Z35541	Rat, Wistar	5/sex/2500 5/sex/3500 5/sex/5000 5/sex/7000 5/sex/9900	99	Water	>5000	Mortalities: 1/10 1/10, 3/10,7/10, 10/10 at 2500, 3500, 5000, 7000 and 9900 mg/kg bw; clinical signs: ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen
Doyle, 1996 (Syngenta) TOX2000-1982	Rat	5/sex/5000	95.6	Water	>5000	No findings
Arcelin, 2007 (Syngenta) ASB2012-11391	Rat	3 ♀/5000	96.1	Water	>5000	Ruffled fur, hunched posture
Tavaszi, 2011 (Syngenta) ASB2012-11392	Rat	3 ♀/5000	96.3	0.5% CMC	>5000	No findings
Pooles, 2014 (Albaugh Europe Sàrl) ASB2014-9147	Rat	5 ♀/2000	85.8	DMS	>2000 (fixed dose method)	Hunched posture
Komura, Hitoshi, 1995 (Arysta) ASB2012-11383	Mouse, ICR	5/sex/5000	95.68	0.5% CMC	>5000 (limit test)	Decreased spontaneous motor activity, sedation and crouching position
Suresh, 1991 (FSG, now ADAMA) TOX9551089	Mouse, Swiss albino	5/sex/2500 5/sex/5000 5/sex/7500	96.8	Peanut oil	>7500	≥2500 mg/kg bw: mortality, lethargy, ataxia, dyspnoe, weight loss

Reference, (Owner), Study identification	Species, Strain	Number of animals / dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Tos et al., 1994 (Industria Prodotti Chimici) TOX9551624	Mouse, Charles River	5/sex/2000	technical	0.5% CMC	>2000 (limit test)	Piloerection, hunched posture, hypoactivity
Dideriksen & Skydsgaard 1991 (Cheminova) TOX9552320	Mouse, Bom:NMRI	5/sex/2000	98.6	Water	>2000 (limit test)	Piloerection, sedation

CMC = carboxymethylcellulose

Frequently occurring signs of oral intoxication were breathing difficulties, diarrhea, reduced activity, ataxia, piloerection, convulsions and hunched posture. Mortality was seen in few studies only and was confined to very high dose levels. The lowest dose causing mortality was 2500 mg/kg bw as reported by Suresh (1991, TOX9551089) for the mouse and by Heenehan et al. (1979, Z35541) for the rat. The number of dead animals at this dose was low and many studies have demonstrated that most animals tolerated the same or much higher doses of 5000 mg/kg bw or even above. Since the oral studies in rats and mice consistently revealed LD_{50} values >2000 mg/kg bw, classification for acute oral toxicity according to CLP regulation is not required.

Acute dermal toxicity

Table 11: Summary of acute dermal toxicity studies with glyphosate acid on rats and rabbits

Reference, (Owner,) Study identification	Species Strain	Number of animals/ Dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Sharp, 1995 (Sanachem) TOX9650910	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	>2000 (limit test)	Splenomegaly, Liver: centri-lobular congestion
Meyer-Carrive, 1994 (Sinon) TOX9500378	Rat, Sprague Dawley	5/sex/2000	95	Suspen-ded (50% w/w) in natrosol (1% w/w in water)	>2000 (limit test)	No findings
Snell, 1994 (Herbex) TOX9500246	Rat, Sprague Dawley	5/sex/2000	95	None	>2000 (limit test)	No findings
Tornai et al, 1994 (ALkaloida) TOX9650143	Rat, Wistar	2/sex/0 5/sex/2000	97.2	Water	>2000 (limit test)	No findings
Walker, 1992 (Barclay) TOX9551813	Rat, Sprague-Dawley	5/sex/2000	> 97	None	>2000 (limit test)	No findings
Suresh, 1991 (FSG, now ADAMA) TOX9551090	Rat, Wistar	5/sex/2500 5/sex/5000	96.8	Water (slurry)	>5000	body weight loss
Brett, 1990 (Agrichem) TOX9551793	Rat, CD	5/sex/0 5/sex/3000 5/sex/5000 5/sex/8000	98.1	0.9% saline	>8000	No findings
Cuthbert & Jackson, 1989 (Cheminova) TOX9300328	Rat, Sprague Dawley	5/sex/2000	98.6	Water for moiste-ning	>2000 (limit test)	No mortalities, body weight loss in one female, scab formation at application site; 0.5 h-1d after dosing reduced activity and piloerection
You, 2009 (Helm) ASB2012-11395	Rat, Sprague Dawley	5/sex/5050	96.4	Water	>5050	body weight loss in 1 male and 1 female
Komura, Hitoshi, 1995 (Arysta) ASB2012-11396	Rat, SD	5/sex/2000	95.68	Water	>2000 (limit test)	No findings
Simon, 2009 (Exxel) ASB 2012-11397	Rat, HanRcc:WIST	5/sex/2000	96.66	Water	>2000	No mortalities, no signs of systemic toxicity; in 4 females slight local signs (erythema, scaling and scabs) at the application sites

Reference, (Owner,) Study identification	Species Strain	Number of animals/ Dose level(s) (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Haferkorn, 2009 (Helm) ASB2012-11398	Rat, CD	5/sex/2000	98.8	Water	>2000	No findings
Haferkorn, 2010 (Helm) ASB2012-11399	Rat, CD	5/sex/2000	96.4	Water	>2000	No findings
Haferkorn, 2010 (Helm) ASB2012-11400	Rat, CD	5/sex/2000	97.3	Water	>2000	No findings
Merkel, 2005 (Helm) ASB2012-11401	Rat, Sprague Dawley	5/sex/5000	97.23	Water	>5000	No findings
Do Amaral Guimaraes 2008 (Helm) ASB2012-11402	Rat, Wistar Hannover	5/sex/2000	98.05	Water (for moisto-ning)	>2000	No findings
Taivioja, 2007 (Nufarm) ASB2012-11403	Rat, HanRcc:WIST	5/sex/2000	95.1	PEG 300	>2000 (limit test)	No findings
Doyle, 1996 (Syngenta) TOX2000-1983	Rat	5/sex/2000	95.6	Moiste-ned with deionised water	>2000	Slight erythema in 1♂, small scabs in 1 ♀
Arcelin, 2007 (Syngenta) ASB2012-11404	Rat	5/sex/5000	96.1	Moiste-ned with purified water	>5000	No findings
Zelenak, 2011 (Syngenta) ASB2012-11405	Rat	5/sex/5000	96.3	Moiste-ned with purified water	>5000	No findings
Reagan and Lavveglia, 1988 (Monsanto) TOX9552325	Rabbit, NZW	5/sex/5000	97.8	Moiste-ned with saline	>5000	Mortality (1 ♀); anorexia, diarrhea, soft stool

Apart from one female rabbit receiving 5000 mg/kg bw (Reagan and Lavveglia, 1988, TOX9552325), there were no deaths. Isolated signs of toxicity comprised body weight loss, diarrhea and slight local effects. Overall, the dermal studies with glyphosate acid in rats and rabbits revealed LD_{50} values of >2000 mg/kg bw or even of >5000 mg/kg bw. Therefore, classification for acute dermal toxicity according to CLP regulation is not required.

Acute inhalation toxicity

Table 12: Summary of acute inhalation toxicity studies with glyphosate acid

Reference, (Owner,) Study identification	Species Strain	Number of animals / Concentrations (mg/L air)	Purity (%)	Exposure conditions; Particle size if given	LC_{50} (mg/L air)	Main effects
Blagden, 1995 (Herbex) TOX9500247	Rat, Sprague Dawley	5/sex/5.35	95	Compressed air; 4 h nose-only	>5.35	Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head)
Tornai, 1994 (Alkaloida) TOX9650144	Rat, Wistar	5/sex/0 5/sex/1.138 5/sex/2.876	97.2	Watery aerosol; 4 h exposure, route not stated	>2.876	Trachea: lymphoid cell infiltration, mucous lung: congestion, haemorrhages, oedema liver: mononuclear cell infiltrations, congestion kidney: congestion, nephrocalcinosis
McDonald & Anderson, 1989 (Cheminova) TOX9552329	Rat, Sprague Dawley	5/sex/4.98	98.6	Dust aerosol; 4 h snout only	>4.98	No adverse findings
Haferkorn, 2010 (Helm) ASB2012-11406	Rat, CD	5/sex/5.18	97.3	4 h nose only (MMAD: 4.63 µm)	>5.18 (limit test)	Slight tremor, slight dyspnoea
Koichi, 1995 (Arysta) ASB2012-11407	Rat, Fischer F344	5/sex/5.48	97.56	Dust,4 h whole body (MMAD: 4.8 µm)	>5.48	Wet and soiled fur (periocular and nasorostral)
Griffith, 2009 (Exxel) ASB2012-11408	Rat	5/sex/5.04	96.66	Dust, 4 h, nose-only, (MMAD 5.25 µm)	>5.04	Increased respiratory rate, hunched posture, pilo-erection, wet fur
Haferkorn, 2009 (Helm) ASB2012-11409	Rat, CD	5/sex/5.12 (dust)	98.8	4h (MMAD: 6.62 µm)	>5.12 (limit test)	Slight dyspnoea and ataxia during exposure
Haferkorn, 2010 (Helm) ASB2012-11410	Rat, CD	5/sex/5.02	96.4	4h (MMAD: 4.2 µm)	>5.02	Slight dyspnoea, slight ataxia and slight tremor during exposure until 3 h after exposure
Carter, 2009 (Helm) ASB2012-11411	Rat, Sprague-Dawley	5/sex/2.24	96.4	4 h (MMAD: 2.6 µm)	>2.24 (limit test)	No findings
Merkel, 2005 (Helm) ASB2012-11412	Rat, Sprague-Dawley	5/sex/2.04	97.23	4 h (MMAD: 2.5 µm)	>2.04 (limit test)	No findings
Decker, 2007 (Nufarm) ASB2012-11414	Rat, albino	5/sex/3.252	95.1	4 h (MMAD: 2.95-3.05 µm)	> 3.252	Salivation in males, breathing effects in both sexes, body

Reference, (Owner,) Study identification	Species Strain	Number of animals / Concentrations (mg/L air)	Purity (%)	Exposure conditions; Particle size if given	LC_{50} (mg/L air)	Main effects
						weight loss
Rattray, 1996 (Syngenta) TOX2000-1984	Rat	5/sex/4.43 5/sex/2.47	95.6	4 h, nose-only, (MMAD: 2.91 and 3.41 µm)	>4.43	Mortality: 2♂ & 2♀ at 4.43 mg/L. Irregular breathing, splayed gait, shaking & reduced righting reflex
Nagy, 2011 (Syngenta) ASB2012-11415	Rat	5/sex/5.04	96.9	4 h nose-only (MMAD: 3.65 µm)	>5.04	Mortality: 1♂ on day 4. Laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, decreased activity and thin body appearance observed until day 3.

Inhalation toxicity of glyphosate was tested in rats and consistently found to be low. In many studies, a concentration ≥5 mg/L was tested. Thus, information on effects of inhaled glyphosate at high concentrations is sufficient even though this limit concentration was not attained in all experiments. Various clinical signs such as irritation of the upper respiratory tract, hyperactivity, increased or decreased respiratory rate, piloerection, loss of hair, wet fur, slight body weight reduction, slight tremor and slight ataxia were observed but were not consistent among the studies. Mortality was confined to the experiments of Rattray (1996, TOX2000-1984) and Nagy (2011, ASB2012-11415) using both test material of the same manufacturer but did not result in an LC50 value below 5 mg/L. Both studies are reported in detail in Volume 3 of the RAR in sub-section B.6.2.3. Since classification for inhalation toxicity is usually based on the LC_{50}, there is no need to classify glyphosate for this endpoint according to the CLP regulation since 5 mg/L air is the trigger concentration for dusts and mists.

4.2.2 Human data

No studies or case reports are available in which humans would have been exposed to the active ingredient itself. However, over the course of time, a number of poisoning incidents have been reported that were due to accidental or intentional (mostly oral, in very few cases inhalative) intake of glyphosate-based herbicides. For summary, see Vol.1, Section 2.6.11, and Vol. 3, B.6.9.4, of the attached RAR. In most cases, actual exposure remained unknown. Furthermore, it is not possible to clearly distinguish between effects due to glyphosate and those caused by co-formulants.

A calculation of ingested doses in a few cases of severe intoxications, including fatalities, suggests that a potentially lethal dose of glyphosate contained in plant protection products to humans will be above 2000 mg/kg bw. According to Lee et al. (2000, ASB2012-11512), Beswick and Millo (2011, ASB2014-9283), Sribanditmongkol et al. (2012, ASB2014-9731) or Zouaoui et al. (2013, ASB2014-9734), ingestion of 300 mL or more of products such as Roundup® containing 36 to 41% glyphosate may result in a fatal outcome, even though most patients survived. A dose of 300 mL of such a formulation would contain up to 123 g glyphosate resulting in a dose of ca 2050 mg/kg bw in a man weighing 60 kg. There is strong evidence that certain co-formulants, e.g., some polyoxethylated alkylamines (POEA, used as surfactants), may either enhance the toxicity of glyphosate or exhibit independent toxic properties resulting in a higher toxicity of many

formulations as compared to the active ingredient (see Vol. 3, B.6.13.3). As far as is known, such surfactants were part of the plant protection products that were ingested in the described clinical cases.

On balance, a higher acute toxicity of glyphosate to humans than to rats is not likely.

Accordingly, poisoning incidents in humans do not support classification and labelling of glyphosate for acute toxicity and are not appropriate for this purpose.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Non-human information

Based on the multitude of acute toxicity studies in rats and mice (see Table 9, Table 10, and Table 11), classification of STOT SE (categories 1 or 2) is not appropriate because non-lethal effects were confined to very high doses and were rather unspecific. This assessment is further supported by the acute neurotoxicity study in rats (Horner, 1996, ASB2012-11500, see Vol. 3, B.6.7) in which no evidence of neurotoxicity was observed at dose levels of 500, 1000, and 2000 mg/kg bw even though unspecific clinical signs occurred and one single female animal was found dead at the top dose level. No clinical evidence of single (i.e., first) dose effects was obtained from the many toxicological studies with repeated administration in which lower doses were applied. Suitable haematological and clinical chemistry data is not available since sampling was not performed during the first days of treatment but, taking into account the toxicological profile of glyphosate, alterations in these parameters are not expected.

With regard to category 3, no evidence of narcotic effects was obtained in any toxicological study. For considerations of respiratory tract irritation, the reader is referred to 4.4.3.

In summary, there is no need to classify glyphosate for STOT SE.

4.3.2 Human data

No appropriate data is available for the active substance. No evidence of organ-specific non-lethal effects (except eye irritation) can be derived from poisoning incidents with formulations.

4.4 Irritation

4.4.1 Skin irritation

In older studies (see Vol. 3, B.6.2.4), either no or only slight/very slight irritation was found. A number of more recent, guideline-compliant studies in rabbits have been submitted for the new EU evaluation and are summarised in Table 13.

Table 13: Summary of most recent skin irritation studies with glyphosate acid

Study (Owner)	Species Strain	Number and sex of animals	Purity [%]	Amount applied / Exposure conditions	Result
Talvioja, 2007 (Nufarm) ASB2012-11418	Rabbit NZW	1 ♂, 2 ♀	95.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Hideo, 1995	Rabbit	6 ♀	97.56	0.5 g moistened with	Non irritant

Study (Owner)	Species Strain	Number and sex of animals	Purity [%]	Amount applied / Exposure conditions	Result
(Arysta) ASB2012-11420	NZW			0.5 mL water; intact skin	
Leuschner, 2009a (Helm) ASB2012-11419	Rabbit Himalayan	3 ♂	96.4	0.5 g moistened with water; intact skin	Non irritant
Leuschner, 2009b (Helm) ASB2012-11421	Rabbit Himalayan	3 ♂	98.8	0.5 g moistened with water; intact skin	Non irritant
Leuschner, 2010 (Helm) ASB2012-11422	Rabbit Himalayan	3 ♂	97.3	0.5 g moistened with water; intact skin	Non irritant
You, 2009 (Helm) ASB2012-11423	Rabbit NZW	1 ♂, 2 ♀	96.4	0.5 g moistened with water; intact skin	Non irritant
Merkel, 2005 (Helm) ASB2012-11424	Rabbit, NZW	3 ♂	97.23	0.5 g moistened with water; intact skin	Slightly irritating
Canabrava Frossard de Faria, 2008 (Helm) ASB2012-11425	Rabbit, NZW	3 ♀	98.05	0.5 g moistened with water; intact skin	Non irritant
Doyle, 1996 (Syngenta) TOX2000-1985	Rabbit, NZW	6 ♀	95.6	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Arcelin, 2007 (Syngenta) ASB2012-11426	Rabbit NZW	1 ♂, 2 ♀	96.1	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
Zelenak, 2011 (Syngenta) ASB2012-11427	Rabbit NZW	3 ♂	96.3	0.5 g moistened with water; intact skin	Slightly irritating

NZW = New Zealand White

Of these 11 studies, 9 were unequivocally negative. Also the remaining two studies do not suggest a need for classification. Merkel (2005, ASB2012-11424) as well as Zelenak (2011, ASB2012-11427) reported very slight erythema in one animal that had, in both studies, cleared within 24 hours.

Thus, when compared to CLP criteria, glyphosate should not be classified and labelled for skin irritation.

In humans, skin irritation was seldom reported (Bradberry et al., 2004, ASB2012-11576). Most likely, the few documented cases were due to co-formulants in glyphosate-containing herbicides. Taking the extensive world-wide use of such products into account, skin irritation by glyphosate is not of concern for humans.

4.4.2 Eye irritation

In 1999, glyphosate was classified by the former European Chemicals Bureau as an eye irritant (Xi) and labelled with the risk phrase R41 (“Risk of serious damage to eyes”). This decision was based

on a German proposal because of several findings of either eye irritation or at least slight irritation in all of a total of six studies that had been reviewed for first evaluation by the EU.

In preparation of the new EU evaluation, a number of studies were submitted that had not been reviewed before at EU level and are compiled in Table 14.

Table 14: Eye irritation tests with glyphosate acid in rabbits that had not been previously reviewed for classification and labelling purposes

Reference; Study identification; owner	Strain, number of Animals	Purity	Amount applied	Effects / Result
Kuhn, 1996; TOX1999-881; Cheminova	NZW, 6 male, 3 females	98.2%	0.1 mL (65 mg)	Severely irritant in unwashed eyes: corneal opacity, conjunctival redness, chemosis, not reversible within 21 days (2 females); moderate irritation in washed eyes, reversible within 21 days Irritant
Talvioja, 2007; ASB2012-11428; Nufarm	NZW, 1 male, 2 females	95.1%	100 mg	Marked, early onset and transient ocular changes (Cornea opacity, conjunctival redness, chemosis), reversible within 10 days, no signs of corrosion or staining Irritant
Leuschner, 2009; ASB2012-11429; Helm	Himalayan, 3 males	96.4%	100 mg rinsed 1h post appl.	Slight signs of ocular changes, reversible within 7 days Non-irritant
Hideo, 1995; ASB2012-11430; Arysta	NZW. 12 females	97.56%	100 mg (pure)	6 females without eye irrigation: Cornea opacity: not reversible within 21 days (3/6 females); iris lesions: all females and reversible within 10 days; conjunctival redness & chemosis: all females and reversible within 16 days; 6 females with eye irrigation (30 sec. & 2 min. post application): reduced effects and faster recovery Irritant
Leuschner, 2009; ASB2012-11432; Helm	Himalayan 3 males	98.8%	100 mg rinsed 1h post appl.	Non-irritant
Leuschner, 2010; ASB2012-11433; Helm	Himalayan 3 males	97.3%	100 mg rinsed 1 h post appl.	Non-irritant
You, 2009; ASB2012-11434; Helm	NZW 2 males 1 female	96.4%	0.1 mL (93.2 mg)	Cornea opacity, iris lesions, conjunctival redness & chemosis reversible within 9 days Irritant
Merkel, 2005; ASB2012-11435; Helm	NZW 3 males	97.23%	0.1 mL (60 mg)	All animals: corneal opacitiy, iris lesions, conjunctival redness & chemosis, reversible within 10 days Irritant
Canabrava Frossard de Faria, 2008; ASB2012-11436; Helm	NZW 1 male 1 female	98.5%	100 mg	Only 2 animals due to severe effects: Corneal opacity, iritis, conjunctival hyperemia, edema and secretion. Effects in female not reversible within 21 days Irritant

Reference; Study identification; owner	Strain, number of Animals	Purity	Amount applied	Effects / Result
Reagan & Laveglia, 1988; Z35395; Monsanto	NZW 6 animals, likely 3/sex	97.76%	100 mg	One rabbit died: considered not treatment related Corneal opacitiy, iritis, conjunctival redness, chemosis in 6/6 animals. Some effects not reversible within 21 days Irritant
Johnson, 1997; TOX2000-1986; Syngenta	NZW 6 females	95.6%	100 mg	Corneal opacity, iritis,conjunctival redness and chemosis. All effects reversible within 8 days Moderately Irritant (according to Kay & Calandra)
Arcelin, 2007; ASB2012-11437; Syngenta	NZW 1male 2 females	96.1%	100 mg	Mild, early-onset and transient ocular changes (reversible within 7 days) Irritant
Tavaszi, 2011 ASB2012-11438; Syngenta	NZW 1 male	96.3%	Glyphosate technical 100 mg	Based on results in one animal, study was terminated at 24 h: corneal opacity & erosion; conjunctiva: redness, chemosis, discharge, few black points; oedema of the eyelids; positive fluorescein staining at 24 h Corrosive

In a total of 13 studies, eye irritation by glyphosate was observed in 9 of them and a further one even revealed corrosive properties. The studies themselves are reported in detail in the attached Volume 3 (B.6.2.5) of the RAR. In contrast, glyphosate proved negative for eye irritation in three studies (Leuschner, 2009, ASB2012-11429; Leuschner, 2009, ASB2012-11432; Leuschner, 2010, ASB2012-11433). However, in these studies, rinsing of the eyes was performed one hour after instillation. This is not in compliance to the current OECD Guideline 405 in which rinsing is scheduled after 24 hours. In many studies, there was no rinsing at all. Thus, it may be assumed that the different outcome was due to this methodological change and that testing in these three experiments by the same researcher was not that rigorous as in the other studies. In three further studies in which test material from the same company (even though of different purity) was applied in another laboratory, the outcome was positive (Merkel, 2005, ASB2012-11435; Canabrava Frossard de Faria, 2008, ASB2012-11436; You, 2009, ASB2012-11434).

In any case, the majority of tests clearly pointed to the risk of eye irritation by glyphosate. Accordingly, the need for classification for eye irritation was confirmed. If category 1 or 2 is more appropriate, depends on the severity and reversibility of effects. Criteria for allocation to category 1 are the following:

- Effects on cornea, iris or conjunctiva at least in one animal that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
- A positive response score (mean following grading at 24, 48, and 72 hours after instillation) for corneal opacity ≥ 3 and/or iritis > 1.5 in at least 2 of 3 animals.

At least one of these criteria was met in the studies by Tavaszi (2011, ASB2012-11438), by Canabrava Frossard de Faria (2008, ASB2012-11436), by Merkel (2005, ASB2012-11435) and by Reagan and Laveglia (1988, Z35395) whereas the other positive studies would instead support classifying glyphosate in category 2.

Since evidence of strong eye irritation was obtained in several (even though not in all) studies, it is proposed to assign category 1.

Accordingly, the current classification "Eye irritation, Category 1" is confirmed. The signal word is "Danger" and the appropriate hazard statement is H318: "Causes serious eye damage".

At least transient eye irritation is a rather frequent symptom in humans following contact with herbicides containing glyphosate (e.g., Acquavella et al., 1999, TOX2002-699). These observations might be due to glyphosate confirming the animal evidence but may be also caused or or enhanced by co-formulants such as POEA surfactants which exhibit a strong eye-irritating potential themselves (see Vol. 3, B.6.13.3).

4.4.3 Respiratory tract irritation

Respiratory tract irritation might be expected because of the eye irritating potential of glyphosate and, in fact, could have actually occurred occasionally in acute inhalation studies (e.g., Tornai, 1994, TOX9650144, see Table 12) but cannot be clearly distinguished from inhalation toxicity. In any case, it would have been confined to high concentrations. In the current CLP guidance, it is stated that evaluation, in the absence of validated animal tests, will be based primarily on human data.

In humans, there is no evidence for respiratory tract irritation by the active substance even though one must acknowledge that such an exposure will seldom occur. For formulations, Burger et al. (2009, ASB2013-11831) reported cases from Germany that might indicate respiratory irritation but, most likely, these findings were due to POEA surfactants.

On balance, there is no sufficient evidence to classify glyphosate for respiratory tract irritation. It should be taken into account that glyphosate is classified and labelled for eye irritation and, thus, irritating properties are already adequately covered.

4.5 Corrosivity

Physico-chemical properties of glyphosate do not suggest corrosive potential. In line with that, evidence of corrosivity coming from the animal studies was confined to a single eye irritation study (Tavaszi, 2011, ASB2012-11438) but was not confirmed in a great number of similar studies for this endpoint or in any of the dermal toxicity or skin irritation studies.

Apart perhaps from the manufacturing process, humans will be always exposed to formulations containing the active ingredient rather than to the pure active ingredient. There were no reports to date pointing to corrosive properties of such formulations, despite clear evidence for eye or mucosal irritation.

Thus, glyphosate should not be considered corrosive and the proposed classification and labelling for eye irritation is adequate and sufficient.

4.6 Sensitisation

4.6.1 Skin sensitisation

There is no animal study suggesting skin sensitisation by glyphosate (see Vol. 3, B.6.2.6). In Table 15, the available and acceptable or at least supplementary maximisation (Magnusson and Kligman) tests and local lymph node assays (LLNA) are listed since they are considered more rigorous and

reliable than the Buehler test. It should be noted that Buehler tests with glyphosate were also consistently negative.

Table 15: Summary of skin sensitisation studies with glyphosate acid

Study	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Result
Snell, 1994 (Herbex) TOX9500250	Guinea pig, Dunkin Hartley	15 ♀	95	Induction: 1% w/v in arachis oil; challenge: 25% w/w or 50% w/w in arachis oil	MK	Not sensitising
Pore et al, 1993 (Luxan) TOX9650652	Guinea pig, English	48 (both sexes)	≥95	Intradermal induction: 5% in propylene glycol; topical: 50% in petrolatum	MK	Not sensitising
Walker, 1991 (Agrichem) TOX9551796	Guinea pig Dunkin Hartley	38 ♀	Not stated	Intradermal induction: 0.1% (w/v) in water; topical: 50% (w/v) in water; challenge: 25% (w/w) in water	MK	Not sensitising
Cuthbert & Jackson, 1989 (Cheminova) TOX9552343	Guinea pig, Dunkin Hartley	46 ♀	98.6	Induction: 10% in water; challenge: 25% in water	MK	Not sensitising
Talvioja, 2007 (Nufarm) ASB2012-11439	Guinea pig	20 ♀/test 10 ♀/control	95.1	Intradermal induction: 3% (w/v) in PEG-300; topical induction: 50% (w/v) in PEG-300; challenge: 25% (w/v) in PEG-300	MK	Not sensitising
Haferkorn, 2010 (Helm) ASB2012-11440	Guinea pig, Dunkin Hartley	15 ♀ (+ 20 for positive control)	96.4	Intradermal induction: 0.01% in water; topical induction: 50%; challenge: 25%	MK	Not sensitising
Hideo, 1995 (Arysta) ASB2012-11441	Guinea pig, Hartley	60 ♀	97.56	Intradermal induction: 5% (w/v) in paraffin oil, topical induction: 25% (w/v) in white petrolatum; challenge: 25% (w/w) in white petrolatum	MK	Not sensitising
Simon, 2009 (Exxel) ASB2012-11442	Guinea pig	15 ♂	96.66	Intradernal induction: 10% (w/w) in purified water; topical induction: 50% (w/w) in purified water; challenge: 15% (w/w) in purified water	MK	Not sensitising
Haferkorn, 2009 (Helm) ASB2012-11443	Guinea pig	15 ♂ (+ 20 for positive control)	98.8	Intradermal induction: 0.01% in water, topical induction: 50%; challenge: 50%	MK	Not sensitising
Haferkorn, 2010 (HAG)	Guinea pig	15 ♂ (+ 20 for	97.3	Intradermal induction: 0.5% in water; topical	MK	Not sensitising

Study	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Result
ASB2012-11444		positive control)		induction: 50%; challenge: 25%		
Richeux, 2006 (Nufarm) ASB2012-11448	Guinea pig	20 ♀/test 10 ♀/control	95.7	Intradermal induction: 0.195% (w/v) in isotonic saline; topical induction: 60% (w/v) in water; challenge: 60% (w/v) & 30% (w/v) in water	MK	Not sensitising
Doyle, 1996 (Syngenta) TOX2000-1987	Guinea pig	20 ♀/test 10 ♀/control	95.6	Intradermal induction: 0.1% (w/v) in water; topical induction: 75% (w/v) in water; challenge: 75% (w/v) & 30% (w/v) in water	MK	Not sensitising
Betts, 2007 (Syngenta) ASB2012-11449	Mouse, CBA	4 ♀/group	96.1	Glyphosate acid dose levels: 0, 10, 25, 45 (% w/v) Hexylcinnamaldehyde (positive control) demonstrated sensitivity of study	LLNA	Not sensitising
Török-Batho, 2011 (Syngenta) ASB2012-11450	Mouse, CBA	4 ♀/group	96.3	Glyphosate acid dose levels: 0, 10, 25, 50 (% w/v) Hexylcinnamaldehyde (positive control) demonstrated sensitivity of study	LLNA	Not sensitising

MK = Magnusson Kligman Maximisation Test
LLNA = Local Lymph Node Assay

Thus, there is unequivocal evidence that glyphosate did not produce skin sensitisation in laboratory animals. Classification and labelling are not needed. To date, there are no reports on skin sensitisation by glyphosate or its formulations in humans.

4.6.2 Respiratory sensitisation

An appropriate animal model is not available. There is no evidence of respiratory sensitisation in humans by contact with formulations containing glyphosate.

4.7 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.7.1 Non-human information

Identification of toxic effects requiring classification and labelling for specific target organ toxicity – repeated exposure (STOT RE) is usually based on short-term (28 days, 90 days, in dogs also 1 year) or lifetime studies. However, other study types, e.g. for reproductive or developmental toxicity, may also provide relevant information (see Guidance on the Application of the CLP

Criteria, Version 4.1 – June 2015, 3.9.2.1.2. Identification of non-human data) and may possibly support a need for classification. The latter case is applicable to glyphosate but a comprehensive picture shall be given. Therefore, in this sub-section, the available short-term toxicity studies with glyphosate are reported first. Thereafter, non-cancer effects in long-term studies are considered. In the third part, maternal toxicity in developmental studies in rabbits is addressed since the new proposal for classification is based on mortality occurring in this animal model.

Short-term studies

A multitude of oral short-term studies with glyphosate was conducted mainly in rats and dogs. In addition, a small number of studies were performed in mice by the oral route or in rats and rabbits by dermal application.

Glyphosate was administered in few subacute studies (duration 14 or 28 days) by the oral route to rats and dogs. Toxicity upon dietary administration to rats was very low with only minor effects such as soft faeces or alterations in some haematological and clinical chemistry parameters at high dose levels (Suresh, 1991a-c, TOX9551095, Z102035, Z102043). The lowest NOAEL of 50 mg/kg bw/day as established by Atkinson et al. (1989, TOX9552351) was mainly based on a higher incidence of nephrocalcinosis in females at 250 mg/kg bw/day and above. However, this finding was not confirmed in a subsequent 90-day study employing more animals that was performed in the same laboratory and rat strain at much higher dose levels (Perry et al., 1991, TOX9552364). Therefore, and since there were no histopathological renal findings in any other short-term study with glyphosate in rats, nephrocalcinosis cannot be attributed to glyphosate administration. In dogs, there were no treatment-related findings observed up to 1000 mg/kg bw/day (Gobordhun and Oshodi, 1989, TOX9552352).

In both Sprague-Dawley (Heath et al., 1993, TOX9552367) and Wistar-derived rats (Pinto, 1996, ASB2012-11461) as well as in NZW rabbits (Johnson, 1982, TOX9552366; Tornai, 1994, TOX9650151), no signs of systemic toxicity became evident following repeated application of glyphosate to the skin over a period of 3 or 4 weeks up to the highest tested dose levels of 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit. However, weak dermal irritation was observed at these high dose levels in both species.

On balance, the subacute studies do not support a classification for STOT RE.

Subchronic studies (90 days or longer) with glyphosate were conducted by the oral route only.

The available studies in rats that are considered acceptable according to today's standards are summarised in Table 16. Taken together, all these studies have demonstrated low toxicity of glyphosate in different rat strains upon repeated oral administration. Soft stools and diarrhoea, together with occasionally reduced body weight gain, might suggest some irritation of the gastrointestinal tract at high dose levels that is not unexpected for a compound of acidic properties and known irritancy at least to the eyes. In the same studies, blood (Parker, 1993, TOX9650149) or haemoglobin (Coles et al., 1996, ASB2012-11451) were observed in urine at high dose levels. A decrease in urine pH was quite frequently noted.

These findings may be assumed to result from physico-chemical properties of glyphosate but this does not necessarily mean that they were not adverse. The same holds true for parotid salivary gland findings reported by Perry et al. (1991, TOX9552364). Histological alterations comprised deep basophilic staining and enlargement of cytoplasm at all dose levels including very few control animals but were clearly more pronounced with regard to incidence and severity at the top dose level in males and females. They were not accompanied by organ weight changes neither of the parotid nor of the sublingual or submaxillary glands. In the latter two glands, no histopathological

changes were noted. The absence of indications for such changes in other studies may be explained by the fact that different or no glands had been examined. Parker (1993, TOX9650149) reported swelling and reddening of sublingual salivary glands in a few animals but no dose response became apparent and histological examination did not reveal any noteworthy findings. Salivary glands were not weighed. Eadie (1989, TOX9551821) and Suresh (1992, TOX9551096) did not report pathological changes in the salivary glands (not further specified). Stout and Johnson (1987, TOX9552362) examined the submaxillary gland only but did not detect any pathological changes. In the more recent studies by Botham (1996, TOX2000-1990) and Coles et al. (1996, ASB2012-11451), salivary glands were reported to be taken but were apparently not weighed or examined histologically. Kinoshita (1995, ASB2012-11452) performed histopathology of the sublingual and submaxillary glands without any noteworthy findings observed but left the parotid gland out of the investigation. Chan and Mahler (1992, TOX9551954), however, published a study in F344 rats in which they reported basophilic changes and hypertrophy of acinar cells in the submaxillary and, more pronounced, in the parotid salivary glands at all dose levels (ranging from 3125 to 50000 ppm). Severity of these findings were clearly related to dose and, based on severity, the NOAEL was set at 6250 ppm, equal to about 400 mg/kg bw/day (JMPR, 2004, ASB2008-6266). These findings directly supported the observations by Perry et al. (1991, TOX9552364).

Alterations in clinical chemistry parameters in the majority of experiments, most often a higher activity of alkaline phosphatase, suggested a weak effect on the liver.

Two studies (Kinoshita, 1995, ASB2012-11452; Coles et al., 1996, ASB2012-11451) identified the caecum as an additional target organ because of certain findings (distention, elevated weight of this part of the intestines and its contents, mucosal atrophy) that had not been noticed before. Even if a specific vulnerability of Sprague-Dawley rats would be assumed, it is difficult to explain why such changes were not observed previously at higher dose levels by Stout and Johnson (1987, TOX9552362), Perry et al. (1991, TOX9552364) or Parker (1993, TOX9650149). One might expect that at least caecal distention would have been observed and reported if it had occurred.

Table 16: Oral subchronic studies in rats

Reference; Study identification; Batch, purity; Owner	Strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Main effects
Botham, 1996; TOX2000-1990; P15, 97.4%; Syngenta	Wistar-derived (Alpk:APfSD), 90 d, feeding	0, 1000, 5000, 20000 ppm	414 mg/kg bw/d (5000 ppm)	1612 mg/kg bw/d (20000 ppm)	Bw gain↓ in m; alterations in some clinical chemistry parameters, in particular AP/ALAT activity↑, urine pH↓
Coles et al., 1996; ASB2012-11451; H95D 161 A, 95.3%; Nufarm	Sprague-Dawley (CD), 90 d, feeding	0, 1000, 10000, 50000 ppm	79 mg/kg bw/d (1000 ppm)	730 mg/kg bw/d (10000 ppm)	Soft faeces, diarrhea; bw gain, food consumption, food efficiency↓ and hemoglobin in urine at top dose level, urine pH↓; alterations in some clinical chemistry parameters, in particular AP activity↑ and Ca↓ at mid and high dose levels; caecum: distention (top

Reference; Study identification; Batch, purity; Owner	Strain, duration, route	Dose levels	NO(A)EL	LO(A)EL	Main effects
					dose groups) and mucosal atrophy (at the two upper dose levels)
Kinoshita, 1995; ASB2012-11452; Batches: 940908, 95.7%; 941209, 95%; T-941209; 97.6%; Arysta	Sprague-Dawley (Crj: CD), 90 d, feeding	0, 3000, 10000, 30000 ppm	168 mg/kg bw/d (3000 ppm)	569 mg/kg bw/d (10000 ppm)	Bw gain↓ in m; alterations in some clinical chemistry parameters, in particular AP activity↑, urine pH↓; caecum: distention and wt (with contents)↑
Perry et al., 1991; TOX9552364; Batch 206-JaK-25-1, 98.6%; Cheminova	Sprague-Dawley, 90 d, feeding	0-20-300-1000 mg/kg bw/d (dietary levels weekly adjusted)	300 mg/kg bw/d	1000 mg/kg bw/d	Bw gain↓ in m, urine pH↓ and some changes in clinical chemistry parameters in f ; m/f: cellular alterations in parotid salivary glands
Parker, 1993; TOX9650149; Lot 46540992, purity not given; Alkaloida#	Sprague-Dawley, 90 d, feeding	0, 2000, 6000, 20000 ppm	371 mg/kg bw/d (6000 ppm)	1262 mg/kg bw/d (20000 ppm)	Diarrhea in m/f; blood in urine; organ wt changes without pathological findings
Suresh, 1992; TOX9551096; Batch 60, 96.8%; ADAMA#	Wistar, 90 d (+28 d recovery, hig dose), feeding	0, 200, 2000, 20000 ppm (+20000 ppm for recovery) group)	147 mg/kg bw/d (2000 ppm)	1359 mg/kg bw/d (20000 ppm)	Bw gain↓ in f; AP activity↑ in m, glucose↑ in f
Eadie, 1989; TOX9551821; Batch L16566, 97.1%; Barclay	Sprague-Dawley (CD), 90-92 d (+35 d recovery for additional control and top dose groups)	0, 2000, 3000, 5000, 7500 ppm (+ 7500 ppm for recovery)	7500 ppm (375 mg/kg bw/d assumed, mean dietary intake not caclculated)	>7500 ppm	No effects up to highest dose
Stout and Johnson, 1987; TOX9552362; Lot XLG 161, 95.2%; Monsanto	Sprague-Dawley, 90 d, feeding	0, 1000, 5000, 20000 ppm	1267 mg/kg bw/d (20000 ppm)	>1267 mg/kg bw/d (20000 ppm)	No effects up to highest dose

[#] supplementary study

It should be explained here that the "main effects" were statistically significant if body weight and organ weights were affected and haematological or clinical chemistry parameters altered. Clinical signs and histological lesions were also reported when occurring in a higher number of animals as in the control group but were not always subject to statistical evaluation or did not gain statistical significance in all cases. Not all of the mentioned findings were observed necessarily at the LOAEL but sometimes only at higher dose levels. This table (as well as Tables 17 and 18 below) is more intended to give an impression of the effect pattern. In any case, statistical significance was taken into account when the NOAELs/LOAELs in the individual studies were established.

In the dog, short-term toxicity (if compared to the life-expectancy of the species) of glyphosate was investigated in a number of studies with oral administration, either via capsules or in the diet. The

valid subchronic dog studies (90 days or 1 year) are summarised in Table 17.

On the whole, the results have shown that the dog is of similar sensitivity as the rat when the NOAELs/LOAELs are considered. There is limited evidence coming from one study that high dose effects may be more severe than in rats or mice but these observations appear somehow inconsistent among the studies.

In the most recent 90-day study by Gaou (2007, ASB2012-11454), severe signs of toxicity were noted in the high dose groups receiving 1000 mg/kg bw/day. The test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) and body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11. Treatment-related histopathological changes in surviving animals consisted of an increased number of adipocytes in the sternal bone marrow in both sexes, as well as prostate and uterine atrophy and other, more infrequent changes in various organs. It is clear that the Maximum Tolerable Dose (MTD) was by far exceeded. In contrast, in the study by Gobordhun (1991, TOX9552384), the same high dose of 1000 mg/kg bw/day was administered also in capsules but for one year causing only minor effects. There is no explanation for this apparent difference although it is known from long-term studies in rats and mice that high-dose effects of glyphosate may differ considerably. A lower purity (and other source) of the test material applied by Gaou (2007, ASB2012-11454) might be relevant.

In 90-day or one-year studies with dietary administration, very few findings were obtained suggesting that glyphosate was better tolerated when administered via the diet than in capsules.

Prakash (1999, ASB2012-11455) reported an initial decline in food consumption and body weight gain but normalisation to control levels was quickly achieved. The only clinical chemistry alteration that was likely related to treatment, i.e., a higher bilirubin concentration, was not accompanied by any pathological change. Thus, these effects were not regarded as adverse.

In the study by Hodge (1996, TOX2000-1991), weak toxic effects were noted at the exaggerated top dose of 50000 ppm, including a decrease in body weight gain and some evidence of liver toxicity. The next lower dietary level of 10000 ppm (approx. 320 mg/kg bw/day) was considered the NOAEL. In line with that, Yoshida (1996, ASB2012-11456) did not find any effects (apart from a reduction in urine pH due to acidic properties of the test substance) in a study in which even higher dietary dose levels of up to 40000 ppm were employed.

Table 17: Subchronic oral studies with glyphosate in dogs

Reference; Study identification; Batch, purity; Owner	Breed, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Gaou, 2007; ASB2012-11454; H05H016A, 95.7%; Nufarm	Beagle, 13 week, oral capsules	0, 30, 300, 1000 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d	Clinical signs (liquid/soft faeces, dehydration, vomi-ting) making termination of high dose groups after 11 wk necessary; bw/bw gain and food consumption↓; clinical chemistry and urine parameters altered; prostate aund uterus atrophy; histological lesions in many organs (such as kidney liver, bone marrow) related to

Reference; Study identification; Batch, purity; Owner	Breed, duration, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
					moribund state
Prakash, 1999; ASB2012-11455; Lots 01/12/1997 and 01/06/1997, >95% both; ADAMA	Beagle, 90 d, dietary	0, 200, 2000, 10000 ppm (equal to 5.2/5.4; 54.2/52.8, 252.4/252.7 mg/kg bw/d in m/f)	252 mg/kg bw/d	>252 mg/kg bw/d	No adverse effects up to highest dose level
Yoshida, 1996; ASB2012-11456); T940308, 94.61%; Arysta	Beagle, 13 week, dietary	0, 1600, 8000, 40000 ppm (approx. 40, 198/201, 1014/1015 mg/kg bw/d in m/f)	1014 mg/kg bw/d	>1014 mg/kg bw/d	Decrease in urine pH in high dose females not regarded as adverse; no further effects
Hodge, 1996; TOX2000-1991; Lots D4490/1, P18, 99.1%; Syngenta	Beagle, 90 d, dietary	0, 2000, 10000, 50000 ppm (68/68, 323/334, 1680/1750 mg/kg bw/d in m/f)	323 mg/kg bw/d	1680 mg/kg bw/d	Bw gain↓; alterations in some clinical chemistry parameters (calcium, albumin↓ in m, AP↑ in f); liver wt↑
Haag, 2008; ASB2012-11457; H05H016A, 95.7%; Nufarm	Beagle, 52 wk, capsules	0, 30, 125, 500 mg/kg bw/d	500 mg/kg bw/d	>500 mg/kg bw/d	No adverse effects, calcium↓ in high dose m
Nakashima, 1997; ASB2012-11458; T-950380, 94.61%; Arysta	Beagle, 12 month, dietary	0, 1600, 8000, 50000 ppm (34/37, 182/184, 1203/1259 mg/kg bw/d in m/f)	182 mg/kg bw/d	1203 mg/kg bw/d	Bw gain↓, loose stool, alterations in some hematological and clinical chemistry parameters
Brammer, 1996; TOX2000-1992; P24, 95.6%; Syngenta	Beagle, at least one year, dietary	0, 3000, 15000, 30000 ppm (*ca* 91, 440/447, 907/926 mg/kg bw/d in m/f)	447 mg/kg bw/d	926 mg/kg bw/d	Bw gain↓ in f
Gobordhun, 1991; TOX9552384; 206-JaK-25-1, 98.6%; 206-JaK-95-5, 99.5%; 229-JaK-5-1, 98.9%; Cheminova (/Monsanto)	Beagle, 52 week, oral capsules	0, 30, 300, 1000 mg/kg bw/d	300 mg/kg bw/d	1000 mg/kg bw/d	Soft/loose/liquid stool, evidence of lower bw gain (not attending statistical significance)

Again, statistical significance was achieved for most effects on body weight, liver weight and laboratory parameters, if not the contrary is indicated. Clinical signs and histological findings were considered on the basis of individual animals affected. In general, statistical considerations are less important for a study with low numbers of individuals per dose level.

Toxicity of glyphosate to mice was investigated in a small number of subchronic studies. The NOAEL in the most recent valid 90-day study was 1221 mg/kg bw/day (Kuwahara, 1995, ASB2012-11453). A very high dose of approx. 6300 mg/kg bw/day caused a reduction in body weight gain, food consumption and efficiency and alterations in some haematological and clinical

chemistry parameters with the latter findings pointing to liver toxicity. Gross necropsy revealed caecum distention that was supported by a higher organ weight but not accompanied by histological lesions. Cystitis of urinary bladder became histologically apparent in some high dose males. Urinary pH (most likely due to acidic properties of the test substance) was noted in all treated male groups. In a previous study (Perry et al., 1991, TOX9552363), no effects were observed up to the highest dose level of 4500 mg/kg bw/day. While these two studies would suggest a lower toxicity in mice than in the rat, a published study from the U.S. NTP (Chan and Mahler, 1992, TOX9551954) provided a lower NOAEL of about 500 mg/kg bw/day in another strain, based on histological changes in the parotid gland at about 1065 mg/kg bw/day and above. The findings comprised increased basophilia but also enlarged cells and acini with relative reduction in the number of acinar ducts. In the studies by Kuwahara (1995, ASB2012-11453) and Perry et al., (1991, TOX9552363), no effects on sublingual or submaxillary glands were noted but the parotid gland was not examined although it is obviously more sensitive to histological changes caused by glyphosate. Taking the salivary gland findings into account, toxicity of glyphosate acid in the mouse appears similar to that in the rat.

Long-term studies

Chronic toxicity, i.e., occurrence of non-neoplastic effects in studies of longer duration, might be also relevant for a STOT RE classification. With glyphosate, a large number of long-term studies have been performed in rats and mice. In a one-year feeding study for chronic toxicity in Wistar-derived rats, Milburn (1996, TOX2000-1998) observed effects on body weight, food consumption and food efficiency as well as an increase in alkaline phosphatase activity and focal basophilia of acinar cells of parotid salivary gland. Unfortunately, the weight of the parotid gland was not determined. Effects occurred from a dietary dose of 8000 ppm (corresponding to 560 mg/kg bw/day in male rats and to 671 mg/kg bw/day in females) onwards with the NOAEL being the next lower dose of 2000 ppm (equal to 141 or 167 mg/kg bw/day).

The long-term (2 years) combined chronic toxicity and carcinogenicity studies in rats and the carcinogenicity studies in mice (18 months or 2 years) are reported in the section on carcinogenicity. Here, it is sufficient to state that an overall NOAEL for the rat studies in the magnitude of 100 mg/kg bw/day may be derived whereas first effects were seen in the range of 300-400 mg/kg bw/day in at least three studies (Stout and Ruecker, 1990, TOX9300244; Atkinson et al., 1993, TOX9750499; Enomoto, 1997, ASB2012-11484) whereas the LOAELs were much higher in the remaining studies. High-dose effects differed considerably among the studies (see Table 25 below). In mice, the overall NOAEL for long-term toxicity in the mouse can be set at 150 mg/kg bw/day, based on the studies by Sugimoto (1997, ASB2012-11493), Kumar (2001, ASB2012-11491) and Knezevich and Hogan (1983, TOX9552381). The overall LOAEL was around 800 mg/kg bw/day. The lowest doses at which effects were observed were 787 mg/kg bw/day in females in the study by Sugimoto (1997, ASB2012-11493) and 814 mg/kg bw/day in males in the study by Knezevich and Hogan (1983, TOX9552381). For details, see Table 30 in the carcinogenicity section. As in rats, the nature of high dose effects in mice was different in the various studies, depending on laboratory, strain, dose selection and, perhaps, purity and impurities profiles of the applied test material.

Reproductive and developmental studies

A large number of multi-generation studies on rats and of developmental (teratogenicity) studies on rats and rabbits is available. These studies are addressed in section 4.10. For possible classification for STOT RE, only the parental or maternal toxicity in these studies might be of interest and

concern. In the rat, treatment-related findings were consistently confined to very high doses. This is shown by NOAELs for parental toxicity in the two-generation studies that range from 197 to approximately 700 mg/kg bw/day. The lowest dose levels at which adverse effects occurred ranged between 668 and > 1000 mg/kg bw/day (see Table 46). In the developmental studies, the lowest NOAEL for maternal toxicity was 300 mg/kg bw/day but, in most studies, no effects were seen up to the limit dose of 1000 mg/kg bw/day (see Table 47).

In contrast, the pregnant rabbit turned out to be the most vulnerable animal model when glyphosate was tested. An "overall" maternal NOAEL of 50 mg/kg bw/day was established in a total of 7 developmental studies, taking into account dose spacing. It was based on mortality, abortions, reductions in body weight (gain) and food consumption and gastro-intestinal clinical signs such as loose stool or diarrhoea. The LOAEL is 100 mg/kg bw/day. At this dose level, there were maternal deaths in the study by Suresh (1993, TOX9551106). An overview on maternal deaths and non-lethal effects in the rabbit studies is provided in Table 18. It should be emphasised that the studies by Bhide and Patil (1989, TOX9551960) and by Suresh (1993, TOX9551106) are only supplementary due to inferior quality but for the endpoint under consideration (maternal toxicity and mortality) they may be taken into consideration. Only those fatalities are listed in the table that can be attributed to treatment. Additional cases are indicated by asterisks. Some of the maternal deaths (the single mortalities in the studies by Hojo and by Brooker, 3 out of 8 at the high dose level in the study by Suresh and one in the study by Coles and Doleman) occurred after cessation of treatment. Nonetheless, it seems reasonable to consider them treatment-related.

Table 18: Maternal mortality and toxicity in the developmental studies with glyphosate in rabbits (all by oral gavage)

Reference; Study identification; Batch, purity; Owner	Strain, duration of treatment	Dose levels	Number of does per group	Premature deaths and dose level(s) at which they occurred	Further maternal effects	Maternal NOAEL / LOAEL (mg/kg bw/d)
Tasker et al., 1980; TOX9552390; Lot XHJ-64, 98.7%; Monsanto	Dutch Belted rabbit, d 6-27 p.c., gavage	0, 75, 175, 350 mg/kg bw/d	16	1 at 175, 7 at 350 mg/kg bw/d	Soft stool, diarrhea	75 / 175
Bhide & Patil, 1989; TOX9551960; Lot 38, 95%; Barclay, Luxan	NZW rabbit, d 6-18 p.c., gavage	0, 125, 250, 500 mg/kg bw/d	15	None	Food con-sumption, bw↓, abortion	250 / 500
Brooker et al., 1991; TOX9552391; 206-Jak-25-1, 98.6%; Cheminova	NZW rabbit, d 7-19 p.c., gavage	0, 50, 150, 450 mg/kg bw/d	16 – 20	1 at 450 mg/kg bw/d	Soft/liquid stool, food consump-tion and bw gain ↓, abortion	50 / 150
Suresh et al., 1993; TOX9551106; Batch 60, 96.8%; ADAMA	NZW rabbit, d 6-18 p.c., gavage	0, 20, 100, 500 mg/kg bw/d	15 – 17 in treated groups, 26 in control	4 at 100, 8 at 500 mg/kg bw/d**	Soft/liquid stool	20 / 100
Hojo, 1995, ASB2012-11498; T-041209, 97.56%;	Japanese White rabbits	0, 10, 100, 300 mg/kg bw/d	18	1 at 300 mg/kg bw/d	Loose stool, abortion	100 / 300

Reference; Study identification; Batch, purity; Owner	Strain, duration of treatment	Dose levels	Number of does per group	Premature deaths and dose level(s) at which they occurred	Further maternal effects	Maternal NOAEL / LOAEL (mg/kg bw/d)
Arysta	(Kbl:JW), d 6-18 p.c., gavage					
Coles & Doleman, 1996; ASB2012-11499; H95D161A, 95.3%; Nufarm	NZW rabbit, d 7-19 p.c., gavage	0, 50, 200, 400 mg/kg bw/d	18	2 at 400 mg/kg bw/d	Food con-sumption, bw gain ↓, scours	50 / 200
Moxon, 1996; TOX2000-2002; Y04704/034, 95.6%; Syngenta	NZW rabbit, d 8-20 p.c., gavage	0, 100, 175, 300 mg/kg bw/d	20	None***	Food con-sumption, bw gain ↓, diarrhea	100 / 175

*Five additional deaths (one in the control and mid dose group each and 3 at the top dose level were attributed to diseases such as pneumonia or gastroenteritis but not to treatment.
** Two deaths in the control group were due to misdosing and clearly not treatment-related.
***In fact, there were 1, 2, 2, and 2 intercurrent deaths in the four groups, mostly related to abortion. Since no dose response was seen, mortality and abortions were not considered treatment-related.

The majority of the maternal deaths did not reflect an acutely toxic effect since they occurred after some days of treatment at least or even around the end of the administration period. A few early deaths were confined to the study by Suresh (1993, TOX9551106) in which 3 does died on the first day of treatment. Two of these deaths were noted in the mid dose group but only one after administration of the high dose. If they were in fact due to acute oral toxicity of glyphosate to pregnant female rabbits, one would have expected a higher number to occur at the top dose level. In contrast, these early deaths rather suggest misgavaging even though this was not reported by the study author. The other four studies in which does died suggest a different time pattern of mortality supporting the assumption of an effect of repeated administration. With regard to the individual studies, the days on which does died or were found dead are depicted in Table 19.

Table 19: Temporal occurrence of treatment-related maternal deaths in the developmental studies with glyphosate in rabbits

Reference; Study identification	Strain, duration of treatment	Dose levels	Day of first death with dose level	Days of further deaths with dose level
Tasker et al., 1980; TOX9552390	Dutch Belted rabbit, d 6-27 p.c.	0, 75, 175, 350 mg/kg bw/d	14 (350 mg/kg bw/d)	17, 18, 21 (350 mg/kg bw/d); 25 (175mg/kg bw/d)
Brooker et al., 1991; TOX9552391	NZW rabbit, d 7-19 p.c.	0, 50, 150, 450 mg/kg bw/d	20 * (450 mg/kg bw/d)	None
Suresh et al., 1993; TOX9551106	NZW rabbit, d 6-18 p.c.	0, 20, 100, 500 mg/kg bw/d	7 (2x 100 mg/kg bw/d; 1x 500 mg/kg bw/d)	11, 14, 15, 18, 19* (500 mg/kg bw/d) 9, 18 (100 mg/kg bw/d)
Hojo, 1995, ASB2012-11498	Japanese White rabbits (Kbl:JW),	0, 10, 100, 300 mg/kg bw/d	20* (300 mg/kg bw/d)	None

Reference; Study identification	Strain, duration of treatment	Dose levels	Day of first death with dose level	Days of further deaths with dose level
	d 6-18 p.c.			
Coles & Doleman, 1996; ASB2012-11499	NZW rabbit, d 7-19 p.c.	0, 50, 200, 400 mg/kg bw/d	19 (400 mg/kg bw/d	20* (400 mg/kg bw/d

*mortality occurring after cessation of treatment

4.7.2 Human information

Not available.

4.7.3 Other relevant information

There are some publications of varying quality describing studies of different types and duration. These studies were performed with formulations and not with the active substance. Therefore this information is not considered for the classification and labelling proposal for glyphosate itself. However, this published information is reported in the attached RAR.

4.7.4 Summary and discussion

In short-term and chronic studies in rats, mice, and dogs, toxic effects of glyphosate were confined to rather high doses. The large differences in the NOAELs/LOAELs in the individual studies are due to dose spacing but it seems clear that in no species effects below 300 mg/kg bw/day should be anticipated. Even effects at higher dose levels are relatively minor in nature but may differ among the studies or the same endpoint and in the same species, depending on strain, laboratory and perhaps also test material (e.g., impurities). Compound–related findings comprised lower body weight gain, rather slight alterations in clinical chemistry and haematological parameters as well as a lower urine pH and clinical signs that indicate gastrointestinal irritation or disturbances. More pronounced toxicity was only seen in a single dog study with capsule administration at the high dose level of 1000 mg/kg bw/day.

Low toxicity of glyphosate upon repeated administration was confirmed in reproduction and developmental studies in rats. In contrast, the pregnant rabbit was much more vulnerable with a much lower maternal NOAEL of 50 mg/kg bw/day and an LOAEL of 100 mg/kg bw/day at which already mortality occurred in at least one study.

4.7.5 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Based on the nature and severity of toxic effects of glyphosate and the NOAELs and LOAELs for the different endpoints in the different species, it may be concluded that only maternal toxicity as observed in the developmental studies in rabbits is of concern with regard to classification as STOT RE. Accordingly, comparison with criteria should be confined to this endpoint and data.

4.7.6 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The following criteria for classification for specific target organ toxicity – repeated exposure are

given in CLP regulation:

CLP criteria
Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case): Rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d Category 2 (H373) Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be Harmful to human health following repeated exposure. Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case): Rat: 28-day: ≤ 300 mg/kg bw/d 90-day: ≤ 100 mg/kg bw/d

For an exposure period of shorter duration as is the case in a developmental study, at least the guidance value for the 28-day study should be considered. Even though the guidance values refer to studies in rats, there is no reason not to take into account effects that had occurred in the rabbit.

Based on the NOAEL of 50 mg/kg bw/day and the LOAEL of 100 mg/kg bw/day for maternal toxicity, category 2 seems most appropriate because these dose levels were clearly below the 28-day guidance values for category 2 but higher than those that would qualify for category 1.

Since the proposal is based on mortality, no organ can be mentioned in brackets as it is recommended but not strictly required by the CLP regulation.

4.7.7 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

It is proposed to classify glyphosate as STOT RE, Category 2. The signal word is "Warning" and the appropriate hazard statement would be H373 (May cause damage to organs through prolonged or repeated exposure).

4.8 Germ cell mutagenicity (Mutagenicity)

4.8.1 Non-human information

In a narrow sense, this hazard classification relates to the ability of a substance to induce heritable mutations, i.e., in germ cells. As compared to the extremely large database on toxicity and also genotoxicity of glyphosate, the available information to directly address this endpoint is scarce. Glyphosate has been shown to be devoid of mutagenic activity in dominant lethal assays when applied as a single oral dose of up to 2000 mg/kg bw to CD-1 mice (Wrenn et al., 1980, TOX9552377) and of up to 5000 mg/kg bw to Wistar rats (Suresh, 1992, TOX9551102).

Thus, as for most substances, evaluation of a mutagenic potential must mainly rely on studies that address mutagenicity and genotoxicity of the active substance glyphosate in somatic cells. A broad spectrum of mutagenicity and genotoxicity tests in vitro and in vivo is available for glyphosate and glyphosate based formulations which is summarised in the following sub-sections with regard to gene mutations in bacteria and somatic cells, chromosome aberrations *in vitro* and in intact animals and direct interaction with the DNA (comprising, *e.g.*, UDS or Comet assays).

The DS is aware that, in addition to the studies with glyphosate, a large number of published studies with formulations containing glyphosate are available which were tested for different mutagenicity and genotoxicity endpoints in a variety of *in vitro* and *in vivo* mammalian and non-mammalian test systems. A part of these studies revealed positive or at least equivocal results in particular when testing was performed in non-standard systems and when so-called “indicator tests” were employed. It is likely that such results were rather due to co-formulants than to glyphosate. Therefore, they cannot be taken into account for classification of glyphosate for mutagenicity. Furthermore, against the background of an extremely large database using standard test systems (bacteria, mammalian cells and mammals), data obtained in non-standard test systems (e.g. plant, insect, worm, fish etc.) was not considered for classification of health related endpoints even if performed with the active ingredient. Therefore, all this information is not provided in this CLH report but may be found in the attached RAR.

Table 20: Summary of germ cell mutagenicity tests in mammals, *in vivo*

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, mating period	Results by authors	GLP, Test guideline	Result details	Comments
Wrenn et al. 1980, TOX9552377	Mouse, Dominant lethal test	Glyphosate, 98.7 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 8 successive one-week mating periods (1 male/2 females)	Negative	GLP, no reference to TG	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	Only 10 males per group. Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice.
Suresh, 1992, TOX9551102	Rat, Dominant lethal test	Glyphosate, 96.8 % oral, 1x 0, 200, 800 or 2000 mg/kg bw 10 successive one-week mating periods (1 male/1 female)	Negative	GLP, OECD 478 (1984)	No increase in post-implantation loss in treated groups. PosControl: stat. significant increase in post-implantation loss.	30 males per group (Control: 10 males, PosControl: 2 x 5 males). Post-implantation loss evaluated after mating of non-treated females with glyphosate-treated male mice.

4.8.1.1 *In vitro* data

The ability of glyphosate to cause gene/point mutations in bacteria was investigated in numerous studies by means of the reverse mutations ("Ames") test giving consistently negative results. The available studies were all run with and without metabolic activation, using liver S9 mix to mimic in vivo liver metabolism. The available valid studies, 16 in total, are compiled in Table 21, along with a Rec assay in *Bacillus subtilis* for investigations of a possible interaction with bacterial DNA.

Table 21: Summary of *in vitro* mutagenicity and genotoxicity tests with glyphosate acid in bacteria

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; purity; metabolic activation	Results
Jensen, 1991; TOX9552371; Cheminova	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate; + S9: 310 – 5000 (plate-incorporation and pre-incubation test); 98.6%	Negative
Shirasu et al., 1978; TOX9552368; Monsanto	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538 and *E. coli* WP2 hcr	10 – 5000 µg/plate (plate-incorporation assay); 98.4%; +/- S9	Negative (supplementary study)
Akanuma, 1995a; ASB2012-11462: Arysta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	156-5000 µg/plate (pre-incubation test); 95.68%; +/- S9	Negative (supplementary study)
Sokolowski, 2007a; ASB2012-11463; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation), 33 – 5000 µg/plate (pre-incubation test); 95.1%; +/- S9	Negative
Sokolowski, 2007b; ASB2012-11464; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test); 97.7%; +/- S9	Negative
Sokolowski, 2007c; ASB2012-11465; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test); 95.0%; +/- S9	Negative
Riberri do Val, 2007; ASB2012-11466; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	648 – 5000 µg/plate (plate–incorporation); 98.01%; +/- S9	Negative (supplementary study)
Flügge, 2009a; ASB2012-11468; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate-incorporation and pre-incubation test); 98.8%; +/- S9	Negative
Flügge, 2010; ASB2012-11469; Helm	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test); 96.4%; +/- S9	Negative
Sokolowski, 2010; ASB2012-11470; Helm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate incorporation and pre-incubation test); 97.16% technical a.i. containing 0.63% glyphosine; +/- S9	Negative
Wallner, 2010;	Ames test	*S. typhimurium* TA 98,	31.6 – 5000 µg/plate (plate	Negative

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels; purity; metabolic activation	Results
ASB2012-11471; Helm		100, 102, 1535, 1537	incorporation and pre-incubation test); 98.2%; +/- S9	
Thompson, 1996; ASB2012-11472; Nufarm	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	0 – 5000 µg/plate (plate–incorporation); 95.3%; +/- S9	Negative (supplementary study)
Callander, 1996; ASB2012-11473; Syngenta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2P *uvrA* and WP2P	100 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 95.6%; +/- S9 (for pre-incubation test only with S9 mix)	Negative
Sokolowski, 2009; ASB2012-11474; Syngenta	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA* pKM 101and WP2 pKM 101	3 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 96.3%; +/- S9	Negative
Schreib, 2012; ASB2014-9133; Industria Afrasa	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	10 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 97%; +/- S9	Negative
Thompson, 2014; ASB2014-9148; Albaugh	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA*	1.5 or 5 – 5000 µg/plate (plate-incorporation and pre-incubation assays); 85.79%; +/- S9	Negative
Akanuma, 1995b; ASB2012-11477; Arysta	Rec assay	*B. subtilis* strains H17 and M45 (+/- S9)	+/- S9 : 7.5 – 240 µg/disk; Lot 940908-1; 95.68%	Negative (supplementary study)

Absence of mutagenicity *in vitro* was further confirmed in a number of studies for point (gene) mutations in mammalian cells, i.e., in two mouse lymphoma assays (Jensen, 1991, TOX9552372; Clay, 1996, TOX2000-1994) and an HPRT test (Li, 1983, TOX9552369). No evidence of clastogenicity was obtained in four valid in vitro studies in human lymphocytes (Van de Waart, 1995, TOX9651525; Fox, 1998, TOX2000-1995) or Chinese hamster lung cells (Kyomu, 1995, ASB2012-11475; Wright, 1996, ASB2012-11476). The conclusion that glyphosate was not clastogenic in vitro was also supported by the negative outcome of the two mouse lymphoma assays (Jensen, 1991, TOX9552372; Clay, 1996, TOX2000-1994). In an UDS assay in rat hepatocytes (Rossberger, 1994, TOX9400697), there was no impact on DNA damage and repair.

Other studies in mammalian cells, in contrast, revealed positive results or contradictory findings. On one hand, Lioi et al. (1998a, ASB2013-9836; 1998b, ASB2013-9837) reported higher rates of SCE and chromosome aberrations when glyphosate (purity ≥98%) was tested in human and bovine lymphocytes in vitro at the maximum concentrations of 51 or 170 µM. Bolognesi et al. (1997, Z59299) found evidence of increased sister chromatid exchange (SCE) in human lymphocytes for 99.9% pure glyphosate at dose levels of 1 mg/mL up to 6 mg/mL. Mladinic et al. (2009a, ASB2012-11907) reported an increase in micronucleus formation in human lymphocytes at the highest and already cytotoxic concentration of 580 µg/mL (approx. 3.43 mM) when S9 mix had been added. Koller et al. (2012, ASB2014-7618) observed an increase in micronucleus frequency in human cells of buccal origin (carcinoma cell line TR146) after treatment with an aqueous solution of 95% technical grade glyphosate for 20 minutes. For this investigation, the cytokinesis-block micronucleus cytome assay was employed. A significant (Chi-square test with Yate's correction, $p \leq 0.001$) and dose-related increase was seen at the upper concentrations of 15 and 20 µg/mL. On the other hand, chromosome aberrations in human lymphocytes could not be reproduced by Mañas et

al. (2009, ASB2012-11892) who tested 96% analytical grade glyphosate up to a higher concentration of 6 mM.

Positive *in vitro* results were also reported when glyphosate was tested by means of (alkaline) single cell gel electrophoresis, i.e., in the Comet assay. In a study with "technical grade" glyphosate and a maximum concentration of 6.5 mM, Monroy et al. (2005, ASB2012-11910) observed an effect on the DNA in human fibroblasts and fibrosarcoma cells. Mañas et al. (2009, ASB2012-11892) found DNA damage in Hep-2 cells of human epithelial origin at glyphosate concentrations between 3 and 7.5 mM with the highest one being already cytotoxic. Mladinic et al. (2009b, ASB2012-11906) reported a similar effect in human lymphocytes without S9 mix at the highest concentration of 580 µg/mL (approx. 3.43 mM). With metabolic activation, tail length and intensity were increased even at a low concentration of 3.5 µg/mL and above. However, these findings were always accompanied by a high rate of early apoptotic and necrotic cells pointing to cytotoxicity. Alvarez-Moya et al. (2014, ASB2014-6902) who tested 96% glyphosate in human lymphocytes observed an increase in tail length at all tested concentrations from 0.7 up to 700 µM but the differences between the concentrations were surprisingly small and there was no clear dose response relationship. Koller et al. (2012, ASB2014-7618) investigated the effects of technical grade (95%) glyphosate in a carcinoma cell line (TR146) of human buccal epithelial origin and reported an increase in tail intensity as compared to the controls at concentrations from 20 up to 2000 µg/mL but there was no dose response relationship indicating that the outcome was equivocal.

An overview on these studies is given in Table 22.

Table 22: Summary of *in vitro* tests for mutagenicity, clastogenicity or DNA damage/repair with glyphosate acid in mammalian cells

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels*; test conditions; purity	Results
Li, 1983; TOX9552369; Monsanto (also published by Li and Long, 1988, TOX9500253)	Mammalian cell gene mutation	Chinese hamster ovary (CHO) cells; HGPRT assay	- S9: 2 – 22.5 mg/mL + S9: 5 – 22.5 (25 ??) mg/mL; Lot XHJ-64; 98.7%	Negative
Jensen, 1991; TOX9552372; Cheminova	Mammalian cell gene mutation	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	- S9: 0.61 – 5.0 mg/mL, + S9: 0.52 – 4.2 mg/mL; 98.6%	Negative
Clay, 1996, TOX2000-1994; Syngenta	Mammalian cell gene mutation	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	+/- S9: 296 – 1000 µg/mL; P24; 95.6%	Negative
Van de Waart, 1995; TOX9651525; Agrichem	Chromosomal aberration	Peripheral human lymphocytes (-S9: 24, 48 h exposure; +S9: 3 h, harvest after 24 or 48 h)	- S9: 33 – 333 µg/mL + S9: 237 – 562 µg/mL; 96%	Negative (supplementary study)
Kyomu, 1995; ASB2012-11475; Arysta	Chromosomal aberration	Chinese hamster lung (CHL) cells	- S9: 62.5 – 500 µg/mL, + S9: 255 – 1000 µg/mL; 95.68%	Negative
Wright, 1996; ASB2012-11476; Nufarm	Chromosomal aberration	CHL cells	+/- S9: 312.5 - 1250 µg/mL; 95.3%	Negative
Fox, 1998; TOX2000-	Chromosomal	Human lymphocytes	- S9: 100 – 1250 µg/mL	Negative

Reference; Study identification; Owner	Type of study	Test organism / test system	Dose levels*; test conditions; purity	Results
1995; Syngenta	aberration		+ S9: 100 – 1250 µg/mL; 95.6%	
Lioi et al., 1998, ASB2013-9836	Chromosomal aberration	Bovine lymphocytes	-S9: 17 - 170 µM (3 - 30 µg/mL) +S9: not tested ≥ 98%	Positive (-S9)
Mladinic et al., 2009a, ASB2012-11907	Micronucleus formation	Human lymphocytes	-S9/+S9: 0.5 - 580 µg/mL 98%	Negative (-S9) Positive (+S9)
Mañas et al., 2009, ASB2012-11892	Chromosomal aberration	Human lymphocytes	-S9: 0.2-6.0 mM (34 - 1015 µg/mL) +S9: not tested 96%	Negative
Koller et al., 2012, ASB2014-7618	Micronucleus formation	Buccal carcinoma TR146 cells	10-20 µg/mL 95%	Positive
Rossberger, 1994; TOX9400697; Feinchemie (ADAMA)	UDS assay	Primary rat (Sprague-Dawley) hepatocytes	0.20 – 111.69 mM; >98%	Negative
Bolognesi et al., 1997, Z59299	Sister-chromatid exchange	Human lymphocytes	-S9: 0.33 and 6 mg/mL +S9: not tested 99.9%	Positive
Monroy et al., 2005, ASB2012-11910	Comet assay	Human fibroblast GM 39 and Human fibrosarcoma HT1080 cells	-S9 (GM39): 4.0-6.5 nM, -S9 (HT1080): 4.5-6.5 nM +S9: not tested Purity: not given	Positive
Mañas et al., 2009, ASB2012-11892	Comet assay	Human liver Hep-2 cells	-S9: 3 - 7.5 mM (507.2 - 1268 µg/mL) +S9: not tested 96%	Positive
Mladinic et al., 2009b, ASB2012-11906	Comet assay	Human lymphocytes	-S9/+S9: 0.5-580 µg/mL 98%	Positive
Koller et al., 2012, ASB2014-7618	Comet assay	Buccal carcinoma TR146 cells	10-2000 µg/mL 95%	Positive
Alvarez-Moya et al., 2014, ASB2014-6902	Comet assay	Human lymphocytes	-S9: 0.0007-0.7 mM (0.118- 118 µg/mL) +S9: not tested 96%	Positive

* Sometimes, higher concentrations were included in testing but these were the dose levels up to which analysis was carried out or reported.

On balance, regarding the *in vitro* studies with glyphosate, standard bacterial assays and mammalian cell gene mutation tests gave consistently negative results. Also, the majority of *in vitro* chromosomal aberration tests and micronucleus tests were negative, and in particular, all of the studies performed under GLP conditions resulted in negative findings. More important, no evidence of chromosome aberration was obtained in a large number of higher tier *in vivo* studies that are described in the next sub-section. *In vitro* indicator tests gave positive results for induction of SCE and DNA strand breaks (comet assay) but a negative result for induction of DNA repair (UDS).

4.8.1.2 *In vivo* data

Extensive testing of glyphosate for mutagenicity was performed in vivo by means of micronucleus assays or chromosome aberration studies that all examined the bone marrow of either mice or rats after oral or intraperitoneal application. All these studies are summarised in Table 23, separated for the application route and the test species.

General suitability of the bone marrow examinations is shown by the affinity of glyphosate to bone tissue as shown in the ADME studies (see attached RAR, Vol. 3, B.6.1), by the occasional observation of bone marrow toxicity in the tests themselves (e.g., by Suresh et al, 1994, TOX9400323) and by the occurrence of hypoplasia in bone marrow in a long-term study in rats although this latter finding was confined to a very high dose (Wood et al., 2009; ASB2012-11490). Thus, there is sufficient evidence that the target tissue in these studies was actually exposed to the test compound.

In a total of 7 out of the 8 valid studies in Table 23, glyphosate of different manufacturing sources proved clearly negative. The only exception was a micronucleus test performed by Suresh (1993, TOX9551100) which demonstrated a statistically significant increase in the incidence of micronuclei in females but not in males at the very high dose of 5000 mg/kg bw that was administered on two consecutive days. In contrast, a cytogenetic study conducted in the same laboratory and the same mouse strain under nearly identical conditions did not provide any evidence of chromosome aberrations even though test material of the same purity was applied at the same dose levels (Suresh, 1994, TOX9400323). In this second study of the same group, a certain degree of cytoxicity to bone marrow cells at the highest dose level became apparent since the mitotic index was reduced. Although not measured in the preceding micronucleus test, such an effect could be expected to have occurred in the previous experiment, too, and cytotoxicity might have contributed to micronucleus formation. Last but not least, the study author also concluded that, under the conditions of the experiment, glyphosate was not mutagenic in the micronucleus test in mice.

A small number of manufacturers studies had been rejected by the DS because they were considered “not acceptable” due to serious deficiencies. One of these studies had caused some discussion during the ongoing evaluation process of glyphosate in the EU, in particular during the public consultation in 2014, since a “positive” result has been claimed. For consistency, this study is briefly reported here. Zoriki Hosomi (2007, ASB2012-11480) administered 98% pure glyphosate from a Brazilian manufacturer to male Swiss mice (six per dose level). The animals were dosed twice with a 24-hour interval between by oral gavage. Sampling took place 24 hours after the second dose. The dose levels were 8, 15, and 30 mg/kg bw, based on toxicity observed in a range-finding test. On bone marrow slides, 3000 PCE per animal were scored for micronuclei. At the highest dose level, there was a statistically significant increase in micronucleus frequency (Chi-square test, $p = 0.02$). Against the large database that is available for glyphosate, this finding is surprising, as well as the high toxicity. In the range finding experiment, two animals that had been administered 2000 mg/kg bw died on day 3 after having shown ataxia and prostration before. The same observations were made in 3 animals which received an oral dose of 320 mg/kg bw. They all died on day 2. Even at a dose level of 50 mg/kg bw, one out of three treated animals died on day 1. The occurrence of deaths and clinical signs at relatively low dose levels was obviously in contradiction to the available acute toxicity tests with glyphosate in the mouse (Komura, 1995, ASB2012-11382; Suresh, 1991, TOX9551089; Dideriksen and Skydsgaard, 1991, TOX9552329; Tos, 1994, TOX9551624) revealing an LD50 higher than 2000 or even 5000 mg/kg bw. In line with that, much higher dose levels were employed in the other (negative) micronucleus assays or cytogenetic studies in mice with substance administration by the oral route (see Table 23). To conclude, this study by Zoriki Hosomi (2007) was seriously flawed by severe toxicity that was

completely unexpected and cannot be explained if the whole toxicological profile of glyphosate is taken into consideration. Either serious methodical mistakes have been made when the study was conducted or the test material was not glyphosate even though it was claimed as such. Both possibilities would turn the study completely unreliable and make it unsuitable for any regulatory use.

Some more studies were performed by intraperitoneal application.

A statistically significant increase in micronucleated PCEs was observed by Durward (2006, ASB2012-11478) after single i.p. injection of 600 mg/kg bw to CD-1 mice. However, this response was modest and within the historical range for vehicle control animals and, therefore, was not considered biologically significant.

Mañas et al. (2009, ASB2012-11892) reported a positive result in a micronucleus test in bone marrow erythrocytes of *Balb* C mice (5 per dose, sex not stated). There was a statistically significant increase ($p < 0.01$ in Dunnett's test) in micronucleated cells at 24 hours after the animals had received two i.p. doses of 200 mg/kg bw, administered 24 h apart, of 96% analytical grade glyphosate. Two i.p. doses of 100 mg/kg bw each were without an effect. The result of this study is, however, flawed by major deviations from internationally agreed test guidelines: a) the sex of the animals was not reported, b) only 1000 (instead of 2000) erythrocytes per animal were scored, and c) "erythrocytes" instead of immature or "polychromatic erythrocytes" (PCE) were scored for micronuclei. In an assay with the reported treatment and sampling times, scoring of all erythrocytes instead of polychromatic erythrocytes is not appropriate according to OECD test guideline 474.

Bolognesi et al. (1997, Z59299) found a weak increase in micronuclei in mouse bone marrow following two i.p. doses of 150 mg/kg bw on two consecutive days. The test material was 99.9% (analytical grade) glyphosate. However, since only 3 or 4 animals were used in the dosed groups and no data for individual animals were provided, it is not possible to assess whether an outlier would have disproportionately influenced the result. In contrast, Rank et al. (1992, Z82234) did not observe an increase in micronucleated PCEs after single i.p. administration of up to 200 mg/kg bw of the glyphosate isopropylammonium (IPA) salt to mice with sampling after 24 and 48 hours. Similarly, Chruscielska et al. (2000, ASB2013-9830) reported a negative micronucleus assay in which glyphosate from Polish production was applied via the i.p. route at a single dose of 300 mg/kg bw to mice. All these studies had methodological deficiencies. The dose levels were lower than those used in the manufacturer's studies which were negative.

Furthermore, the oral route in the micronucleus assay or cytogenetic study is of higher relevance for risk assessment.

An overview of the valid micronucleus tests and cytogenetic studies *in vivo* is given in Table 23.

Table 23: Summary of somatic cell mutagenicity tests in mammals, *in vivo*

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
Jensen, 1991, TOX9552374	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.6% oral, 1x 0 or 5000 mg/kg bw, sampled after 24, 48 and 72 h	Negative	GLP, OECD 474 (1983)	*MN/2000 PCE [mean (range)]:* Control: 2.7 (1-4) 24h, 5000 mg/kg: 3.2 (1-5) 48h, 5000 mg/kg: 2.8 (1-6) 72h, 5000 mg/kg: 1.7 (0-4) PosControl: 48.2 (32-58)	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.
Suresh, 1993, TOX9551100	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.8% oral, 2x 0, 50, 500 or 5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Weakly positive for top dose females	GLP, OECD 474 (1984)	*% MNPCE [mean (range)], male/female:* Control: 0.69 (0.1-1.6)/0.51 (0.2-1.0) 50 mg/kg: 0.84 (0.2-1.4)/0.28 (0.0-0.5) 500 mg/kg: 0.73 (0.4-1.6)/0.52 (0.2-1.3) 5000 mg/kg: 0.89 (0.7-1.1)/1.05*(0.4-1.6) PosControl: 2.33* (1.5-3.2)/2.39* (1.4-3.4) *p<0.05	5 animals per sex and dose (Control: 10/sex). 2000 PCE scored/animal. PCE/NCE: no effect (but PosControl).
Suresh, 1994, TOX9400323	Mouse, Chromosome aberration test, bone marrow	Glyphosate, 96.8% oral, 2 x 0-5000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 475 (1984)	*No. of aberrations per 250-250-500 metaphases (male/female/total)* Control: 12/10/22 5000 mg/kg: 10/11/21 PosControl: 139*/155*/294* *p<0.05	5 animals per sex. 50 metaphases/animal examined. *Mitotic index (%) (male/female/total)* Control: 13.3/17.4/15.3 5000 mg/kg: 8.9*/9.5*/9.2* PosControl: 14.7/5.5*/10.1*
Fox & Mackay, 1996, TOX2000-1996	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.6% oral, 1x 0 or 5000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/1000 PCE (mean±SD), male/female:* 24h, Control: 1.6±0.8/1.4±0.7 24h, 5000 mg/kg: 2.1±1.6/2.1±2.5 24h, PosControl: 22.2±6.1*/23.3±4.9* 48h, Control: 1.7 ±1.3/0.7±0.6 48h, 5000 mg/kg: 2.1±1.9/0.8±0.8 *p<0.01	5 animals per sex and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect.
Honarvar, 2008, ASB2012-11483	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.1% oral, 1x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 h 1x 0 or 2000 mg/kg bw, sampled after 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)]:* 24h, Control: 1.4 (0-3) 24h, 500 mg/kg: 1.6 (1-2) 24h, 1000 mg/kg: 1.6 (1-2) 24h, 2000 mg/kg: 1.4 (0-2) 24h, PosControl: 63.0 (44-92)* 48h, Control: 1.4 (0-3)	5 males per group and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (293 studies): *% MNPCE [mean±SD, (range)]:*

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
					48h, 2000 mg/kg: 1.6 (0-3) *p<0.01	*0.084±0.031 (0.01 – 0.18)*
Patel, 2012, ASB2014-9277	Mouse, Micronucleus test, bone marrow	Glyphosate, 98.9% oral, 2 x 0 or 2000 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean (range)]:* Control: 0.033 (0-0.05) 2000 mg/kg: 0.0 (0-0) PosControl: 2.49* (1.1-3.7) *p<0.01	6 males per group. 2000 PCE scored/animal. PCE/NCE: no effect at 2000 mg/kg, increased in PosControl. Historical control data (of 73 studies) *% MNPCE [mean±SD (range)]: 0.02±0.02 (0.0-0.07)*
Roth, 2012, ASB2014-9333	Mouse, Micronucleus test, bone marrow	Glyphosate, 96.3% oral, 1 x 0 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean±SD, (range)]:* 24h, Control: 3.2±3.6 (0-8) 24h, 2000 mg/kg: 2.3±0.5 (2-3) 24h, PosControl: 40.2±18.2* (16-67) 48h, Control: 1.4±1.1 (0-3) 48h, 2000 mg/kg: 1.1±1.3 (0-3) *p<0.01	7 males per group (Control and PosControl: 5 males each). 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (of 219 studies) *% MNPCE [mean±SD (range of mean group value)]:* 0.108±0.039 (0.01-0.25)
Flügge, 2009, ASB2012-11479	Rat, Micronucleus test, bone marrow	Glyphosate, 98.8% oral, 1 x 0, 500, 1000 or 2000 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE (mean±SD), male/female:* 24h, Control: 1.6±1.1/1.8±0.4 24h, 500 mg/kg: 1.0±1.2/1.2±1.3 24h, 1000 mg/kg: 0.8±0.4/1.6±0.9 24h, 2000 mg/kg: 1.2±0.8/0.8±0.8 24h, PosControl: 30.2±10.5*/24.0±4.9* 48h, Control: 2.0 ±1.9/2.2 ±1.3 48h, 2000 mg/kg: 1.6±0.9/0.8±0.8 *p<0.05	5 animals per sex and dose and sampling time. 2000 PCE scored/animal. PCE/NCE: no effect. Historical control data (24, 48 and 72 h samplings combined): *MN/1000 PCE [mean and (range):* Males: 1.97 (0.4 – 5.7) Females: 1.86 (0.4 – 4.7)
Li and Long, 1988, TOX9500253 Li, 1983, TOX9552369	Rat, Chromosome aberration test, bone marrow	Glyphosate, 98% i.p., 1 x 0 or 1000 mg/kg bw, sampled after 6, 12 and 24 h	Negative	No GLP, no reference to TG	*% aberrant cells (mean), male/female/total:* 6h, Control: 1.3/2.7/2.0 6h, 1000 mg/kg: 2.3/3.0/2.7 12h, Control: 1.0/1.5/1.2 12h, 1000 mg/kg: 2.0/2.5/2.3 24h, Control: 1.3/2.3/1.8 24h, 1000 mg/kg: 1.0/3.7/2.6	Consistent with OECD 475 (1984): 6 animals per sex and sampling time. Ca 50 metaphases/animal examined. Slides were coded and scored "blind". Original study reported in RAR as

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
					PosControl: 42.2*/23.8*/40.8* * p < 0.05	Li, 1983 (TOX9552375).
Rank et al., 1993, Z82234	Mouse, Micronucleus test, bone marrow	Glyphosate isopropylamine salt, purity not stated i.p., 1 x 0, 100, 150 or 200 mg/kg bw sampled after 24 and 48 h	Negative	No GLP, no reference to TG	*% MNPCE (mean±SD):* 24h, Control: 0.27±0.11 24h, 100 mg/kg: 0.20±0.13 24h, 150 mg/kg: 0.2±0.13 24h, 200 mg/kg: 0.25±0.10 24h, PosControl: 2.53±0.59 48h, 150 mg/kg: 0.13±0.09 48h, 200 mg/kg: 0.12±0.09	Consistent with OECD 474 (1983): Mostly 5 animals per sex and dose and sampling time. 1000 PCE scored/animal. Slides were scored randomly. PCE/NCE: no effect.
Bolognesi et al., 1997, Z59299	Mouse, Micronucleus test, bone marrow	Glyphosate, 99.9% i.p., 2 x 150 mg/kg bw (24 h interval), sampled 6 or 24 h after second dose	Positive	No GLP, no reference to TG	*MN/1000 PCE (mean±SD):* Control: 0.75±0.46 6h, 2x 150 mg/kg: 1.4±0.9 24h, 2x 150 mg/kg: 2.4±1.5* 24h, PosControl: 80.0±8.5* * p < 0.05	6 males in Control and PosControl group. 3000 PCE scored/animal. PCE/NCE: 0.73±0.06 in Control, 0.6±0.05 at 6h, 0.5±0.2 at 24h. *Deviations from OECD 474 (1997):* *Only 3(4) males examined per sampling time.* *Sampling time of Control not stated.* *Independent coding of slides not stated.*
Mañas et al., 2009a, ASB2012-11892	Mouse, Micronucleus test, bone marrow	Glyphosate, 96% i.p., 2 x 50, 100 or 200 mg/kg bw (24 h interval), sampled 24 h after second dose	Positive	No GLP, OECD 474 (1997)	*MN/1000* ***Erythrocytes*** *(mean±SD):* Control: 3.8 ±0.8 2x 50 mg/kg: 3.7±0.5 2x 100 mg/kg: 4.2±0.5 2x 200 mg/kg: 13.0±3.5* PosControl: 19.2±3.9* * *P* < 0.01	5 animals per dose. PCE/NCE no effect. *Deviations from OECD 474 (1997):* *Sex of animals not reported.* *1000* ***erythrocytes*** *(not* ***PCE****) scored/animal.* *Independent coding of slides not stated.*
Carvalho and Marques, 1999, ASB2012-11482	Mouse, Micronucleus test, bone marrow	Glyphosate, 95% i.p., 2 x 0, 187.5, 375 or 562.5 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, internal SOP	*MN/1000 PCE [mean (range)], male/female:* Control: 0.4 (0-1)/0.8 (0-2) 188 mg/kg: 0.0 (0)/0.6 (0-3) 375 mg/kg: 0.6 (0-3)/0.6 (0-2) 563 mg/kg: 0.4 (0-2)/0.6 (0-1) PosControl: 4.8* (4-7)/4.8* (2-12)	5 animals per sex and dose. 1000 PCE and 1000 NCE scored per animal. PCE/NCE: no effect (but PosControl). MN/1000 NCE: no effect (but

Reference	Species, test, tissue	Test substance, purity, application route, dose levels, sampling time	Results	GLP, Test guideline	Result details	Comments
					*p<0.05	PosControl). *$LD50_{i.p.}$=750 mg/kg*
Durward, 2006, ASB2012-11478	Mouse, Micronucleus test, bone marrow	Glyphosate, 95.7% i.p., 1 x 0, 150, 300 or 600 mg/kg bw, sampled after 24 and 48 h	Negative	GLP, OECD 474 (1997)	*% MNPCE [mean±SD, (range)]:* 24h, Control: 0.06±0.06 (0.0-0.15) 24h, 150 mg/kg: 0.07±0.04 (0.0-0.10) 24h, 300 mg/kg: 0.06±0.05 (0.0-0.15) 24h, 600 mg/kg: 0.19±0.07* (0.05-0.25) 24h, PosControl: 3.03±0.49*** (2.20-3.35) 48h, Control: 0.1±0.12 (0.0-0.35) 48h, 600 mg/kg: 0.09±0.11 (0.0-0.30) *p<0.05, ***p<0.001	7 males per group and sampling time. 2000 PCE scored/animal. *Pre-test: Mortality at 800-1000 mg/kg, clinical signs at 150 mg/kg and above.* PCE/NCE: reduced at 600 mg/kg (not in PosControl). Stat. sign. increase in MNPCE at 600 mg/kg (24 h), within historical control. *Control data from 60 groups (24h):* *0.0-0.9 MN/1000 PCE: 40x (67%)* *1.0-1.4 MN/1000 PCE: 14x (23%)* *1.5-2.0 MN/1000 PCE: 3x (5%)* *2.1-2.5 MN/1000 PCE: 3x (5%)*
Costa, 2008, ASB2012-11481	Mouse, Micronucleus test, bone marrow	Glyphosate, 98% i.p., 2 x 0, 15.6, 31.3 or 62.5 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)], male/female:* Control: 0.0 (0)/0.0 (0) 15.6 mg/kg: 0.0 (0)/0.0 (0) 31.3 mg/kg: 0.0 (0-1)/0.0 (0) 62.5 mg/kg: 0.6 (0-3)/0.0 (0) PosControl: 23.0* (8-30)/12.2* (7-26) *p<0.01	5 animals per sex and dose. 2000 PCE scored/animal. *Pre-test: Mortality at 500-1000 mg/kg, decreased PCE/NCE at 250 mg/kg and above.* PCE/NCE no effect. Historical control: ca. 3 MN/1000 PCE
Costa, 2010, ASB2014-9284	Mouse, Micronucleus test, bone marrow	Glyphosate, 98% i.p., 2 x 0, 125, 250 or 375 mg/kg bw (24 h interval), sampled 24 h after second dose	Negative	GLP, OECD 474 (1997)	*MN/2000 PCE [mean (range)], male/female:* Control: 0.4 (0-2)/0.4 (0-1) 125 mg/kg: 0.2 (0-1)/0.0 (0-1) 250 mg/kg: 0.0 (0)/0.0 (0) 375 mg/kg: 0.2 (0-1)/0.0 (0-1) PosControl: 8.0* (5-11)/6.4* (5-9) *p<0.01	5 animals per sex and dose. 2000 PCE scored/animal. *Clinical signs at 125 mg/kg and above.* PCE/NCE: slight increase at 250 and 375 mg/kg and in PosControl. Historical control: ca. 3 MN/1000 PCE

NCE, normochromatic erythrocytes; MN, micronucleus; MNPCE%, percent of micronucleated polychromatic erythrocytes; PCE, polychromatic erythrocytes; SD, standard deviation

Table 24: Summary of tests on DNA adducts and DNA strand breaks in mammals, *in vivo*

Reference	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments
Bolognesi et al., 1997, Z59299	Mouse DNA adduct (8-OHdG by LC/UV), liver	Analytical grade glyphosate (purity 99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	- (4 h) + (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.9 moles 8-OHdG/10^5 moles dG 24 h: approx. 3.6 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments
Bolognesi et al., 1997, Z59299	Mouse DNA adduct (8-OHdG by LC/UV), kidney	Analytical grade glyphosate (purity 99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 8 and 24 h	- (4 & 24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 0.6 moles 8-OHdG/10^5 moles dG 4 h: approx. 0.5 moles 8-OHdG/10^5 moles dG 24 h: approx. 0.4 moles 8-OHdG/10^5 moles dG*	3 male animals per group, at least 3 independent repeat experiments
Peluso et al., 1998, TOX1999-318	Mouse DNA adduct (^{32}P-DNA post labelling), kidney	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear
Peluso et al., 1998, TOX1999-318	Mouse DNA adduct (^{32}P-DNA post labelling), liver	Glyphosate isopropylammonium salt i.p.; 1 × 0, 130 or 270 mg/kg bw; sampled after 24 h	–	No GLP, no reference to TG	Not reported	6 animals in control group, 6 in low dose group and 3 in high dose group, sex of animals not clear
Bolognesi et al., 1997, Z59299	Mouse DNA strand breaks (alkaline elution assay), liver	Analytical grade glyphosate (purity 99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	+ (4 h) - (24 h)	No GLP, no reference to TG	(Estimated from figure in report) Control: approx. 15 *10^3/mL 4 h: approx. 47 *10^3/mL* 24 h: approx. 20 *10^3/mL	3 male animals per group, at least 4 independent repeat experiments
Bolognesi et al.,	Mouse	Analytical grade glyphosate (purity	+ (4 h)	No GLP,	(Estimated from figure in report)	3 male animals per

Reference	Species, test, tissue	Test substance, purity, route, dose levels, sampling time	Results by authors	GLP, Test guideline	Result details	Comments
1997, Z59299	DNA strand breaks (alkaline elution assay), kidney	99.9%) i.p.; 1 × 300 mg/kg bw; sampled after 4 and 24 h	- (24 h)	no reference to TG	Control: approx. 17 *10^3/mL 4 h: approx. 55 *10^3/mL* 24 h: approx. 25 *10^3/mL	group, at least 4 independent repeat experiments
Manas et al., 2013, ASB2014-6909	Mouse comet assay, blood cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 2.98±1.08 40 mg/kg bw per day: 8.54***±7.82 400 mg/kg bw per day: 9.06***±5.15	6 animals per group sex of animals not clear
Manas et al., 2013, ASB2014-6909	Mouse comet assay, liver cells	Glyphosate (96%) Drinking water, 14 days, 0, 40 or 400 mg/kg bw per day; sampled after treatment period	+	No GLP, no reference to TG	Tail moment (mean ± SEM): Control: 7.14±3.41 40 mg/kg bw per day: 7.92*±3.99 400 mg/kg bw per day: 20.59***±15.47	6 animals per group sex of animals not clear

8-OHdG, 8-hydroxy-2' -deoxyguanosine; dG, deoxyguanosine; SEM, standard error of the mean; SCGE, single cell gel electrophoresis

Apart from this study type, there is some rather equivocal published information that was gained by other methods.

A possible impact on the DNA was investigated by Bolognesi et al. (1997, Z59299) also in vivo. A transient but significant effect towards DNA damage in liver and kidney was noted in the alkaline elution assay after glyphosate (300 mg/kg bw) had been administered once by the i.p. route to mice. This assay may indicate the induction of DNA single-strand breaks and alkali labile sites. A test for DNA oxidative damage suggested glyphosate to stimulate oxidative metabolism in the liver at 24 hours after application. This data is not easy to interpret since the results are given in summary figures only which are based on pooled individual data. There are reporting inconsistencies, e.g., it is not clear how many animals were actually used for testing. A positive control substance was not included. In contrast, no evidence for DNA adduct formation was reported following intraperitoneal administration of glyphosate isopropylammonium salt to mice at a single dose of 270 mg/kg bw (Peluso et al., 1998, TOX1999-318).

More recently, Mañas et al. (2013, ASB2014-6909) reported a positive Comet assay in liver and blood cells of *Balb C* mice after glyphosate (96% analytical grade) administration at dose levels of 40 and 400 mg/kg bw/day for 14 days in drinking water. A clear dose response was seen only in the liver. The authors also reported evidence of oxidative stress.

Taking into account that glyphosate proved negative in the UDS assay (Rossberger, 1994, TOX9400697), the published findings in this indicator test are not considered to provide convincing evidence of an interaction with the DNA. Positive results in the alkaline elution assay may also occur as a result of toxic but non-mutagenic effects. In general, DNA damage end points such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects end point categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. Stimulation of oxidative metabolism is not a sign of mutagenicity but may elucidate a possible mechanism behind toxic effects.

4.8.2 Human information

There is (partly contradictory) epidemiological data available that should be used, however, with some reservation. It must be taken into account that the study participants had been always exposed to plant protection products containing glyphosate but never to the active substance itself. Furthermore, there must have been parallel exposure to many other environmental chemicals. Thus, the situation resembles that one for many chemicals. In the “Guidance on the Application of the COP Criteria (Version 4.1, June 2015), it is stated therefore: “Epidemiological studies have been to date unable to provide evidence to classify a substance as a Category 1A mutagen.”

For the available data, the reader is referred to Vol. 3 of the attached RAR, Section B.6.4.8.7.

4.8.3 Other relevant information

Not available.

4.8.4 Summary and discussion of mutagenicity

Glyphosate has been tested in an adequate range of mutagenicity and genotoxicity tests.

In vitro bacterial assays and mammalian cell gene mutation assays gave consistently negative results. Also, results from *in vitro* mammalian chromosome aberration tests and *in vitro* micronucleus tests were negative when the studies were conducted according to internationally agreed test guidelines. *In vitro* indicator tests for induction of SCE and DNA strand breaks gave positive results.

In vivo, 11 micronucleus tests or cytogenetic studies in somatic cells that were conducted according to internationally agreed test guidelines gave negative results, while in only one test a weakly positive effect was seen in female mice receiving a very high and likely cytotoxic dose. Published studies with methodological limitations revealed contradictory results. In most of these studies, relatively low dose levels were employed and the intraperitoneal route was used which does not properly reflect the human exposure. When the weight of evidence is considered, it can be concluded that glyphosate was devoid of a clastogenic potential. Evidence of DNA damage such as strand breaks was observed in several published indicator tests following a high i.p. dose or repeated oral (via drinking water) doses. In contrast, an UDS was negative. Usually, standard mutagenicity tests such as cytogenicity or micronucleus assays are considered more important than indicator tests.

As reported in the beginning of this section, there was no evidence for mutagenic activity in germ cells of mice and rats at oral doses up to 2000 mg/kg bw.

In summary, taking a weight of evidence approach, glyphosate (active substance) is considered not mutagenic.

4.8.5 Comparison with criteria

The following criteria for classification for germ cell mutagens are given in the CLP regulation:

CLP regulation
The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: — positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or — positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells *in vivo*, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from: — somatic cell mutagenicity tests *in vivo*, in mammals; or — other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. Note: Substances which are positive in *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

There is no positive evidence of mutagenicity/genotoxicity coming from epidemiological studies. Accordingly, category 1A is clearly not appropriate. Likewise, because of the negative results in the majority of the *in vitro* and *in vivo* mutagenicity tests including nearly all guideline-compliant standard assays and since positive findings were mainly confined to indicator tests, categories 1B and 2 also do not apply.

4.8.6 Conclusions on classification and labelling

No hazard classification of glyphosate for mutagenicity is warranted according to the CLP criteria.

4.9 Carcinogenicity

4.9.1 Non-human information

Long-term toxicity and carcinogenicity of glyphosate were investigated in a large number of studies in rats and mice that are all tabulated in this section, first those in rats and subsequently those in mice. Published data is reported below the tables. Thereafter, tumour types of which the incidence was increased in at least one study in the respective species are considered in detail.

Studies in rats

The DS is aware of a total of 9 unpublished long-term feeding studies with the technical active ingredient in rats (Table 25) of which 6 were performed in compliance with OECD TG 453 whereas the remaining three were flawed by serious deficiencies. The main effects as summarised in this table were statistically significant and either dose-related or observed at the top dose level only. However, they were not necessarily all noted at the LOAEL. Two more (published) studies with a glyphosate salt and a formulation are briefly reported below the table.

Table 25: Long-term feeding studies with glyphosate in rats (deficient studies on bottom)

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration	Dose levels	NOAEL	LOAEL	Targets / Main effects
Wood et al., 2009; ASB2012-11490; H05H016A, 95,7%; Nufarm	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Wistar	0, 1500, 5000, 15000 ppm (progressively increased up to 24000 ppm), equal to 86/105, 285/349, and 1077/1382 mg/kg bw/d (m/f)	285 mg/kg bw/d	1077 mg/kg bw/d	Bw gain↓, transient increase in AP activity, changes in distribution of renal mineralisation, adipose infiltration of bone marrow (indicative of hypoplasia)↑, slight increase in cutaneous alterations
Brammer, 2001; ASB2012-11488; P30, 97.6%; Syngenta	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Wistar-derived	0, 2000, 6000, 20000 ppm (121/145, 361/437, 1214/1498 mg/kg bw/d in m/f)	361 mg/kg bw/d	1214 mg/kg bw/d	Bw, food consumption and (initially) utilization↓, clinical chemistry findings (AP and ALAT activity↑, bilirubin↑, urine pH↓), kidney papillary necrosis, prostatis and periodontal inflammation↑ in high-dose males
Enomoto, 1997; ASB2012-11484, 11485, 11486, 11487; T-941209, 97.56% and T-950308, 94.61%; Arysta	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Sprague-Dawley	0, 3000, 10000, 30000 ppm (104/115, 354/393, 1127/1247 mg/kg bw/d in m/f)	104 mg/kg bw/d	354 mg/kg bw/d	Bw/bw gain, food consumption (initially) and utilization↓, loose stool↑, tail masses↑ due to follicular hyperkeratosis and abscesses, caecum: distention and wt↑, pH↓ and dark appearance of urine
Suresh, 1996; TOX9651587; 2 batches used, 96.8/96.0%; ADAMA	Combined chronic toxicity/ carcino-genicity (OECD TG 453); 2 yr; Wistar	0, 100, 1000, 10000 ppm (6.3/8.6, 59.4/88.5, 595.2/886 mg/kg bw/d in m/f)	59 mg/kg bw/d	595 mg/kg bw/d	AP activity↑ (f), slight in-crease in cataracts (m, no clear dose response in f)
Atkinson et al., 1993; TOX9750499; 229-JaK-5-1, 98.9% and 229-JaK-142-6, 98.7%; Cheminova	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Sprague-Dawley	0, 10, 100, 300, 1000 mg/kg bw/d (dietary levels regularly adjusted)	100 mg/kg bw/d	300 mg/kg bw/d	Bw gain↓, AP activity↑, urine pH↓, salivary glands: wt↑ and histological findings, liver wt↑
Stout and Ruecker, 1990; TOX9300244; XLH-264, 96.5%; Monsanto	Combined chronic toxicity/ carcinoge-nicity (OECD TG 453); 2 yr; Sprague-Dawley	0, 2000, 8000, 20000 ppm (89/113, 362/457, 940/1183 mg/kg bw/d in m/f)	89 mg/kg bw/d	362 mg/kg bw/d	Bw and bw gain↓ in f, liver wt↑, stomach mucosal inflammation, cataracts in m, urine pH↓, survival <50% in all groups incl. controls
Bhide, 1997*; ASB2012-11489	Combined chronic	0, 3000, 15999, 25000 ppm (150/210,	150 mg/kg bw/d	780 mg/kg bw/d	AP activity↑ (m/f), bw gain↓ in m, equivocal alterations in

Reference; Study identification; Batch, purity; Owner	Study type, strain, duration	Dose levels	NOAEL	LOAEL	Targets / Main effects
	toxicity/ carcinoge-nicity; 2 yr; Sprague-Dawley	780/1060, 1290/1740 mg/kg bw/d in m/f)			organ weights (testis, brain, liver, kidneys) mostly at interim sacrifice (after 1 yr)
Lankas, 1981**; TOX2000-595 and TOX2000-1997; XHJ-64, 98.7%; Monsanto	Combined chronic toxicity/ carcinoge-nicity; 26 months; Sprague-Dawley	0, 3/3.4, 10.3/11.2, 31.5/34 mg/kg bw/d in m/f (dietary levels adjusted according to values as measured in the 1st week)	31.5 mg/kg bw/d (NOEL)	Not estab-lished	No effects observed
Calandra, 1974***; Z35230; Monsanto	Chronic toxicity study; 2 yr; "Charles River albino rat"	0, 30, 100, 300 ppm	100 ppm	300 ppm	Liver (lipidosis)

*poor study with many serious reporting deficiencies including lacking information on test material, surprisingly low spontaneous tumour incidences in the controls but the number of animals undergoing histopathology was also low; study rejected for EU risk assessment process; **study flawed by serious reporting deficiencies and employment of too low dose levels far below an MTD, not acceptable according to current standards but previously often used for regulatory purposes; ***deficient IBT study, not guideline-compliant, dose levels much too low for meaningful evaluation, not used for any regulatory assessment during the last decades

In a published study (Chruścielska et al., 2000a; ASB2013-9829), administration of glyphosate was also oral but via drinking water. A 13.85% aqueous solution of glyphosate ammonium salt (purity and batch not given in the article) was administered for two years to Wistar-RIZ outbred rats at concentrations of 300, 900, or 2700 mg/L. The initial group size was very large with 85 male and female rats per dose level of which 30 animals in total (i.e., 10 per timepoint) per dose and sex were used for interim sacrifices after 6, 12, or 18 months of treatment. It was stated that the study was conducted in compliance with OECD 453 but the report is very brief and no raw data is available. There was no increase in neoplastic lesions neither in males nor in females at any dose level as demonstrated in two tables displaying the cancer incidences. Due to reporting deficiencies and because a glyphosate salt solution but not the acid was tested, this study if of very limited value with regard to classification and labelling.

A further two-year study in rats was published by Séralini et al. (2012, ASB2012-15514) but a formulation and not the active substance was tested. Its main objective was to investigate a possible impact of long-term feeding of genetically modified (glyphosate-resistant) maize to rats but three of the test groups were administered a commercially available formulation (Roundup GT Plus, apparently authorised at least in Belgium) containing 450 g glyphosate/L at different concentrations ranging from 0.1 ppb (50 ng glyphosate/L) to 0.5% (2.25 g glyphosate/L) in drinking water. In these groups, the authors reported alterations in some clinical chemistry (blood and urine) parameters and hormone levels and histopathological lesions concerning the liver and the gastrointestinal tract but also a higher incidence of mammary tumours in females resulting in a shorter lifespan. This study was heavily discussed in the scientific community as well as in the general public where it gained notable attention due to massive promotion although it was clearly flawed by many serious deficiencies. A major point of concern was the small group size of only 10 males and 10 females

per dose, i.e., the test design was that of a subchronic study. Such a small number of animals is not sufficient for a long-term study because age-related changes cannot be adequately taken into account. A comprehensive critical assessment of this study was published by EFSA (2012, ASB2012-15513). The conclusion was that: "the currently available evidence does not impact on the ongoing re-evaluation of glyphosate […]". Later on, the paper was withdrawn by the journal in which it had been first published but was re-published in another one. In any case, this study is not suitable for classification and labelling purposes.

Because of the strong limitations of the two published studies, evaluation of carcinogenicity of glyphosate to rats can be based only on the studies that are summarised in Table 25. Due to their deficiencies, also the studies by Bhide (1997, ASB2012-11489), by Calandra (1974, Z35230) and by Lankas (1981, TOX2000-595 and TOX2000-1997) cannot be considered suitable for this purpose. However, since the latter study was subject to debate with regard to certain tumour types, it is taken here into consideration, along with the 6 guideline-compliant studies.

According to the evaluation by the DS, no evidence of carcinogenicity was obtained in any of the long-term studies in rats. Chronic toxicity was confined to high dose levels in all the studies but clear differences became apparent in what was actually observed (see Table 25). For more information, the reader is referred to the attached RAR (Volume 1, 2.6.6.1; Volume 3, B.6.5.1).

However, in the public debate on glyphosate but also in the IARC evaluation (IARC, 2015, ASB2015-8421), some neoplastic findings in two older studies have been subject to discussion. These findings comprised:

- an increase in islet cell tumours of the pancreas in both of these studies (Stout and Ruecker, 1990, TOX9300244; Lankas, 1981, TOX2000-595, TOX2000-1997)
- an increase in liver tumours in the study by Stout and Ruecker (1990, TOX9300244);
- an increase in C-cell adenoma of the thyroid in the same study; and
- an increase in interstitial cell tumours of the testis in the study by Lankas (1981, TOX2000-595, TOX2000-1997).

In the following, all these tumour types are considered in greater detail. That means also that the statistical calculations were repeated. In the original study reports, mostly pairwise comparisons had been made. In the 2015 IARC evaluation, trend tests were the preferred statistical tool. The DS re-calculated the statistical significance of the observed tumour incidences by taking both approaches.

For overall assessment, however, it must be further acknowledged that glyphosate is different from most other active substances in plant protection products because a number of comprehensive and high quality studies are available for nearly all toxicological endpoints. If dose levels are comparable, it would be expected that adverse effects were, at least to a certain extent, reproducible in other studies. A "weight of evidence" approach should and may be applied, therefore, as a general principle. Findings (including neoplastic) will be considered to have occurred by chance if they are not dose-related or cannot be confirmed at higher dose levels in other studies.

Pancreatic islet cell tumours

IARC noted that, based to the tumour incidences reported by Stout and Ruecker (1990, TOX9300244), a significant increase in pancreatic islet cell adenoma in male rats was observed at two dose levels but there were neither a statistically significant positive trend nor a progression to carcinoma. When the DS re-evaluated the reported incidences using Cochran-Armitage trend testing, the absence of a statistically positive trend was confirmed (Table 26).

The pairwise comparison by Fisher's exact test, in contrast, revealed a significant increase over the control incidence but only for the low dose group. Apparently, there was no clear dose response, which one would expect. Indeed, there was no progression towards malignancy since the only carcinoma in this study was found in a control male.

Table 26: Pancreatic islet cell tumours in SD rats (Stout and Ruecker, 1990, TOX9300244). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values for the pairwise comparison reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males/Group	Animals with islet cell adenoma
0	43	1
89	45	8 (0.030)
362	49	5 (0.209)
940	48	7 (0.062)
Trend test (p-value)		0.1687

In addition, IARC reported a significant increase in the incidence of pancreatic tumours in a second study in SD rats, i.e., in one of the treated male groups in the study of Lankas (1981, TOX2000-595, TOX2000-1997). However, according to IARC, there was no positive trend over all dose groups and, again, no indication for progression to carcinoma. Re-evaluation by the DS confirmed a significant increase in adenomas and for adenomas and carcinomas combined for the male low dose group when compared to the concurrent controls. Pairwise comparison did not reveal statistical significance for the pancreatic islet cell adenoma at the two upper dose levels. However, a significantly positive trend for carcinomas in male animals was found that has not been previously reported (Table 27). There was no increase in pancreatic tumours in the females.

Table 27: Pancreatic tumours in male SD rats (Lankas, 1981, TOX2000-595, TOX2000-1997). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males/Group	Adenoma	Carcinoma	Adenoma + Carcinoma
0	50	0	0	0
3	49	5 (0.027)	0 (1.000)	5 (0.027)
10.3	50	2 (0.495)	0 (1.000)	2 (0.495)
31.5	50	2 (0.495)	1 (1.000)	3 (0.242)
Trend test (p-value)		0.5284	0.0496	0.3207

This situation is similar as in the study by Stout and Ruecker (1990, TOX9300244). There was evidence of an increase in pancreatic tumours in treated males but, again, the difference to the control group was strongest in the low dose group and a clear dose response was missing. The

positive trend for carcinoma in this study is due to the rare occurrence of this tumour and the incidence of a single carcinoma in the high dose group compared to the absence of this tumour type in the control and lower dose groups.

For overall assessment, it must be taken into consideration that in the five more recently conducted and guideline-compliant rat studies summarised in Table 25, even at very high dose levels, no increase in pancreas tumours was seen (Table 28). In four of them, incidence was highest in the control group. In the two studies discussed above, the incidences were elevated in treated groups but without a clear dose response.

Table 28: Pancreatic islet-cell tumours in long-term studies with glyphosate in male rats

Study	Control	Low dose	Mid dose	Second mid dose	High dose
Wood et al., 2009, ASB2012-11492	4 / 51	1 / 51 (86 mg/kg bw/day)	2 / 51 (285 mg/kg bw/day)	-	1 / 51 (1077 mg/kg bw/day)
Brammer et al., 2001, ASB2012-11488	1 / 53	2 / 53 (121 mg/kg bw/day)	0 / 53 (361 mg/kg bw/day)	-	1 / 52 (1214 mg/kg bw/day)
Enomoto, 1997, ASB2012-11484, 11485, 11486, 11487; T-941209	4 / 50	1 / 50 (104 mg/kg bw/day)	2* / 50 (354 mg/kg bw/day)	-	1 / 50 (1127 mg/kg bw/day)
Suresh, 1996, TOX9651587	3 / 48	0 / 30 (6.3 mg/kg bw/day)	0 / 32 (59.4 mg/kg bw/day)	-	1 / 49 (595.2 mg/kg bw/day)
Atkinson et al., 1993, TOX9552382	7 / 50	1 / 24 (10 mg/kg bw/day)	2 / 17 (100 mg/kg bw/day)	2 / 21 (300 mg/kg bw/day)	1 / 49 (1000 mg/kg bw/day)
Stout and Ruecker, 1990, TOX9300244	2* / 43	8 / 45 (89 mg/kg bw/day)	5 / 49 (362 mg/kg bw/day)		7 / 48 (940 mg/kg bw/day)
Lankas, 1981, TOX2000-595, TOX2000-1997	0 / 50	5 / 49 (3 mg/kg bw/day)	4 / 50 (10.3 mg/kg bw/day)	-	3* / 50 (31.5 mg/kg bw/day)

*including one carcinoma

To conclude, an (occasionally significant) increase in pancreatic tumours in male rats was confined to two studies of which one is now considered insufficient due to the very low doses employed and because of reporting deficiencies. In both cases, a dose-response was lacking and there was no tendency of progression to malignant neoplasia. A higher incidence of pancreatic tumours was not reproducible in five more recent, guideline-compliant studies with a spontaneous incidence in untreated control animals that sometimes resembled the frequencies that were reported by Stout and Ruecker (1990, TOX9300244) or Lankas (1981, TOX2000-595, TOX2000-1997).

Liver tumours

In the study of Stout and Ruecker (1990, TOX9300244), again, IARC reported a significantly

positive trend for hepatocellular adenoma in males (Table 29). When the reported incidences were re-evaluated by the DS using Cochran-Armitage trend testing and Fisher's exact test, the statistically positive trend was confirmed for adenomas but no positive trend was observed for adenoma and carcinoma combined. In particular for combined incidence, a dose response was hardly to be seen and the pairwise comparison failed to reveal a statistically significant difference between any of the treated groups and the control group.

Table 29: Liver cell tumours in male SD rats (Stout and Ruecker, 1990, TOX9300244). Fisher's exact test was used to compare each treatment group to control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Male rats	Liver adenoma	Liver adenoma + carcinoma
0	44	2	5
89	45	2 (1.000)	4 (0.739)
362	49	3 (1.000)	4 (0.732)
940	48	7 (0.162)	9 (0.392)
Trend test (p-value)		0.0171	0.0752

Moreover, no increase in liver tumours was reported in any other long-term study in rats. In general, hepatotoxicity of glyphosate is very limited. In fact, absolute and relative liver weight was increased in high dose males in the study by Stout and Ruecker (1990, TOX9300244) but there were no pre-neoplastic findings that might progress to liver tumours. Based on the lack of increased liver tumour rates in all other long-term/carcinogenicity studies in two rat strains (Wistar and SD), the DS interpreted the increased incidence of liver tumours, mainly due to increased rates of liver adenomas, in one study as not attributable to glyphosate but to have occurred by chance.

Thyroid C-cell tumours

In the study of Stout and Ruecker (1990, TOX9300244), there was an increase in C-cell adenoma in female rats. This tumour was detected in 2 control and 2 low dose females but in 6 animals of the mid and high dose group each. In contrast to the (negative) pairwise comparison, the Cochran-Armitage trend test was weakly positive ($p = 0.0435$). In the absence of such a finding in any of the other rat studies, this increase in C-cell tumours is also considered a chance event. In addition, the thyroid is not a target organ of glyphosate. There were neither an increase in pre-neoplastic histological lesions nor an organ weight change noted in any other study with glyphosate even though distribution of radiolabelled glyphosate to the thyroid has been demonstrated in ADME studies by Ridley and Mirly (1988, TOX9552356) and by McEwen (1995, ASB2012-11379).

Interstitial cell tumours of the testes

In the study by Lankas (1981, TOX2000-595, TOX2000-1997), an increase of interstitial testicular tumours was observed. The actual incidences were 0/50, 3/50, 1/50, and 6/50 animals in the control group and at the three dose levels, respectively. Apparently, there was no clear dose response but in the top dose group receiving ca 31.5 mg glyphosate/kg bw per day, the difference to the control was statistically significant (Fisher's exact test, $p < 0.05$). In the original study report, it was argued that the absence of this tumour type in the control group was unusual and that the top dose incidence

was only marginally above the historical control range. Reliability of this information could not be verified and, even if correct, this explanation would not be convincing. However, and more important, no increase in testicular tumours was observed in any other long-term study with glyphosate in rats even though much higher doses were administered.

Studies in mice

In total, five long-term studies are available that may be considered valid according to current standards and were performed in compliance with OECD TG 451. They are summarised in Table 30. As in rats, chronic toxicity was confined to high dose levels in all the studies but some differences became apparent in what was actually observed. For more information, the reader is referred to the attached RAR (Volume 1, 2.6.6.2, Volume 3, B.6.5.2).

The DS is aware of two further long-term studies in mice which have been very briefly reported in an older EU evaluation report (Germany, 1998, ASB2010-10302). These studies by Vereczkey and Csanyi (1982, TOX9650154) and by Bhide (1988, TOX9551831) did not comply with current standards. In both of them, the top dose level was 300 ppm and, thus, much too low for meaningful evaluation. No increase in any tumour type had been reported but these studies are not suitable for the purpose of classification and labelling. The same holds true for a published study on skin tumour promotion (George et al., 2010, ASB2012-11829). This experiment was performed with a commercial product that most likely contains irritating co-formulants. It cannot contribute to a decision on the classification of glyphosate. Furthermore, the up- and down-regulation of protein expression is not sufficient to prove a carcinogenic effect. Apart from that, there are no published studies on carcinogenicity in mice.

Thus, evaluation of a carcinogenic potential of glyphosate in mice is based on the five available, guideline-compliant studies. In line with the approach taken for the rat studies, the main effects as summarised in this table were statistically significant and either dose-related or observed at the top dose level only. This approach implies that these findings were not necessarily all noted at the LOAEL.

Table 30: Long-term feeding studies with glyphosate in mice

Reference; Study identifi-cation; purity; Owner	Study type, strain, duration, route	Dietary dose levels and corresponding mean daily intake	NOAEL	LOAEL	Targets / Main effects
Wood et al., 2009, ASB2012-11492; 95.7%; Nufarm	Carcinogenicity (OECD TG 451); 18 mo; CD-1 (ICR), feeding	0, 500, 1500, 5000 ppm (71/98; 234/299; 810/1081 mg/kg bw/d in m/f)	810 mg/kg bw/d	Not established	No effects observed
Kumar, 2001, ASB2012-11491; >95.14%; ADAMA	Carcinogenicity (OECD TG 451); 18 mo, Swiss albino	0, 100, 1000, 10000 ppm (15; 151; 1460 mg/kg bw/d, sexes combined since values were similar)	151 mg/kg bw/d	1460 mg/kg bw/d	Higher incidence of malignant lymphoma at top dose level (outside historical control range for males); cystic glands in stomach in m↑ (equivocal toxicological relevance)
Sugimoto, 1997, ASB2012-11493; 97.56% or 94.61% (2 lots	Carcinogenicity (OECD TG 451); 18 mo; CD-1 (ICR)	0, 1600, 8000, 40000 ppm (165/153; 838/787; 4348/4116 mg/kg bw/d in m/f)	153 mg/kg bw/d	787 mg/kg bw/d	Bw gain, food consumption and efficiency↓, loose stool, caecum distended and

Reference; Study identifi-cation; purity; Owner	Study type, strain, duration, route	Dietary dose levels and corresponding mean daily intake	NOAEL	LOAEL	Targets / Main effects
used); Arysta					organ wt↑, prolapse and ulceration of anus in m
Atkinson et al., 1993; TOX9552382; 98.6%; Cheminova	Carcinogenicity (OECD TG 451); 2 yr, CD-1	0, 100, 300, 1000 mg/kg bw/d (dietary levels regularly ad-justed)	1000 mg/kg bw/d	Not established	Equivocal evidence of enlarged/firm thymus and increase in mineral deposition in the brain, not regarded as adverse
Knezevich and Hogan, 1983; TOX9552381; 99.7%; Monsanto	Carcinogenicity with chronic to-xicity elements (OECD TG 451/453); 2 yr, CD-1	0, 1000, 5000, 30000 ppm 157/190; 814/955; 4841/5874 mg/kg bw/d in m/f)	157 mg/kg bw/d	814 mg/kg bw/d	Bw (gain) ↓ in high dose males, histological findings in liver (centrolobular hy-pertrophy), kidney (histological changes) and bladder (epithelial hyperplasia) in males

In these studies, there was evidence of increases in three types of tumours, all in males: malignant lymphoma, renal tumours, and haemangiosarcoma, however, there was no consistency between the studies. In the following, all these three types are addressed in detail. That means also that the statistical calculations were repeated. In the original study reports, mostly pairwise comparisons had been made. In the 2015 IARC evaluation, in contrast, trend tests were the preferred statistical tool. The DS re-calculated the statistical significance of the observed tumour incidences by taking both approaches.

Malignant lymphoma

The total numbers of affected animals in the various mouse studies are given in Table 31.

Table 31: Total incidence of malignant lymphoma in long-term studies with glyphosate in different mouse strains and appropriate historical control (HC) data from the performing laboratory if available

Study, Strain		Males				Females			
Wood et al, 2009, ASB2012-11492 Crl:CD-1 (ICR) BR	Dose (ppm)	0	500	1500	5000	0	500	1500	5000
	Affected	0/51	1/51	2/51	5/51	11/51	8/51	10/51	11/51
Kumar, 2001, ASB2012-11491 HsdOLA:MF1 (Swiss albino)	Dose (ppm)	0	100	1000	10000	0	100	1000	10000
	Affected	10/50	15/50	16/50	19/50*	18/50	20/50	19/50	25/50*
	HC	Study range: 6–30% Study mean: 18.4% Basis: 250 male mice in 5 studies (1996-1999 covering the in-life phase of the actual study)				Study range: 14–58% Study mean: 41.6% Basis: 250 female mice in 5 studies (1996-1999)			

Study, Strain		Males				Females			
Sugimoto, 1997, ASB2012-11493 Crj:CD-1 (ICR)	Dose (ppm)	0	1600	8000	40000	0	1600	8000	40000
	Affected	2/50	2/50	0/50	6/50	6/50	4/50	8/50	7/50
	HC	Study range: 3.85–19.23% Study mean: 6.33% Basis: 458 male mice in 12 studies (1993-1998)				Study range: 7.84–26.92% Study mean: 15.03% Basis: 459 female mice in 12 studies (1993-1998)			
Atkinson et al., 1993, TOX9552382, CD-1 (not further specified)	Dose (mg/kg bw/d)	0	100	300	1000	0	100	300	1000
	Affected[#]	4/50	2/50	1/50	6/50	14/50	12/50	9/50	13/50

* increase statistically significant according to original study report, for females based on percentage and not on total number of affected mice
[#] based on histological examination of lymph nodes with macroscopic changes

Obviously, the carcinogenicity study in Swiss albino mice by Kumar (2001, ASB2012-11491) revealed an increase in malignant lymphoma incidence over the control at the top dose level of around 1460 mg/kg bw/day in both sexes but the background (control) incidence was also quite high. In fact, at least in males, the number of affected animals in the control groups was markedly higher in this strain than in three studies in CD-1 mice. It must be emphasised that this tumour is quite common in ageing mice and that Swiss mice are frequently affected (for details, see below). In this study, malignant lymphoma accounted for 54.6% of the total number of tumours when all groups are considered together.

In the most recent study in CD-1 mice by Wood et al. (2009, ASB2012-11490), there was a higher incidence of the same tumour type in high dose males (5/51 vs. 0/51 in the control group). Likewise, in the study by Sugimoto (1997, ASB2012-11493), there were a higher number of male mice affected at the exaggerated dose level of 40000 ppm (approx. 4350 mg/kg bw/day) than in the control group (6/50 vs. 2/50). In the study by Atkinson et al. (1993, TOX9552382), in contrast, there was no dose response and the incidence in the control group was similar to that at the top dose level.

In the earliest study in CD-1 mice by Knezevich and Hogan (1983, TOX9552381), malignant lymphoma was not mentioned as a separate entity but malignant lymphoblastic tumours of the lymphoreticular system in male mice did not show an increase with dose (Table 33) even though the maximum mean daily dose of 4841 mg/kg bw/day was higher than in any other study.

Table 32: Lymphoreticular neoplasia in male CD-1 mice in the study by Knezevich and Hogan (1983, TOX9552381)

Tumour type / dose (ppm	Males			
	0	1000	5000	30000
Lymphoblastic lymphosarcoma with leukaemia	1	4	3	2
Lymphoblastic lymphosarcoma without leukaemia	0	1	0	0
Composite lymphosarcoma	1	0	1	0

Tumour type / dose (ppm	Males			
Lymphoreticular neoplasms (total)	2 / 48	5 / 59	4 / 50	2 / 49

If a more recent histopathological nomenclature would have been used, malignant lymphoma was covered by this data.

The data on malignant lymphoma became subject to statistical re-evaluation by means of different methods. It must be emphasised that in the first evaluation by the DS in 2013 only the statistical evaluation by the study authors according to the original study plans had been taken into account resulting in a weak but significant increase in this tumour type in high dose males and females in the study in Swiss mice but not in CD-1 mice as given in Table 31.

- For the study by Kumar (2001, ASB2012-11491), a significantly increased incidence of malignant lymphoma in males and females of the high dose group was mentioned in the study report. For analysis, the Z-test had been employed revealing a significance level of 0.002. Interestingly, when the more usual Fisher's exact test had been used, p-values of 0.077 or even 0.225 would have been obtained and the significance lost in both sexes. The trend test also provided a p-value above the significance level of 0.05, most probably because of the high control incidence (see Table 33).

Table 33: Malignant lymphoma in Swiss albino mice (Kumar, 2001, ASB2012-11491). Fisher's exact test was used to pairwise compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	50	10	50	18
15	50	15 (0.356)	50	20 (0.837)
151	50	16 (0.254)	50	19 (1.000)
1460	50	19 (0.077)*	50	25 (0.225)*
Trend test (p-value)		0.0655		0.068

* The original study report indicated a statistically significant increase ($p<0.05$), using the Z-test.

- In contrast, re-analysis of the studies by Wood et al. (2009, ASB2012-11490) and Sugimoto (1997, ASB2012-11493) showed statistically significant increases with dose for male CD-1 mice in the trend test (Table 34 and Table 35) but a rather low or even "zero" incidence in the control groups might be behind this finding. For the data from the Wood et al. (2009, ASB2012-11490) study, a first pairwise comparison by Fisher's exact test suggested a borderline increase at the top dose level but statistical significance was not achieved ($p = 0.056$). This result was confirmed by the chi-square test. Also for this comparison, the very low control incidence (0/51) should be taken into consideration. No evidence of an increase in malignant lymphoma was found in females.

Table 34: Malignant lymphoma in CD-1 mice (Wood et al., 2009, ASB2012-11490). Chi square test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	51	0	51	11
71	51	1 (1.000)	51	8 (0.611)
234	51	2 (0.475)	51	10 (1.000)
810	51	5 (0.067)[#]	51	11 (1.000)
Trend test (p-value)		0.0037		0.3590

[#] Chi –square test was chosen in accordance to the recommendations of the statistics package used. Using Fisher's exact test, a p-value of 0.056 (two-sided) was calculated. Depending on the tool used for calculation, the two-tailed Z-test produced p-values of 0.0220, 0.0219 and 0.067.

Table 35: Malignant lymphoma in CD-1 mice (Sugimoto, 1997, ASB2012-11493). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	50	2	50	6
165	50	2 (1.000)	50	4 (0.741)
838	50	0 (0.495)	50	8 (0.774)
4348	50	6 (0.269)	50	7 (1.000)
Trend test (p-value)		0.0085		0.2971

No evidence of an increase in malignant lymphoma was obtained upon statistical re-evaluation for the study by Atkinson et al. (1993, TOX9552382) confirming the prior assumption (Table 36).

Table 36: Malignant lymphoma in CD-1 mice (Atkinson et al., 1993, TOX9552382). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each sex, a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Males with malignant lymphoma	Females on study	Females with malignant lymphoma
0	50	4	50	14
100	50	2 (0.678)	50	12 (0.657)
300	50	1 (0.362)	50	9 (0.342)
1000	50	6 (0.741)	50	13 (1.000)
Trend test (p-value)		0.0760		0.4831

It may be concluded that the statistical significance of the suspected increase in malignant lymphoma in the various studies depends very much on the statistical method that is used for data analysis. When the trend test is applied, the studies by Wood et al. (2009, ASB2012-11490) and Sugimoto (1997, ASB2012-11493) provide evidence of an effect which was not the case when pairwise comparison was performed. In contrast, the increase in the study of Kumar (2001, ASB2012-11491) was not confirmed neither by the trend test nor by a different pairwise test than the Z-test that had been used first.

According to OECD criteria (OECD 116), significance in either kind of test (i.e., trend test or pair-wise comparison) was sufficient to reject the hypothesis of a chance event. However, statistical significance is not the only criteria to decide whether or not an increase in a certain tumour type should be assumed as treatment-related. For a firm conclusion on the likeliness of an increase in malignant lymphoma in mice due to glyphosate exposure, the biological significance of a numerically higher tumour rate, the whole database in the species and the respective strains (i.e., historical control data on the background incidence of a given tumour type) and more aspects such as dose selection and dose response must be taken into consideration.

At first, dose selection and dose response in the individual studies might be of importance. In the studies by Wood et al. (2009, ASB2012-11490) and by Atkinson et al. (1993, TOX9552382) in CD-1 mice, comparable top doses of 810 or 1000 mg/kg bw/day were administered and a similar incidence of malignant lymphoma was noted in high dose males (5/51 or 6/50, respectively). However, the control group incidences were clearly different (0/51 vs. 4/50) resulting in a positive trend test in the study by Wood et al. (2009, ASB2012-11490) only. A dose of 4348 mg/kg bw/day was actually applied in the study by Sugimoto (1997, ASB2012-11493) as a maximum. The study was also performed in CD-1 mice and the malignant lymphoma incidence of 6/50 at the top dose level was similar to what was seen in the two studies mentioned before even though the applied dose was by four to five times higher. This is surprising since a further increase would be expected if it was a treatment-related effect. These doubts are further supported by the long-term study by Knezevich and Hogan (1983, TOX9552381) in which an even still higher dose of 4841 mg/kg bw/day was fed without an increase in lymphoreticular tumours in general. Unfortunately, malignant lymphoma was not mentioned as a particular pathological entity but it can be reasonably assumed that such tumours have been reported as “lymphoreticular neoplasia”.Thus, if all four studies in CD-1 mice are taken together, there is no consistent dose response.

Then, the huge variability of spontaneous incidences of malignant lymphoma in mice as suggested by historical control data must be taken into consideration. This holds true for both Swiss and CD-1 mice as well as for other strains (Wogan and Pattengale, 1984, ASB2016-889). Unfortunately, reliable historical control data on malignant lymphoma incidence from the performing laboratories are available only for two of the glyphosate studies (Sugimoto, 1997, ASB2012-11493, and Kumar, 2001, ASB2012-11491). Therefore, it is necessary to use also data from the open literature or from industry databases even though such information is usually considered less relevant.

In the study in Swiss mice by Kumar (2001, ASB2012-11491), the historical control incidence from the performing laboratory was in a very wide range from 6 to 30% in male mice (study mean 18.4%) and from 14 to 58% in females (study mean 41.6%). Thus, the actual malignant lymphoma incidence in this study of 38% in males and 50% in females was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Of course, the relevance of this data is questionable since it was based on observations in only five studies employing in total 250 untreated control animals per sex. Nonetheless, it seems well in line with information that was found in the literature providing confirmation that Swiss mice are prone to developing lymphoreticular tumours. According to older articles, control incidences in male mice of

Swiss or Swiss-derived strains may reach 18–27.5% and exceed 36% in females (Sher, 1974, Z22020; Roe and Tucker, 1974, ASB2015-2534; Tucker, 1979, Z83266). In a more recent publication, Tadesse-Heath et al. (2000, ASB2015-2535) even mentioned a nearly 50% lymphoma (mostly of B cell origin) incidence in a colony of CFW Swiss mice but also emphasised the contribution of widespread infections with murine oncogenic viruses to the high but remarkably variable incidence of tumours of the lymphoreticular system in this species. This problem is known for long and was often addressed in the past in textbooks of virology or mouse pathology. Already more than 30 years ago, Wogan and Pattengale (1984, ASB2016-889) described the contradictory situation as follows: "The role of oncogenic viruses in many hematopoietic tumours in mice is well established. Virtually all spontaneous or induced lymphomas which have been studied in mice contain oncogenic viruses. It is also recognized that oncogenic viruses and chemicals can act synergistically on cells in vitro and in vivo to cause tumour formation. This can be manifested by either increased incidence, decreased latency, or both. This raises the important issue as to whether a chemical which induces lymphoma in mice requires the presence of a murine oncogenic virus. If so, perhaps the induction of this tumour in mice would not be relevant to human carcinogenic risk. However, since it is possible that many other species, including man, carry undetected oncogenic virus which may act with chemicals to increase tumour burdens, considerations of viral carcinogenesis do not totally resolve the questions concerning the significance of mouse lymphoma in safety testing, except to point out that the prevalence of oncogenic viruses in mice may make them highly susceptible to the induction of lymphoma, leukaemia, and perhaps other neoplasms." No information is available on possible abundance of oncogenic viruses in the mouse colonies from which the animals used in the glyphosate studies were obtained. During a teleconference (TC 117) on carcinogenicity of glyphosate hold by EFSA (EFSA, 2015, ASB2015-12200), it was mentioned by an U.S. EPA observer that the Kumar (2001, ASB2012-11491) study had been excluded from U.S. EPA evaluation due to the occurrence of viral infection that could influence survival as well as tumour incidences, especially those of lymphomas. However, in the study report itself, there was no evidence of health deterioration due to suspected viral infection and, thus, the actual basis of EPA's decision is not known.

On request of the DS, reliable historical control data was provided by the Japanese laboratory in which the study by Sugimoto (1997, ASB2012-11493) had been run. In male Crj:CD-1 (ICR) mice, incidence of malignant lymphoma in this laboratory varied very much. It ranged from 3.85% to 19.23% in the control groups from 12 studies that had been performed between 1992 and 1998 (Kitazawa, 2013, ASB2014-9146). Thus, the 12% incidence at the top dose level in the study with glyphosate was well covered by the range even though it was above the mean value of 6.33%. (In females, control incidences in the comparison studies ranged from 7.84 to 26.92% with a mean of 15.03%.)

Unfortunately, for the study of Wood et al. (2009, ASB2012-11492), the submitted historical control data was not particularly useful for the assessment. In fact, control data from a total of nine studies were submitted (Wood, 2015, ASB2015-2531) but were of not much use because incidences in male and female mice were not reported separately and since the data were apparently from the same contract research organisation but not from the same test facility. However, the mentioned study incidences ranging from 0% up to 32% (both sexes combined) show the large variability of malignant lymphoma frequency and would, theoretically, cover all male and female groups in the studies in CD-1 mice. This assumption is supported by further historical control data for CD-1 mice collected from industry databases (Giknis and Clifford, 2005, ASB2007-5200; Anonym, 2015, ASB2015-2532) or open literature (Son and Gopinath, 2004, ASB2015-2533). According to these data collections, malignant lymphoma is quite common in CD-1 mice but the reported incidences in different CD-1 strains and among the laboratories were extremely variable. Mostly, they were higher in females than in males but even in males may reach rates between 10% and 20%. The

Charles River database (Giknis and Clifford, 2005, ASB2007-5200) includes data obtained in a total of 59 studies (duration 78 to 104 weeks) in CD-1 mice. The animals were bred in four different Charles River facilities in the United States and the studies were performed in 11 laboratories in North America and Europe between 1987 and 2000. The diagnosis "malignant lymphoma" was used in 42 studies revealing study incidences ranging from a minimum of 1.45 up to a maximum of 21.67% with a total mean in all untreated animals of 4.5%. The malignant lymphoma incidences in male mice receiving the highest doses in the studies by Atkinson et al. (1993, TOX9552382), Sugimoto (1997, ASB2012-11493), and Wood et al. (2009, ASB2012-11490) accounted for not more than 12% and would fit into this range even though the mean was exceeded.

On balance, based on uncertainties with regard to partly contradictory study outcomes depending on the statistical method applied, inconsistent dose response in the individual studies, and a highly variable tumour incidence as suggested by historical control data, it is not likely that glyphosate has induced malignant lymphoma in mice. A possible role of oncogenic viruses should not be ignored. Moreover, human relevance of such an effect, if occurring only as a high-dose phenomenon as it was the case here, is considered equivocal.

Renal tumours in male mice

In the IARC evaluation (IARC, 2015, ASB2015-8421), a positive trend for renal (tubular) adenoma and carcinoma in males in the study by Knezevich & Hogan (1983, TOX9552381) was highlighted. This increase had been subject to discussion already in the 1980s when this study was evaluated for the first time by U.S. EPA. At that time, re-evaluation of the histopathological findings by a "Pathology working group (PWG)" had been requested and was performed. By the DS, the positive trend can be confirmed (Table 37) even though a pairwise comparison did not indicate a statistically significant difference to the control, neither for the adenoma nor for the carcinoma or both combined.

Table 37: Renal adenoma and carcinoma in male CD-1 mice (Knezevich and Hogan 1983, TOX9552381), based on originally reported data and re-evaluation by PWG. Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. For each endpoint a Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	N	Original report	Re-evaluation by PWG		
		Adenoma	Adenoma	Carcinoma	Combined
0	49	0	1	0	1
157	49	0 (1.000)	0 (1.000)	0 (1.000)	0 (1.000)
814	50	1 (1.000)	0 (0.495)	1 (1.000)	1 (1.000)
4841	50	3 (0.242)	1 (1.000)	2 (0.495)	3 (0.617)
Trend test (p-value)		0.0080	0.2473	0.0370	0.0339

For a more comprehensive assessment and to provide a broader view, the incidence of renal tumours in all long-term studies in male CD-1 mice was considered (Table 38). From this overview, it becomes clear that such tumours are rare but still may also occur in untreated animals. A numerically higher incidence in adenoma was seen in the study by Sugimoto (1997, ASB2012-11493) and, again, this increase was confined to male mice receiving the highest dose. Thus, there

was an increase in renal tumour incidence over the overall control level in the two studies in which extremely high dose levels of 4841 or 4348 mg/kg bw/day) had been administered. The top dose levels in the studies by Wood et al. (2009, ASB2012-11490) and by Atkinson et al. (1993, TOX9552382) were much lower and no increase in renal tumours was seen. However, it must be emphasised that the same number of animals was affected in the study by Atkinson et al. (1993, TOX9552382) in the control and low dose groups as in the study by Sugimoto (1997, ASB2012-11493) at the top dose level and that the difference to 3/50 affected mice in the study by Knezevich and Hogan (1983, TOX9552381) was only marginal. Even though no historical control data from the performing laboratories was provided, a simple comparison of the control groups in the individual studies with glyphosate suggests that renal tumours may occur in untreated control males at a similar incidence than in the groups receiving very high doses.

Table 38: Incidences of renal tubule tumours in the four available glyphosate studies in male CD-1 mice

Study	**Knezevich and Hogan, 1983, TOX9552381**	**Atkinson et al., 1993, TOX9552382**	**Sugimoto, 1997, ASB2012-11493**	**Wood et al., 2009, ASB2012-11490**
Dose levels	**0, 1000, 5000, 30000 ppm**	**0, 100, 300, 1000 mg/kg bw/d**	**0, 1600, 8000, 40000 ppm**	**0, 500, 1500, 5000 ppm**
Control	1 / 49	2[#] / 50	0 / 50	0 / 51
Low dose	0 / 49	2[#] / 50	0 / 50	0 / 51
Mid dose	1[#] / 50	0 / 50	0 / 50	0 / 51
High dose	3[##] / 50	0 / 50	2 / 50	0 / 51

[#] including one carcinoma; [##] including two carcinomas

With regard to malignancy, carcinoma were reported by the PWG when re-evaluating the study by Knezevich and Hogan (1983, TOX9552381) and also by Atkinson et al. (1993, TOX9552382). In contrast, both renal tumours found by Sugimoto (1997, ASB2012-11493) were benign. It should be kept in mind that it is difficult to discriminate between benign and malignant renal tubule tumours and, thus, combined incidence might provide the most appropriate figure.

No renal tubule tumours were seen in female mice in any of these studies.

In order to provide a complete picture, renal tumour incidences in male mice in the study by Kumar (2001, ASB2012-11491) in Swiss mice are given in Table 39 even though this study is not being considered further since another strain was employed. In total, 3 renal tumours (described as adenoma) were observed, affecting both the mid and high dose groups. According to the original study report, all neoplasia were assessed for statistical significance by means of the Z-test which was apparently negative. A Cochran-Armitage test for trend and a Peto test were also mentioned by the study author, however, it is not clear if trend analysis has been actually performed. When the renal tumours were re-analysed by the DS, there was a positive linear trend whereas Fisher's exact test failed to indicate a significant difference. No renal tumours were seen in female Swiss albino mice and there was no evidence of concomitant kidney pathology neither in males nor in females.

Table 39: Renal tubular tumours adenoma in male Swiss mice (Kumar 2001, ASB2012-11491). Fisher's exact test was used to compare each treatment group to the

respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Adenoma
0	50	0
15	50	0 (1.000)
151	50	1 (1.000)
1460	50	2 (0.495)
Trend test (p-value)		0.0390

Even if not fully comparable because of the strain differences, it should be remembered that the top dose incidence of 2/50 in this study was the same as seen in CD-1 mice in the study by Atkinson et al. (1993, TOX9552382) in the control and low dose groups.

With respect to CD-1 mice, the finding in the study by Sugimoto (1997, ASB2012-11493) was also subject to statistical re-evaluation for trend by the DS revealing a positive result (Table 40), most probably due to the "zero" incidence in the control group. As to be expected because of the low number of affected mice at the top dose level, the pairwise comparison (as performed also according to the original report) did not indicate a statistically significant difference.

Table 40: Renal tubular tumours adenoma in CD-1 mice (Sugimoto, 1997, ASB2012-11493). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	Males on study	Adenoma
0	50	0
165	50	0 (1.000)
838	50	0 (1.000)
4348	50	2 (0.495)
Trend test (p-value)		0.0078

On the basis of this data, it cannot be clearly distinguished whether the small increase in a rare renal tumour in mice at exaggerated dose levels that have been applied for 2 years or at least 18 months could be attributed to glyphosate itself and its toxicity, was due to long-lasting renal excretion of large amounts of an otherwise more or less inert substance or rather a chance event. The whole database, quantitative (dose) and mechanistic considerations as well as historical control data should be taken into account.

It must be emphasised that a higher number of male CD-1 mice bearing renal tumours as compared to the concurrent controls were only seen in the studies by Sugimoto et al. (1997, ASB2012-11493) and by Knezevich and Hogan (1983, TOX9552381) at the maximum doses of 4348 or even 4841 mg/kg bw/day and, therefore, cannot be either supported or contravened by the other studies in which lower maximum doses of up to 1000 mg/kg bw/day had been applied, i.e., those of Atkinson et al. (1993, TOX9552382) and Wood et al. (2009, ASB2012-11490). For the study in Swiss mice, there is no other study to match it. If increased tumour incidences are found only at the highest dose levels in a lifetime study, the occurrence of a confounding effect of excessive toxicity

should be regarded very critically. Dose levels of >4000 mg/kg bw per day were well in excess of the limit dose for carcinogenicity testing (1000 mg/kg bw per day) as recommended by OECD guidance document 116. The OECD test guideline 451 for carcinogenicity studies does not give a precise recommendation but states that the highest dose level should elicit signs of minimal toxicity, with depression of body weight gain of less than 10%. However, in the studies by Sugimoto et al. (1997, ASB2012-11493) and by Knezevich and Hogan (1983, TOX9552381), however, the body weight gain in high dose males was decreased by more than 15% compared to controls. Mean terminal body weight of top dose males in the Knezevich and Hogan (1983, TOX9552381) study was by 11% lower than in the controls. In addition, there were gastrointestinal signs and lesions in the first and a significant increase in central lobular hepatocyte hypertrophy and central lobular hepatocyte necrosis suggesting some liver toxicity in the second study (see Table 30). Of particular interest was the observation of some kidney pathology in the study by Knezevich and Hogan (1983, TOX9552381). There was a positive trend for chronic interstitial necrosis in males with 12/50 affected in the high dose group versus 5/49 in the control. In females, there was a dose-related increase in proximal tubule epithelial basophilia and hypertrophy which were not seen among untreated control animals at all. Another finding in the urogenital tract in the same study was slight to mild urothelial hyperplasia in the bladder in mid and high dose males. The percentage of affected animals accounted for 6% in both the control and low dose groups but for 20% in the mid dose and for 16% in the high dose group. Even though there was no clear dose response, it may be assumed that glyphosate (acid) when administered at high doses might produce mucosal irritation. To conclude, there is some evidence that the MTD was exceeded in both studies at the highest dose level at which the number of tumour-bearing mice was slightly increased.

As outlined above in the section on mutagenicity, a genotoxic mode of action is unlikely. Occurrence of non-neoplastic lesions in the kidney was confined to an exaggerated dose level in the study by Knezevich and Hogan (1983, TOX9552381) in mice (see paragraph above) and papillary necrosis in a long-term study in male Wistar rats receiving more than 1200 mg/kg bw/day (Brammer, 2001, ASB2012-11488). On the other hand, the orally absorbed amount of ingested glyphosate is virtually completely and chemically unchanged eliminated in the urine (see section on toxicokinetics and metabolism above) and glyphosate acid is a known irritant to the eyes (see section above). However, it is questionable if irritation would sufficiently explain tumour formation in the kidney.

Historical control data from the Charles River Laboratories is available for Crl:CD1 (ICR) mice, based on 52 studies of at least 78 weeks duration that were performed between 1987 und 2000. From this data, it becomes clear that renal tumours are quite rare since adenoma were seen in five and carcinoma in four studies only. The maximum incidence for adenoma was 4% and for carcinoma 2% (Giknis and Clifford, 2005, ASB2007-5200). The top dose finding of 2/50 in the study by Sugimoto (1997, ASB2012-11493) is at the upper edge of adenoma frequency. In the study by Knezevich and Hogan (1983, TOX9552381) which is not actually covered by the timeframe of the historical database, the adenoma incidence (2%) at the top dose level would be inside the historical range whereas a carcinoma incidence of 4% was above. However, it is very difficult to distinguish between malign and benign kidney tumours and progression is frequent.

To conclude, it is not likely that the renal tumours in male mice are treatment-related for the following considerations:

- Even the incidences of affected animals at exaggerated doses exceeding the OECD-recommended limit of 1000 mg/kg bw/day and also the MTD were not statistically significantly increased when compared with the concurrent controls.
- If the whole database is taken into account, it becomes apparent that the top dose incidences in the studies by Sugimoto (1997, ASB2012-11493) and by Kumar (2001, ASB2012-11491) are the same as in the study by Atkinson et al. (1993, TOX9552382) in both the control and low dose groups and the number of affected males in the study by Knezevich and Hogan (1983, TOX9552381) was only slightly higher (3 vs. 2).
- Even the incidences at exaggerated doses are covered by the historical control range.
- No pre-neoplastic kidney lesions have been observed in treated animals.
- There is no plausible mechanism.

Haemangiosarcoma in male mice

Another tumour type was observed by Atkinson et al. (1993, TOX9552382) and highlighted by IARC. Again, the trend test was positive even though a pairwise comparison failed to indicate statistical significance. This holds true also for the study by Sugimoto (1997, ASB2012-11493) when re-evaluated by the DS (Table 41).

Table 41: Haemangiosarcoma in male CD-1 mice (Atkinson et al., 1993, TOX9552382; Sugimoto, 1997, ASB2012-11493). Fisher's exact test was used to compare each treatment group to the respective control group, with p-values reported in brackets. A Cochran-Armitage trend test was performed, with p-values reported in a separate row.

Dose (mg/kg bw/day)	N	Haemangiosarcoma	Dose (mg/kg bw/day)	N	Haemangiosarcoma
Atkinson et al. (1993, TOX9552382)			**Sugimoto (1997, ASB2012-11493)**		
0	50	0	0	50	0
100	50	0 (1.000)	165	50	0 (1.000)
300	50	0 (1.000)	838	50	0 (1.000)
1000	50	4 (0.059)	4348	50	2 (0.495)
Trend test (p-value)		0.0004			0.0078

With regard to the other studies in CD1 mice, there were no haemangiosarcoma in the study by Wood et al. (2009, ASB2012-11490) in the vascular system up to the highest dose level of approx. 810 mg/kg bw/day. However, if also tumours of this type in the liver and/or kidney were taken into account, the incidence was 2/51 (control), 1/51 (71 mg/kg bw/day), 2/51 (234 mg/kg bw/day), and, again, 1/51 at the top dose level of 810 mg/kg bw/day. In the earliest study by Knezevich and Hogan (1983, TOX9552381), haemangiosarcoma was not listed as a particular histopathological entity but was observed in the spleen of one mid-dose male animal (1/50). Incidence in females, in all studies in CD-1 mice, varied between 0 and 2 but there was no dose response and the tumour occurred also in the controls (1/51 in the study by Wood et al., 2009, ASB2012-11490).

In the study by Kumar (2001, ASB2012-11491) in Swiss mice, there was no evidence of a treatment-related increase in haemangiosarcoma. This tumour type was found in one mid dose male and one control female only. Thus, this study in another strain does not need to be considered in this context.

Despite the positive trend test in two studies in CD-1 mice, this finding is not considered treatment related. According to Atkinson et al. (1993, TOX9552382), the historical control incidence in the performing laboratory ranged from 0/50 to 4/50 and, thus, would cover the incidence at the top dose level. This historical data was based on a total of six 2-year studies in CD-1 mice from the same laboratory and had been accepted by the JMPR in its 2004 evaluation of glyphosate although it was not mentioned in the study report when these studies had been performed. For the other studies with glyphosate, no historical data on haemangiosarcoma incidence in the performing laboratories is available.

Historical control data provided by Charles River indicate a very variable incidence of haemangiosarcoma. On different sites of the body, tumours of this type were seen in untreated control animals in 8 of 52 studies. The incidence varied between 1.67 and 12% (Giknis and Clifford, 2005, ASB2007-5200) covering the top dose findings in the glyphosate studies. .in mice

Furthermore, since Sugimoto (1997, ASB2012-11493) employed a more than four times higher top dose than Atkinson et al. (1993, TOX9552382), a markedly higher haemangiosarcoma incidence would have been expected if this tumour was in fact treatment-related.

Thus, there is not sufficient and convincing evidence to consider haemangiosarcoma in male mice treatment-related and sufficient for classification.

In Table 42, incidences of the three tumour types under discussion in male CD-1 mice in the four glyphosate studies are summarised with regard to dose response. This compilation allows a comparative view on all four studies in male CD-1 mice. It becomes apparent that all these tumours were present over the whole dose spectrum and in were observed in the control groups as well. No consistent increase was seen. If historical control data from the Charles River Laboratories is taken into account, all tumour incidences in all control and treated groups were below the maxima of the historical control data even though the mean values were always exceeded and, with regard to renal tumours, the top dose incidence in the study by Knezevich and Hogan (1983, TOX9552381) was at the upper boundary of the range when adenoma and carcinoma were combined.

The highest incidences were observed in groups receiving very high doses of glyphosate, i.e., 4841 mg/kg bw/day in case of renal tumours, 1000 and 4348 mg/kg bw/day in case of malignant lymphoma and 1000 mg/kg bw/day with regard to haemangiosarcoma. These dose levels were at or far above the recommended limit for testing of 1000 mg/kg bw/day. It is noteworthy that no similar or stronger increase of the latter two tumour types was seen in concurrent studies in which similar or even higher doses were administered. Concerning renal tumours, it should be acknowledged that in fact 3/50 animals were affected at a dose level of 4841 mg/kg bw/day but the number of cases in untreated controls or at a dose level of ca 100 mg/kg bw was 2/50 in another study suggesting that this tumour, even if rare, is not uncommon in male CD-1 mice. To conclude, over a wide dose range, there is no evidence of a consistent increase in any tumour type in male CD-1 mice.

Table 42: Summary of selected tumour incidences in male CD-1 mice from four studies with glyphosate and historical control data.

Dose (mg/kg bw per day)	***HC, Maximum % found***	**0**	**0**	**0**	**0**	**71**	**100**	**157**	**165**	**234**	**300**	**810**	**814**	**838**	**1000**	**4348**	**4841**
Study		A	B	C	D	D	B	A	C	D	B	D	A	C	B	C	A
Study duration (months)		24	24	18	18	18	24	24	18	18	24	18	24	18	24	18	24
Survival		20/50	26/50	26/50	39/51	41/51	25/50	16/50	34/50	39/51	29/50	35/51	17/50	27/50	25/50	29/50	26/50
Renal tumours[#]	*4 (adenoma) 2 (carcinoma)*	1/49	2/50	0/50	0/51	0/51	2/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	0/50	2/50	3/50
Malignant lymphoma*	*21.7*	2/48	4/50	2/50	0/51	1/51	2/50	5/49	2/50	2/51	1/50	5/51	4/50	0/50	6/50	6/50	2/49
Haemangiosarcoma**	*12.0*	0/48	0/50	0/50	2/51	1/51	0/50	0/49	0/50	2/51	0/50	1/51	1/50	0/50	4/50	2/50	0/49

Study: A = Knezevich and Hogan (1983, TOX9552381), PWG re-evaluation; B = Atkinson et al. (1993, TOX9552382); C = Sugimoto (1997, ASB2012-11493); D = Wood et al. (2009, ASB2012-11492).

Renal tumours: combined incidence of adenoma and carcinoma given for individual studies.

* Study A: Malign lymphoblastic tumours (3 categories) instead of malignant lymphoma which was not mentioned as a pathological entity.

** Whole body/multiple organ.
Highlighted in grey – dosage exceeded the OECD-recommended limit dose of 1000 mg/kg bw/day and the MTD.
HC: Historical control data for Crl:CD-1 (ICR) mice from Charles River Laboratories (Giknis and Clifford, 2005, ASB2007-5200)

4.9.2 Human information

The only source of human information on carcinogenicity of glyphosate is epidemiology. However, it is not possible to distinguish between effects of the active substance glyphosate and its co-formulants since humans are always exposed to plant protection products and their residues but hardly ever to the active substance alone. Furthermore, it is difficult if not impossible to attribute health effects including cancer to glyphosate-containing products since humans are exposed to a great number of environmental chemicals. Therefore, the actual value of such data for classification is questionable and in any case limited.

A number of epidemiological studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the scope of their conclusions regarding either pesticides in general, certain classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. An essential consideration in both, risk assessment and interpreting the relevance of toxicology data, is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculative if exposure cannot be confirmed and quantified.

Moreover, only a small number of cancer cases are observed in all the individual studies, making it difficult to obtain clear results. There are a lot of problems with confounders: in most studies, glyphosate is included together with several other pesticides/insecticides so that the specific effects of each individual substance are difficult if not impossible to determine with any certainty. Farmers who use one chemical substance may also use another. It is not clearly stated which formulation of glyphosate is used; that is, different brands may have been used which have slightly different chemical mixtures and co-formulants, which themselves may have carcinogenic effects. The exposure cannot be easily measured. For example, no measures from biomarkers from the blood are used. Exposure is measured through interviews or questionnaires. Here, the problem is in reliance on memory to accurately determine the amount of exposure to the chemicals. Furthermore, there may be a recall biases since individuals with cancer are more likely to think about possible reasons for their cancer than healthy individuals. Moreover, in these studies we find a problem with the classification of the cancers. Non-Hodgkin's lymphomas (NHLs) have been not consistently defined over time. The definition has changed over time due to the use of different diagnostic methods: first morphological methods, then modern immunological methods were applied. Therefore, the NHLs reported do not always comprise the same cancers. For instance, some include, others exclude hairy cell leukaemia. Multiple myelomas may also be considered presently as NHL but not previously. Some studies are thus not comparable and some comparisons are difficult because of the in- and exclusion of certain subtypes which are not the same. This may skew the picture. IARC notes in quite a number of studies that there is limited information on glyphosate exposure. On the other hand, evidence from epidemiological studies has to be considered with all necessary care since at least uncertainties due to extrapolating from animal to human toxicology is avoided in this approach.

The largest and most convincing epidemiological study of pesticide exposure and health outcomes in the United States was the Agricultural Health Study (AHS) in which glyphosate was also addressed and included. Dozens of publications have resulted from data generated in this study of approx. 57,000 enrolled farmers (applicators). Blair et al. (2009, ASB2012-11566) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukaemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005, ASB2012-11605) used

data from the AHS in order to compare glyphosate use and multiple cancer endpoints. No association was noted for glyphosate with all cancers types under investigation, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, NHL and leukaemia. In an earlier publication based on a different data set, however, De Roos et al. (2003, ASB2012-11606) had reported an association between NHL and glyphosate use. Likewise, McDuffie et al. (2001, ASB2011-364) mentioned a non-significant positive association between self-reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009, ASB2012-11566), in contrast, did not report an association between glyphosate use and NHL in the AHS data but a "possible association" between glyphosate use and multiple myeloma was mentioned making reference to a "suggested association" between glyphosate use and multiple myeloma suggested by De Roos et al. (2005, ASB2012-11605). However, in this paper, no significant increase in relative risk for multiple myeloma was demonstrated. Both papers by De Roos et al. will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009, ASB2012-11623) specifically referenced De Roos et al. (2005 ASB2012-11605) to provide no evidence of cancers of any type to be associated with glyphosate.

Lee et al. (2005, ASB2012-11882) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern about higher positive associations observed for proxy respondents with glyphosate and several other pesticides. They suggested perhaps more accurate reporting of proxies for cases and underreporting by proxies for controls.

Monge et al. (2007, ASB2012-11909) investigated associations between parental pesticide exposures and childhood leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalonil and "others". No association was noted for paternal exposures, but elevated incidence of leukaemias was associated with maternal exposures to "other pesticides" during pregnancy.

Some further epidemiological studies have focused on an association between pesticide exposure and Non-Hodgkin`s Lymphoma (NHL). Hardell and Eriksson (1999, ASB2012-11838) investigated in a case-control study the incidence of NHL in relation to pesticide exposure in Sweden. 404 cases and 741 controls have been included. The authors discussed an increased risk for NHL especially for phenoxyacetic acids. Glyphosate was included in the uni-variate and multi-variate analyses. However, only 7 of 1145 subjects in the study gave exposure histories to this agent. The authors reported a moderately elevated odds ratio (OR) of 2.3 for Glyphosate. This OR was not statistically significant and was based on only 4 "exposed" cases and 3 "exposed" controls. The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43% of the pesticide use information, and the difficulty in the controlling for potential confounding factors given the small number of exposed subjects.

A further study was submitted by Hardell et al. (2002, ASB2012-11839). This study pools data from the above mentioned publication by Hardell and Eriksson (1999, ASB2012-11838) with data from a previously submitted publication from Nordström et al. (1998, TOX1999-687).

The authors found increased risks in a uni-variate analysis for subjects exposed to herbicides, insecticides, fungicides and impregnating agents. Among herbicides, significant associations were found for glyphosate and MCPA. However, in multi-variate analyses, the only significantly increased risk was found with a heterogeneous category of "other herbicides" and not for glyphosate. No information is given about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g., smoking, use of prescribed drugs etc.). In all, the above

mentioned limitations of the publication of Hardell and Eriksson (1999, ASB2012-11838) are also applicable to the publication by Hardell et al. (2002, ASB2012-11839).

Fritschi et al. (2005, ASB2012-11624) submitted a case-control study with 694 cases of NHL and 694 controls in Australia. Substantial exposure to any pesticide was associated with an increase in NHL. However, no association between NHL and glyphosate can be made on the basis of this study. No information was given about exposure duration, glyphosate products used, and application rates. Therefore, the documentation is considered to be insufficient for assessment.

Eriksson et al. (2008, ASB2012-11614) reported a case-control study which included 910 cases of NHL and 1016 controls living in Sweden. The highest risk was calculated for MCPA. Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02. Results and reliability of the study are discussed below.

Alavanja et al. (2013, ASB2014-9174) reviewed studies on cancer burden among pesticide applicators and others due to pesticide exposure. In this article, the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, NHL, leukaemia, multiple myeloma, and breast cancer were integrated. Glyphosate was reported to be the most commonly used conventional pesticide active ingredient worldwide. However, the only association between the use of glyphosate and cancer burden mentioned in this review was the observation of Eriksson et al. (2008, ASB2012-11614, see above).

The following epidemiological studies did not reveal an association between glyphosate and specific cancer types.

- Alavanja et al. (2003, ASB2012-11535) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigner et al. (2008, ASB2012-11917) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong et al. (2009, ASB2012-11922).
- The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study in farmers in British Columbia, Canada, by Band et al. (2011, ASB2012-11555).
- Lee et al. (2004, ASB2012-11883) reported a lack of association between glyphosate use and stomach and oesophageal adenocarcinomas.
- Carreon et al. (2005, ASB2012-11585) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel et al. (2005, ASB2012-11613) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.
- Flower et al. (2004, ASB2012-11620) reported AHS data on parental use of specific pesticides and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti et al. (2009, ASB2012-11544) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren et al. (2009, ASB2012-11875) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake et al. (2011, ASB2012-11865) reported a lack of association between

glyphosate and Hodgkin's lymphoma.

- Pahwa et al. (2011, ASB2012-11987) reported a lack of association between glyphosate and multiple myeloma.
- Schinasi and Leon (2014, ASB2014-4819) published the results of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to pesticides. Phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and lindane were positively associated with NHL. However, no association between NHL and glyphosate was reported.
- Kachuri et al. (2013, ASB2014-8030) investigated an association between lifetime use of multiple pesticides and multiple myeloma in Canadian men. Excess risks of multiple myeloma were observed among men reported to be using other pesticides such as carbamates, phenoxy herbicides or organochlorines. However, no excess risk was observed for glyphosate.
- Cocco et al. (2014, ASB2014-7523) investigated the role of occupational exposure to agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes. No increased CLL risk in relation to glyphosate became evident.
- Alavanja and Bonner (2012, ASB2014-9173) reviewed studies on occupational pesticide exposure and cancer risk. Twenty one pesticides identified subsequent to the last IARC review showed significant exposure-response associations in studies of specific cancers. No significant association was observed for glyphosate.
- El-Zaemey and Heyworth (2013, ASB2014-9473) reported a case control study on the association between pesticide spray drift from agricultural pesticide application areas and breast cancer in Western Australia. The findings support the hypothesis that a woman who ever noticed spray drift or who first noticed spray drift at a younger age had increased risk of breast cancer. However, it was not possible to examine whether the observed associations are related to a particular class of pesticides.
- Pahwa et al. (2011, ASB2014-9625) investigated the putative association of specific pesticides with soft-tissue sarcoma (STS). A Canadian population-based case-control study conducted in six provinces was used for this analysis. A higher incidence of STS was associated with the insecticides aldrin and diazinon after adjustment for other independent predictors. However, no statistically significant association between STS and exposure to glyphosate or other herbicides was observed.
- Koutros et al. (2011, ASB2014-9594) studied associations between pesticides and prostate cancer. No statistically significant positive association between pesticides and prostate cancer were observed. There was suggestive evidence on an increased risk (OR>1.0) with an increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate and methyl bromide. However, no increased risk was observed for glyphosate.

In a comprehensive review of the AHS publications and data, Weichenthal et al. (2010, ASB2012-12048) noted that increased rates in the following cancers were not associated with glyphosate use: overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukaemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, oesophagus cancer and thyroid cancer.

Mink et al. (2012, ASB2014-9617) submitted a comprehensive review of epidemiologic studies of

glyphosate and cancer. To examine potential cancer risks in humans they reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. They also reviewed relevant methodological and biomonitoring studies of glyphosate. The review found no consistent pattern of positive associations indicating a causal relationship between total cancer (in adults or in children) or any site-specific cancer and exposure to glyphosate.

Unfortunately, there was no overview table of epidemiologal studies in the RAR. However, more information is given in the addendum on carcinogenicity that is attached to this CLH report. The tables there were related to the evaluation of epidemiological studies by the IARC and have been copied into this CLH dossier, with few amendments, for the sake of transparency.

Table 43: Cohort studies which were considered in the IARC Monograph.

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Alavanja et al., 1996, ASB2015-7849	The Agricultural Health Study (AHS), large prospective cohort study	The only cohort study to date to have published findings on exposure and the risk of cancer at many different sites.	The data of this study were used in further studies. Conclusions are described there.	The AHS study was described in the RAR as basis for a number of publications.	Data of this publication were used for further studies. Conclusions on glyphosate are presented with these studies.
Alavanja et al., 2003, ASB2012-11535	Use of pesticides and prostate cancer risk (based on AHS)	No significant exposure-response association of glyphosate with cancer of prostate was found.	Agreement	Yes	No significantly increased risk of prostate cancer.
Andreotti et al., 2009, ASB2012-11544	Pesticide use and risk of pancreatic cancer (based on AHS)	The odds ratio for ever- versus never-exposure to glyphosate was 1.1 (0.6-1.7) while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (0.6-2.6)	Agreement	Yes	No significantly increased risk of pancreatic cancer.
Blair et al., 2011, ASB2015-7868	Impact of pesticide exposure misclassification on estimates of relative risks in the AHS	Nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study power.	Glyphosate was not assessed in this study.	No, no assessment of glyphosate in this study	No assessment of glyphosate in this study
Dennis et al., 2010, ASB2015-8439	Pesticide use and risk of melanoma (based on data of AHS)	Exposure to glyphosate was not associated with cutaneous melanoma within the AHS.	Agreement	No	No increased risk of melanoma.
De Roos et al., 2005a, ASB2012-11605	Cancer incidence among glyphosate-exposed pesticide applicators (based on data of the AHS)	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and of melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. For multiple myeloma the relative risk was 1.1 (0.5-2.4) when adjusted for age, but was 2.6 (0.7-9.4), when adjusted for	Agreement with the reported results and the conclusion on limited power of the study. Further discussion of multiple myeloma in this study see also re-evaluation by Sorahan (2015,	Yes	No increased risk of all cancers and of cancers in lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and of

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
		multiple confounders. The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	ASB2015-2284), below		melanoma, all lympho-haematopoietic cancers, NHL and leukaemia. Interpretation of multiple myeloma is limited.
De Roos et al., 2005b, ASB2015-8437	Response in the discussion on the study of De Roos et al., 2005a, ASB2012-11605 (see above)	The study had limited power for the analysis of multiple myeloma. Missing data limit the interpretation of the findings.	Agreement	No, the paper is no study but only a response in the discussion on study of De Roos et al., 2005a, ASB2012-11605 (see above).	See De Roos et al., 2005a, ASB2012-11605
Engel et al., 2005, ASB2012-11613	Pesticide use and breast cancer risk	No difference in incidence of breast cancer for women who reported ever applying glyphosate (odds ratio 0.9 (0.7-1.1); Women who never used glyphosate but whose husband had used (no information on duration of use): odds ratio 1.3 (0.8-1.9)	Agreement	Yes	No significantly increased risk of breast cancer.
Flower et al., 2004, ASB2012-11620	Parental pesticide application and cancer risk in children; (based on data of AHS)	"For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population." Limited power of the study for glyphosate exposure.	The cited IARC conclusion considers the risk for children of all pesticide applicators. However, this statement is not relevant for the assessment of glyphosate. There was an increased odds ratio in result of application of pesticides aldrin, dichlorvos and ethyl dipropylthiocarbamate. However, the results for glyphosate did not demonstrate any risk for childhood cancer. The odds ratios for maternal	Yes	No increased risk of childhood cancer.

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
			use and paternal use of glyphosate are even clearly below 1. Agreement with the limited power of the study.		
Landgren et al., 2009, ASB2012-11875	Pesticide exposure and risk of monoclonal gammopathy (based on data of AHS)	No association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance, a premalignant plasma disorder that often precedes multiple myeloma; odds ratio 0.5 (0.2-1.0)	The study authors conclude a nonsignificant decrease of monoclonal gammopathy of undetermined significance (MGUS), on the large data base of the AHS.	Yes	Nonsignificant decrease of risk of MGUS which usually precedes multiple myeloma
Lee et al., 2007, ASB2015-8228	Pesticide use and risk of colorectal cancer (based on data of AHS)	Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (0.9-1.6), 1.0 (0.7-1.5) and 1.6 (= 0.9-2.9) for cancers of the colorectum, colon and rectum respectively.	Agreement	No	No significantly increased risk of colorectal cancers.
Sorahan, 2015, ASB2015-2284	Glyphosate and multiple myeloma, re-analysis of AHS data; data of the study of De Roos et al., 2005a, ASB2012-11605 (see above) are reanalysed	Sorahan confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information.	The author concluded that "*this secondary analysis of AHS data does not support the hypothesis that glyphosate use is a risk factor for multiple myeloma*".	No, study was published after completion of the RAR.	No significantly increased risk of multiple myeloma based on the AHS data

Table 44: Case-control studies on Non-Hodgkin lymphoma (NHL), multiple myeloma and leukaemia which were considered in the IARC Monograph.

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Brown et al., 1990, TOX2003-999	Pesticide exposure and other agricultural risk for leukaemia	The odds ratio for glyphosate was 0.9 (0.5-1.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	No increased risk of leukaemia, limited power of the study.
Brown et al., 1993, TOX2002-1000	Pesticide exposure and multiple myeloma	The odds ratio for glyphosate was 1.7 (0.8-3.6). The study had limited power to assess effects of glyphosate.	Agreement	No, because released before 2000	Limited power of the study to assess effects of glyphosate.
Cantor et al., 1992, ASB2015-7885	Pesticides and other agricultural risk factors for non-Hodgkin lymphoma	The odds ratio for men who ever handled glyphosate was 1.1 (0.7-1.9), low power of the study to assess risk of NHL associated with glyphosate	Agreement	No, because released before 2000	No significantly increased risk of non-Hodgkin lymphoma, limited power of the study
Cocco et al., 2013, ASB2014-7523	Pesticide exposure and lymphoma risk	Odds ratio for glyphosate exposure was 3.1 (0.6-17.4); the study had a very limited power to assess the effects of glyphosate on risk of NHL	Agreement with the reported results and the conclusion on limited power of the study. Only 4 exposed cases and 2 control subjects have been considered in this study.	Yes	Very limited power of the study (only 4 exposed cases and 2 control subjects)
De Roos et al., 2003, ASB2012-11606	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Eriksson et al., 2008, ASB2012-11614	Pesticide exposure and risk of non-Hodgkin lymphoma	See separate assessment in this addendum	See separate assessment in this addendum	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Hardell and Eriksson, 1999, ASB2012-11838	Pesticide exposure and risk of non-Hodgkin lymphoma	The odds ratio for ever-use of glyphosate was 2.3 (0.4-13.4) in a univariate analysis, and 5.8 (0.6-54) in a multivariable analysis. The exposure frequency was low for glyphosate, and the study had limited power to detect an effect.	Agreement with the reported results and the conclusion on limited power of the study. Only 4 exposed cases and 3 control subjects have been considered in this study.	Yes	no conclusion possible because of limited power of the study (only 4 exposed cases and 3 control subjects)

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Hardell et al., 2002, ASB2012-11839	Pesticide exposure and risk of non-Hodgkin lymphoma and hairy cell leukaemia	The study is a pooled analysis of two case-control studies (see Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum). Increased risk was found for glyphosate only in univariate analysis (odds ratio, 3.04 (1.08-8.52)), however, the odds ration decreased in multivariate analysis to 1.85 (0.55-6.20). The exposure frequency for glyphosate was low and the study had limited power.	Agreement with the presented results and the conclusion on limited power of the study. The study is a pooled analysis of two case-control studies (see separate discussion on studies of Hardell and Eriksson, 1999, TOX1999-686, ASB2012-11838 and Nordström et al., 1998, TOX1999-687 in this addendum).	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Kachuri et al., 2013, ASB2014-8030	Pesticide exposure and risk of multiple myeloma	The odds ratio for ever-use of glyphosate was 1.19 (0.76-1.87); no association was found for light users (≤ 2 days per year, odds ratio 0.72 (0.39-1.32), the odds ratio in heavier users (>2 days per year) was 2.04 (0.98-4.23). The study had relatively low response rates.	Agreement	Yes	No increased risk of multiple myeloma for ever use of glyphosate, higher (not significant) OR if mixing or applying glyphosate >2 days per year, low response rate
Karunanayake et al., 2012, ASB2012-11865	Pesticide exposure and risk of non-Hodgkin lymphoma	Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (0.74-1.76) adjusted for age and province, and 0.99 (0.62-1.56) when additionally adjusted for medical history variables.	Agreement	Yes	No increased risk of non-Hodgkin lymphoma
Lee et al., 2004a, ASB2015-8238	Pesticide exposure and risk of non-Hodgkin Lymphoma among asthmatics	Subject with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics. The odds ratio associated with glyphosate use was 1.4 (0.98-21.) among non-asthmatics and 1.2 (0.4-3.3) among asthmatics.	Agreement	No	No significantly increased risk of non-Hodgkin lymphoma for asthmatics and non-asthmatics; non-significantly lower risk of NHL for asthmatics than non-asthmatics

Study (Author/year)	Subject	Evaluation by IARC	Comment by RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
McDuffie et al., 2001, ASB2011-364	Pesticide exposure and risk of non-Hodgkin lymphoma	Odds ratio of 1.26 (0.87-1.80) and 1.20 (0.83-1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with 2+ days of exposure per year had an odds ratio of 2.12 (1.2-3.73) compared with those with some but ≤ 2 days of exposure. The study was large, but had relatively low participation rates.	See separate assessment in this addendum	Yes	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Nordström et al., 1998, TOX1999-687	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia	An age-adjusted odds ratio of 3.1 (0.8-12) was observed for exposure of glyphosate. However, the study had limited power, only 4 exposed cases and there was no adjustment for other exposures.	Agreement with reported results and conclusions on limited power, only 4 exposed cases and 5 exposed controls are considered in this study	Yes	Limited power of the study (only 4 exposed cases and 5 exposed controls)
Orsi et al., 2009, ASB2012-11985	Pesticide exposure and risk of lymphoid neoplasms	The odds ratios associated with any exposure to glyphosate were 1.2 (0.6-2.1) for all lymphoid neoplasms, 1.0 (0.5-2.2) for NHL, 0.6 (0.2-2.1) for lymphoproliferative syndrome, 2.4 (0.8-7.3) for multiple myeloma, and 1.7 (0.6-5.0) for Hodgkin lymphoma.	Agreement with reported results. It should be considered in the discussion on an association between glyphosate and NHL that the OR of NHL in this study (12 exposed cases and 24 exposed controls) was 1.0.	No	Please refer to Table 2.2-2 given in Addendum 1 to RAR, 2015
Waddell et al., 2001, ASB2015-8037	Use of organophosphate pesticides and risk of non-Hodgkin lymphoma	IARC compared the numbers of cases and controls in this study with the study of De Roos et al., 2003; however, no information on glyphosate in this study	No information on glyphosate	No, no information on glyphosate	no information on glyphosate
Zahm et al., 1990, ASB2013-11501	Exposure to 2,4-D and risk of non-Hodgkin Lymphoma	The study was mentioned by IARC because data were used in the study of De Roos et al., 2003	No information on glyphosate	No, no information on glyphosate	no information on glyphosate

Table 45: Case-control studies on other cancer types and meta-analyses which were considered in the IARC Monograph.

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
Lee et al., 2004b, ASB2012-11883	Pesticide use and risk of adenocarcinomas of stomach and oesophagus	For ever use of glyphosate, the odds ratio was 0.8 (0.4 - 1.4) for cancer of the stomach, and 0.7 (0.3 - 1.4) for oesophageal cancer; the power of the study was limited.	Agreement	Yes	No increased risk of adenocarcinomas of stomach and oesophagus
Ruder et al., 2004, ASB2015-8078	Pesticide exposure and risk of gliomas	No association was found with any of the pesticides assessed, including glyphosate. Glyphosate use was assessed, but specific results were not presented.	Agreement	No	No increased risk of gliomas
Carreon et al., 2005, ASB2012-11585	Pesticide exposure and risk of gliomas	There was a reduced risk for glyphosate (OR 0.7 (0.4 - 1.3).	Agreement	Yes	Reduced risk of gliomas
Lee et al., 2005, ASB2012-11882	Pesticide use and risk of gliomas	There was a non-significant excess risk with glyphosate use for the overall group, but there was inconsistency between observations for self-responds and observations for proxy respondents. The study had limited power to detect an effect of glyphosate use and was difficult to interpret.	Agreement	Yes	Limited power of the study, difficult to interpret
Pahwa et al., 2011, ASB2014-9625	Pesticide exposure and risk of soft-tissue sarcoma	The fully adjusted odds ratio for glyphosate was 0.90 (0.58 - 1.40).	Agreement	Yes	No increased risk of soft-tissue sarcoma
Monge et al., 2007, ASB2012-11909	Pesticide exposure and risk of childhood leukaemia	Association of childhood cancer with glyphosate were reported only for an “other pesticides” category that also included other chemicals, glyphosate was not specifically assessed.	Agreement	Yes	No specific assessment of glyphosate
Schinasi and Leon, 2014, ASB2014-4819	Meta-analysis, exposure to pesticides and non-Hodgkin lymphoma	The meta-analysis for glyphosate included six studies and yielded a meta-risk ratio of 1.5 (1.1 - 2.0). The working group noted that the most fully adjusted risk estimates from the	Agreement, see separate assessment in this addendum (section 2.4).	Yes	See separate assessment in this addendum (section 2.4).

Study (Author/year)	Subject	Evaluation IARC	Comment RMS on IARC evaluation	Study reported in RAR Draft April 2015	Final conclusion of RMS, considering IARC evaluation
		articles by Hardell et al. (2002, ASB2012-11839) and Eriksson et al. (2008, ASB2012-11614) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta-risk-ratio of 1.3 (1.03 - 1.65).			

OR, odds ratio

4.9.3 Other relevant information

In the IARC Monograph, oxidative stress was discussed as a possible mechanism of carcinogenicity. For detailed mechanistic information on e.g. oxidative stress please refer to the addendum to the RAR or to the RAR, that are both attached to this CLH report. However, with regard to oxidative stress it was concluded in the addendum that from the sole observation of oxidative stress and the existence of a plausible mechanism for induction of oxidative stress through uncoupling of mitochondrial oxidative phosphorylation alone, genotoxic or carcinogenic activity in humans cannot be deduced for the active substance glyphosate and glyphosate based formulations.

4.9.4 Summary and discussion of carcinogenicity

For glyphosate, a large quantity of animal data regarding carcinogenicity was submitted by different applicants and is partly also available from published scientific literature. At least six acceptable chronic toxicity and carcinogenicity studies in rats and five carcinogenicity studies in mice have been evaluated. Therefore, all available data were considered together using a weight of evidence approach with consideration of the biological significance, dose response, relationship of the highest doses used to the maximum tolerated dose and the consistency of the neoplastic findings among the studies.

In the rat, no evidence of carcinogenic effects was evident and only occasional increases in few different tumour types (pancreas, liver, thyroid, and testes) were observed in two older studies which one is considered not acceptable any longer if current standards are applied. These findings were not confirmed in five more recent, guideline-compliant studies employing very high dose levels. Moreover, the pancreatic tumours did not show a dose response. When the whole toxicological profile of glyphosate is taken into consideration, the pancreas, the thyroid and the testes were no target organs of this substance and liver effects of glyphosate were very limited. The overall conclusion can be drawn that glyphosate was not carcinogenic to the rat.

In the mouse, the incidences in malignant lymphoma, in renal tumours and haemangiosarcoma in male animals were considered in detail. Slightly higher incidences when compared with concurrent controls were confined to very high dose levels above the OECD-recommended limit dose of 1000 mg/kg bw/day and exceeding the MTD. In addition, the outcome of statistical tests was contradictory. Mostly, but not always, trend tests revealed statistical significance but pairwise comparisons failed to detect a significant difference relative to the control group. The reported incidences of all three tumour types fell within their historical control range which were, however, of variable reliability. If the four studies in CD-1 mice are considered together, it becomes apparent that all tumours were observed also in the control groups and in some groups receiving lower doses in at least one concurrent study. Furthermore, the results were not consistent with regard to dose responses. To conclude, there is not enough evidence to consider the tumours in mice as treatment-related.
Epidemiological studies revealed partly contradictory results. However, in most studies, no association with an exposure to glyphosate could be established. In particular, the largest study, i.e., the AHS (see above), was negative. Taken together, the epidemiological data does not provide convincing evidence that glyphosate exposure in humans might be related to any cancer type. Epidemiological studies are of limited value for detecting the carcinogenic potential of an active substance in plant protection products since humans are never exposed to a single compound alone. Thus, the results of the studies are associated to different formulations containing glyphosate or mixtures of different active substances.

4.9.5 Comparison with criteria

The following criteria for classification as a carcinogen are given in CLP regulation:

CLP regulation

A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.
The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:

— human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or
— animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals.

The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.
[…]
3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows:

(a) Carcinogenicity in humans
The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

— sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence;
— limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

(b) Carcinogenicity in experimental animals
Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.
The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

— sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;
— limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

CLP regulation
3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here. 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner. 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity *in vivo* may indicate that a substance has a potential for carcinogenic effects.

General remark: For the majority of chemical substances evaluated under the CLP-Regulation, normally one study addressing each endpoint is required and usually sufficient for classification and labelling purposes. In contrast, for glyphosate, a large quantity of animal data regarding carcinogenicity was submitted by different applicants and at least six acceptable chronic toxicity and carcinogenicity studies in rats and five carcinogenicity studies in mice have been evaluated. In such a situation, the criteria of the CLP-Regulation may not be applicable directly to the available information for glyphosate. Instead, all available data should be considered together using a weight of evidence approach with consideration of the biological significance, relationship of the applied doses to the maximum tolerated dose and the consistency of the neoplastic findings. Basing any conclusion only on the statistical significance of an increased tumour incidence identified in a single study should be avoided.

Category 1A is not applicable since epidemiological studies do not suggest a strong link of glyphosate exposure to human cancer. In most studies, including the by far largest one, no association could be established. The DS concluded in accordance with IARC (2015) „*There is limited evidence in humans for the carcinogenicity of glyphosate.*" This is perhaps the best description of the available data since the other IARC categories ("*Evidence suggesting lack of carcinogenicity*"; "*Inadequate evidence of carcinogenicity*"; "*Sufficient evidence of carcinogenicity*") are even less suitable.

Category 1B is also not applicable since experimental evidence in laboratory animals is far from being "sufficient". Furthermore, the active substance glyphosate is devoid of genotoxic potential.

In the rat, tumours were only occasionally seen. For pancreatic tumours, no dose response became apparent in the two studies in which an increase was observed (Lankas, 1981, TOX2000-595,

TOX2000-1997; Stout and Ruecker 1990, TOX9300244). Moreover, these tumours could not be reproduced in any other long-term study. The same holds true for liver and thyroid tumours that were found in one and the same study (Stout and Ruecker 1990, TOX9300244) at the highest dose level. For a substance such as glyphosate for which a large number of independent studies is available, reproducibility is crucial. An increase in testicular tumours in an old and rather deficient study (Lankas, 1981, TOX2000-595, TOX2000-1997) was clearly a chance event since they occurred at a relatively low dose level but were not seen in six other valid studies in which much higher doses were administered. Thus, carcinogenicity to rats can be excluded with a high degree of certainty.

In the mouse, the situation is slightly different and three tumour types were considered in detail.

First, the slightly higher incidences in the rather common malignant lymphoma in three studies (Sugimoto, 1997, ASB2012-11493; Kumar, 2001, ASB2012-11491; Wood et al., 2009, ASB2012-11490) were not considered to be treatment-related when a weight of evidence approach was taken. The very different dose levels in all the studies and the dose-specific incidences were included as well as the high variability in spontaneous occurrence of this tumour type and also the statistical uncertainties.

Renal tumour incidences and haemangiosarcoma incidences in male mice from three or two out of five studies, respectively, were slightly higher when compared to concurrent controls at very high dose levels at or exceeding the OECD-recommended limit of 1000 mg/kg bw/day and sometimes being above the MTD. Statistical significance was only observed with a trend test but not in pair-wise tests. Furthermore, the low incidences even at high doses fell within the historical control ranges and the findings were not consistent among the acceptable studies in mice. Thus, these findings were considered not of relevance for assessment of carcinogenicity.

Category 2 is also not applicable based on haemangiosarcoma incidences and the respective dose response considerations. In addition to being in the historical control range, this tumour type was also seen in the control and treated groups in other studies with glyphosate (Kumar, 2001, ASB2012-11491; Wood et al., 2009, ASB2012-11490), without evidence of a dose response relationship. The difference between these figures and the incidence at the top dose levels in two studies (Atkinson et al., 1993, TOX9552382; Sugimoto, 1997, ASB2012-11493) is small or missing (1 or 2 vs. 4 and 2; see Table 42). Statistical significance with the trend test may be explained by the zero incidence in concurrent controls in the studies by Atkinson et al. (1993, TOX9552382) or Sugimoto (1997, ASB2012-11493). Furthermore, there was no increase in the Sugimoto study even though the dose level was by more than four times higher than applied by Atkinson et al. (1993, TOX9552382).

With regard to the incidences in kidney tumours in the studies by Knezevich and Hogan (1983, TOX9552381) and Sugimoto (1997, ASB2012-11493) at the top dose level, it should be noticed, on one hand, that the MTD was exceeded and, on the other hand, that a similar incidence of renal tumours (2 vs. 3 or 2) had been seen in the study by Atkinson et al. (1993, TOX9552382) in both the control and low dose group (see Table 42). Furthermore, no pre-neoplastic kidney lesions have been observed in treated animals, even at excessive dose levels. Thus, also for this tumour type, there is no convincing evidence that it is related to glyphosate administration.

On balance, this inconsistent data is not sufficient for classification and labelling of glyphosate as a category 2 carcinogen.

Based on the available data no mode of action could be identified. Mechanistic data, e.g., providing evidence of oxidative stress are partly contradictory but should not be given much weight in a situation where a very comprehensive database of high quality long-term studies in laboratory animals is available.

4.9.6 Conclusions on classification and labelling

Based on the epidemiological data as well as on data from long-term studies in rats and mice, taking a weight of evidence approach, no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria.

4.10 Toxicity for reproduction

4.10.1 Effects on fertility

4.10.1.1 Non-human information

The reproductive toxicity of glyphosate was tested in a large number of two-generation studies in rats of which 6 may be considered fully valid or at least supplementary from a current point of view. These studies are summarised in Table 46, along with a (deficient) three-generation study.

The DS is aware of three further reproduction studies which have been referred to in an older EU evaluation (Germany, 1998, ASB2010-10302). No adverse effects were reported in any of these studies but they are not considered to be suitable for the purpose of classification and labelling. In three-generation studies by Schroeder and Hogan (1981, TOX9552385) and by Bhide (1988a, TOX9551965), the top dose levels of 30 or approx. 15 mg/kg bw/day were much too low and could not be expected to reveal any toxic effect. The same holds true for a non-guideline "segment I" study with gavage administration of up to 10 mg/kg bw/day by Bhide (1988b, TOX9551832). A published reproduction study (Dallegrave et al., 2007; ASB2012-2721) was performed with a commercial formulation and, thus, is also not useful for classification and labelling of the active substance.

Table 46: Reproductive (two-generation) studies with glyphosate in rats

Reference; Study identification; Purity; Owner	Study type, strain, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Dhinsa et al., 2007; ASB2012-11494; 95.7%; Nufarm	Two-gen., Sprague-Dawley, diet	0, 1500, 5000, 15000 ppm	Parental, reproductive, offspring: 5000 ppm (351 mg/kg bw/d)	Parental, reproductive, offspring: 15000 ppm (1000-1600 mg/kg bw/d)	Parental.: liver, kidney wt↑; Repro: homogenisation resistant spermatid count↓; Off-spring: delay in preputial separation in F1 males
Moxon, 2000; TOX2000-2000; 97.6%; Syngenta	Two-gen., Wistar-derived AlpK, diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm (293 mg/kg bw/d); Reproductive: 10000 ppm (985 mg/kg bw/d)	Parental, offspring: 10000 ppm (985 mg/kg bw/d); Reproductive: not established	Parental, offspring: bw↓ (F1 pups & F1-adults)
Takahashi, 1997; ASB2012-11495; 94.61%; Arysta	Two-gen., Sprague-Dawley,	0, 1200, 6000, 30000 ppm	Parental, offspring: 6000 ppm (417 mg/kg bw/d);	Parental, offspring: 30000 ppm	Parental: loose stool, bw↓, caecum distention, organ wt

Reference; Study identification; Purity; Owner	Study type, strain, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
	diet		Reproductive: 30000 ppm (>2000 mg/kg bw/d)	(>2000 mg/kg bw/d); Reproductive: not established	changes; Offspring: bw↓, caecum distention
Suresh, 1993*; TOX9300009; 96.8%; ADAMA	Two-gen., Wistar rat, diet	0, 10, 100, 1000, 10000 ppm	Parental, offspring & reproductive 10000 ppm (700-800 mg/kg bw/d)	-	No treatment related effects
Brooker et al., 1992**; TOX9552389; 99.2%; Cheminova	Two-gen., Sprague-Dawley, diet	0, 1000, 3000, 10000 ppm	Parental, offspring: 3000 ppm (197 mg/kg bw/d); reproductive: 10000 ppm (668 mg/kg bw/d)	Parental, offspring: 10000 ppm (668 mg/kg bw/d); Reproductive: not established	Parental, offspring: bw↓, food & water ↑, cellular alterations of salivary glands in F0/F1 m/f
Reyna, 1990; TOX9552387; 97.67%; Monsanto	Two-gen., Sprague – Dawley rat, diet	0, 2000, 10000, 30000 ppm	Parental, offspring & reproductive: 10000 ppm (720-760 mg/kg bw/d)	Parental, offspring & reproductive: 30000 ppm (~2000 mg/kg bw/d)	Parental: bw gain↓, soft stool; Reproductive: litter size ↓(equivocal); Offspring: bw gain↓
Antal, 1985***; Alkaloida	Three-gen., CD rat, diet	0, 200, 1000, 5000 ppm	Parental, offspring & reproductive: 5000 ppm (462-502 mg/kg bw/d)	-	No treatment related effects

*supplementary study since dose levels might have been too low and no effects were seen at all
**supplementary range-finding one generation study (Brooker et al., 1991, TOX9552388) also available but without impact on classification and labelling (see attached RAR)
***study not valid according to current standards because of major reporting deficiencies

It should be explained here that the "main effects" were statistically significant if body weight and organ weights or reproductive parameters (apart from reduced litter size in the study by Reyna, 1990, TOX9552387) were affected. Clinical signs or macroscopic findings were also reported when occurring in a higher number of animals as in the control group but were not always subject to statistical evaluation or did not gain statistical significance in all cases. Not all of the mentioned findings were observed necessarily at the LOAEL but sometimes only at higher dose levels. In any case, statistical significance was taken into account when the NOAELs/LOAELs in the individual studies were established.

Parental toxicity was confined to minor effects at high dose levels only. Sometimes, the findings were not consistent among the studies. The cellular alterations in parotid (males and females) and submaxillary (females only) salivary glands in F0 and F1 animals as known before from subchronic and long-term studies were reported only by Brooker et al. (1992) and in the preceding range-finding experiment but were presumably not investigated in the other studies. In addition to these histological findings, high dose (approx. 670 mg/kg bw/day) parental effects comprised gastrointestinal disturbances and a decrease in body weight whereas food and water consumption were increased.

Dhinsa et al. (2007, ASB2012-11494) observed higher absolute and relative organ weights of the liver (F0 & F1 females) and the kidneys (F0 females) at the highest dose level of 15000 ppm (1000 – 1600 mg/kg bw/day). The same effect on organ weights had been reported by Takahashi (1997) in F0 and F1 animals of both sexes, along with decreased prostate weight (F1), loose stool (F0/F1, both sexes), reduced body weight (F0/F1 males) and caecum distention (F0/F1, both sexes). All these findings, however, were confined to an exaggerated dose of 30000 ppm (>2000 mg/kg bw/day). At the same, very high dietary dose, a reduction in body weight gain and gastrointestinal effects (soft stool) had been described in adult animals in the earliest reproduction study by Reyna (1990, TOX9552387).

No evidence of reproductive toxicity was observed in any of these studies apart from a rather equivocal reduction in litter size in the study by Reyna (1990, TOX9552387) at a dose level of more than 2000 mg/kg bw/day. In the two litters produced by the F0 generation, a non-significant reduction by up to 10% was observed which was less pronounced in the F1. This dose is far above any limit dose and, furthermore, a lower litter size was not confirmed in the study by Takahashi (1997, ASB2012-11495) in which the same dietary concentration of 30000 ppm had been tested. A decrease in homogenisation resistant spermatids in the Cauda epididymidis has been observed by Dhinsa et al. (2007, ASB2012-11494) after administration of 15000 ppm but had no impact on fertility or reproductive success and, thus, was of questionable relevance. This reduction (Control: 399.9 million/gram; 15000 ppm: 309.0 million/gram) was noted in F0 males but was not reproducible at any dose levels in F1 males.

Weak effects on the offspring were indicated by a reduced pup weight or weight gain in most studies but were confined to very high, parentally toxic dose levels. In addition, a significant delay in sexual maturation in male pups (F1) became apparent at the top dose level of 15000 ppm (~1000 mg/kg bw/day) in the study by Dhinsa et al. (2007, ASB2012-11494) because preputial separation was delayed, occurring after 45.9 days on average versus 43.0 days in the control group. At attainment of sexual maturation as indicated by preputial separation, the mean bodyweight of the male pups was 230 g as compared to 210 g in the control group. This effect was not related to a decrease in the bodyweight and bodyweight gain of the male pups (followed up to day 21). A treatment-related effect on the sexual development of male offspring cannot be excluded although this later onset of sexual maturation had no impact on subsequent reproductive performance. It is important to note that this finding occurred at the limit dose at which parental toxicity was also apparent. Furthermore, it was not confirmed in any of the other reproduction studies.

In summary, rigorous testing of glyphosate up to very high doses in a number of comprehensive studies did not provide evidence of reproductive or offspring toxicity. The few observed effects were small, of equivocal relevance and confined to parentally toxic dose levels. There is no need for classification for effects on sexual function and fertility, based on the animal studies.

4.10.1.2 Human information

Several epidemiological studies are available in which a possible impact of glyphosate exposure on reproductive outcome was investigated. Parameters under study comprised fecundity, miscarriage, pre-term delivery, gestational diabetes mellitus, birth weights, congenital malformations, neural tube defects, or the occurrence of attention-deficit disorder / attention-deficit hyperactive disorder (ADD/ADHD) in children. In most instances, glyphosate and reproductive outcomes lack a statistically significant positive association, as described in a recent review of glyphosate non-cancer endpoint publications (Mink et al., 2011, ASB2012-11904). For ADD/ADHD, a positive association with glyphosate use had been claimed by Garry et al. (2002, ASB2012-11626) but the reported incidence of approx. 1 % in the study population was well below the general population incidence rate of approx. 7 %.

For more information, see Vol. 3 of the attached RAR.

In general, the relevance of epidemiological data to detect effects of glyphosate on fertility or reproductive performance is quite limited. This is mainly due to the fact that operators, bystanders, or residents are exposed to plant protection products containing glyphosate but not to the active substance itself. Furthermore, there is always mixed exposure to a variety of chemicals in the environment or to their residues in our diet. The extent of exposure is mostly unknown.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

The developmental toxicity and teratogenicity of glyphosate were tested in a great number of studies in rats and rabbits.

Rat

The available valid (guideline-compliant) developmental studies in rats are summarised in Table 47 whereas the few published studies are briefly mentioned below.

Table 47: Developmental toxicity studies in rats

Reference; Study identification; Purity; Owner	Strain, route, duration of treatment	Dose levels	NOAEL	LOAEL	Targets / Main effects
Moxon, 1996; ASB2012-10080; 95.6%; Syngenta	Alpk (Wistar derived), gavage, d 7-16 p.c.	0, 250, 500, 1000 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Not applicable	None
Hatakenaka, 1995 ASB2012-11497; 95.68%; Arysta	CD (SD), gavage, d 6-15 p.c.	0, 30, 300, 1000 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal: Loose stool Development: skeletal anomalies↑
Brooker et al., 1991, TOX9552393; 98.6%; Cheminova	CD, gavage, d 6-15 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 300 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal: slight bw gain↓, noisy respiration (2/25); Development: ossification↓, skeletal anomalies
Suresh, 1991, TOX9551105; 96.8%; ADAMA	Wistar, gavage, d 6-15 p.c.	0, 1000 mg/kg bw/d	Maternal: 1000 mg/kg bw/d; Developmental: <1000 mg/kg bw/d	Maternal: not applicable; Developmental: 1000 mg/kg bw/d	Maternal: no effects; Development: ossification↓
Tasker and Rodwell, 1980; TOX9552392; 98.7%; Monsanto	Charles River, gavage, d 6-19 p.c.	0, 300, 1000, 3500 mg/kg bw/d	Maternal & developmental: 1000 mg/kg bw/d	Maternal & developmental. 3500 mg/kg bw/d	Maternal:mortality, soft stool, diarrhea; Development: bw↓, post-implantation losses
Anonym (Author perhaps Antal), 1981; TOX9650160; purity 96.8%; Alkaloida	CFY, diet, d 6-18 p.c.	Calculated to be 0, 22, 103, 544 mg/kg bw/d	Maternal & developmental: 544 mg/kg bw/d	Not applicable	None

It should be explained here that the "main effects" were statistically significant if body weight and organ weights or developmental parameters were affected. Clinical signs were also reported when occurring in a higher number of animals as in the control group but were not always subject to statistical evaluation or did not gain statistical significance in all cases. Not all of the mentioned findings were observed necessarily at the LOAEL but sometimes only at higher dose levels. In any case, statistical significance was taken into account when the NOAELs/LOAELs in the individual studies were established. The same holds true for the studies in rabbits addressed below.

More recently, a developmental toxicity study in outbred Wistar-RIZ rats was published by Chruścielska et al. (2000b, ASB2013-9831). Glyphosate (source and purity not given) was administered to 20 pregnant females per group by oral gavage from day 7 through day 14 of pregnancy at dose levels of 750, 1500 or 3000 mg/kg bw/day. No evidence of maternal or developmental toxicity was observed but reporting of this study was so brief that its quality cannot be assessed.

A further developmental study in Wistar rats was performed by Bhide (1986, TOX9551834) in which no signs of maternal or developmental toxicity were observed up to the highest dose level of 500 mg/kg bw/day but that study was flawed by many deficiencies putting its validity and reliability into question.

Another published developmental study (Dallegrave et al., 2003, ASB2012-11600) was performed with a commercial formulation and, therefore, is not suitable for classification and labelling of the active substance.

Thus, evaluation of glyphosate for a developmental toxicity and possible teratogenicity to rat foetuses is based on the six studies which are compiled in Table 43.

Severe maternal effects (mortality) were confined to the exaggerated dose of 3500 mg/kg bw/day in the study by Tasker and Rodwell (1980, TOX9552392). Up to the limit dose of 1000 mg/kg bw/day there were only rather weak effects such as gastrointestinal signs or a lower body weight gain.

Likewise, no teratogenic potential was seen in these studies. The lowest NOAEL for developmental effects was 300 mg/kg bw/day and the LOAEL was 1000 mg/kg bw/day, based on the studies by Brooker et al. (1991, TOX9552393) and Hatakenaka (1995, ASB2012-11497). In the first study, evidence of delayed ossification and increased incidence of foetuses with skeletal anomalies was observed at 1000 mg/kg bw/day whereas a slight increase in lumbar ribs (11 out of 7 litters compared to 4 out of 2 litters in control animals) was observed in the second. With regard to the single dose study by Suresh (1991, TOX9551105), it was acknowledged that a developmental NOAEL could not be established. At the same dose level, a higher incidence of delayed ossification (caudal vertebral arch, forelimb proximal & hindlimb distal phalanges) was observed and considered adverse, despite the fact that delayed ossification of other parts of the skeleton (skull) was more frequently seen in the control. However, these findings are not of concern because a robust NOAEL for developmental toxicity well below this high dose was established in the other studies.

These previously submitted studies did not show any teratogenic potential in rats. At the very high dose level of 3500 mg/kg bw/day causing maternal toxicity and in one study even mortality, post-implantation loss and both skeletal variations and retardations were observed (Brooker et al., 1991, TOX9552393; Tasker and Rodwell, 1980, TOX9552392). In the most recent study by Moxon (1996, ASB2012-10080), no effects were seen up to 1000 mg/kg bw/day, i.e., the highest dose tested.

No effects were seen in dams or in foetuses when the test substance was administered up to a daily

dose of more than 500 mg/kg bw/day (approx. 10000 ppm) via the diet (Anonym, author perhaps Antal, 1981, TOX9650160).

In summary, the rat studies revealed only slight developmental effects which were confined to very high and already maternally toxic dose levels.

Rabbit

For assessment of developmental toxicity of glyphosate in rabbits, seven studies by oral gavage are available of which one (Bhide and Patil, 1989, TOX9551960) is flawed by serious deficiencies and may be considered with strong reservations only. The studies are summarised in Table 48.

Table 48: Developmental toxicity studies with glyphosate in rabbits

Reference; Study identification; Purity; Owner	Strain, duration of treatment, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Coles and Doleman, 1996; ASB2012-11499; 95.3%; Nufarm	NZW rabbit, d 7-19 p.c., gavage	0, 50, 200, 400 mg/kg bw/d	Maternal & developmental: 50 mg/kg bw/d	Maternal & developmental: 200 mg/kg bw/d	Maternal: mortality (2 deaths at top dose), bw gain↓; Development: post-implantation loss
Moxon, 1996; TOX2000-2002; 95.6%; Syngenta	NZW rabbit, d 8-20 p.c., gavage	0, 100, 175, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Developmental: 300 mg/kg bw/d	Maternal: food intake and bw gain ↓, clinical signs; Development: foetal wt ↓, ossification retarded
Hojo, 1995, ASB2012-11498; 97.56%; Arysta	Japanese White rabbits (Kbl:JW), d 6-18 p.c., gavage	0, 10, 100, 300 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: 300 mg/kg bw/d	Maternal: 300 mg/kg bw/d; Developmental: not applicable	Maternal: mortality (1 death), loose stool, abortion; Development: none
Suresh et al., 1993*; TOX9551106; 96.8%; ADAMA	NZW rabbit, d 6-18 p.c., gavage	0, 20, 100, 500 mg/kg bw/d	Maternal: 20 mg/kg bw/d; Developmental: 100 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: not established due to low number of foetuses at top dose	Maternal: mortality (4 deaths at mid and 8 at high dose), soft/liquid stool; Development: no clear-cut effects up to 100 mg/kg bw/d (high dose group excluded due to low number of foetuses and litters)
Brooker et al., 1991; TOX9552391; 98.6%; Cheminova	NZW rabbit, d 7-19 p.c., gavage	0, 50, 150, 450 mg/kg bw/d	Maternal: 50 mg/kg bw/d; Developmental:1 50 mg/kg bw/d	Maternal: 150 mg/kg bw/d; Developmental: 450 mg/kg bw/d	Maternal: mortality (1 at top dose), clinical signs (GI-tract), food intake and bw gain ↓; Development: late embryonic death, post implantation loss, cardiac malformations
Bhide & Patil, 1989**; TOX9551960; Lot 38, 95%;	NZW rabbit, d 6-18 p.c., gavage	0, 125, 250, 500 mg/kg bw/d	Maternal & developmental: 250 mg/kg bw/d	Maternal & developmental: 500 mg/kg bw/d	Maternal: food intake and bw↓, abortion; Development: dead foetuses, malformations

Reference; Study identification; Purity; Owner	Strain, duration of treatment, route	Dose levels	NOAEL	LOAEL	Targets / Main effects
Barclay, Luxan					(external, visceral & skeletal)
Tasker et al., 1980*; TOX9552390; 98.7%; Monsanto	Dutch Belted rabbit, d 6-27 p.c., gavage	0, 75, 175, 350 mg/kg bw/d	Maternal: 75 mg/kg bw/d; Developmental: 175 mg/kg bw/d	Maternal: 175 mg/kg bw/d; Developmental: not established due to low number of foetuses	Maternal: mortality (1 death at mid, 7 at high dose), soft stool, diarrhea; Development: none up to 175 mg/kg bw/d (high dose group excluded due to low number of foetuses and litters)

* supplementary study since high dose group could not be evaluated for developmental toxicity/teratogenicity

** study with serious deficiencies in conduct and reporting

In addition, the DS is aware of a single study with dietary administration of glyphosate (purity 96.8%, source most likely Alkaloida) to pregnant NZW rabbits. In this poorly reported study (Anonym, author perhaps Antal, 1981, TOX9650160), the test material was fed from gestation day 6 through 19 at three different dietary concentrations corresponding to daily intakes of 10.5, 50.7 or 255.3 mg/kg bw. Maternal toxicity was not observed. Likewise, there were no malformations noted and foetal weight was not affected. However, there was an increase in foetal losses at the two upper dose levels even though there was no the clear dose response (6.06 or 7.03% as compared to 0.93 or 0.79% in the control or low dose groups, respectively) that one would expect if the effect was really treatment-related. From the brief description, it appears that these findings were mostly post-implantation losses and, thus, would be somehow in line with what was observed in guideline-compliant gavage studies.

No published developmental studies in rabbits are available.

Excessive maternal toxicity became apparent mainly by a number of unscheduled, treatment-related deaths in 5 out of 7 studies in dose range from 100 to 500 mg/kg bw/day. In two studies (Tasker et al., 1980, TOX9552390; Suresh et al., 1993, TOX9551106), nearly one half of top dose animals was affected resulting in the loss of these dose groups for evaluation of developmental and teratogenic effects in foetuses. Mortality among pregnant does has been used to justify the proposal for classification of glyphosate for STOT RE and was therefore discussed in the respective section (see Table 18). Maternal toxicity was further characterised by gastro-intestinal clinical signs and reductions in food consumption and body weight or body weight gain. Sometimes, abortions were noted of which it is not clear whether they were due to maternal or instead to foetotoxicity. In any case, it must be acknowledged that all developmental findings in foetuses occurred in a dose range that was clearly toxic to the does even though there were differences among the studies with regard to severity of maternally toxic effects.

In spite of evident maternal toxicity, no developmental effects were observed in the study by Hojo (1995, ASB2012-11498) up to the top dose level of 300 mg/kg bw/day and in the study by Tasker et al. (1980, TOX9552390) up to the mid dose of 175 mg/kg bw/day, i.e., the highest dose at which foetuses could be evaluated. The other five studies deserve more detailed description since, here, developmental effects have been observed.

- In the study by Coles and Doleman (1996, ASB2012-11499), an increase in post-implantation losses was observed at the two upper dose levels, i.e., in the presence of

maternal toxicity. The numbers of affected does were 10/15 at the mid dose and 9/15 at the high dose level as compared to 4/14 in the control group and 4/18 at the low dose level. In contrast, there was no increase in morphological anomalies.

- The study by Moxon (1996, TOX2000-2002), in contrast, revealed different developmental effects. Reduced foetal body weight and retarded ossification were observed at 300 mg/kg bw/day, again in the presence of maternal toxicity. No evidence of teratogenicity was obtained.
- The study by Suresh et al. (1993, TOX9551106) was compromised by high maternal mortality. During treatment, 4 does of the mid and 5 females in the top dose group died. In addition, further three high dose females died after scheduled cessation of substance administration. In principle, the premature death of more than one half of the pregnant rabbits at the high dose level would have required immediate termination of this group. From the beginning of the experiment, there were less does in the treated groups than in the control (15 to 17 mated females vs. 26). Together with the animal losses and a case of complete litter resorption, this difference resulted in a very low number of litters and foetuses from the highest dose group that were available for teratological examination at scheduled sacrifice. An overview of foetal findings is given in Table 49.

  The percentage of foetuses with ‘dilated heart’ was significantly increased at all dose levels. The diagnosis ‘dilated heart’ was not defined in the study report and neither criteria for this diagnosis nor any measurements of the heart and its size were provided. Because of the low number of foetuses and litters, it is hardly possible to interpret any of the results obtained in the top dose group. If only the low and mid dose group are considered and compared to the controls, the absolute number of foetuses and litters with ‘dilated heart’ was quite small and did not show a difference between the two groups although the dose applied to mid dose females was by five times higher. Thus, there was no clear dose response even though just this would be expected if it was a treatment-related effect.

  In the presence of severe maternal toxicity, there was also a slight increase in the percentage of foetuses with extra 13^{th} rib.

  In summary, the study results do not allow meaningful assessment developmental effects for the highest dose level. If assessment is confined to the low and mid dose levels, there was no clear evidence of foetotoxicity or teratogenicity because the finding ‘dilated heart’ was not really substantiated in the study report and because of the lacking dose response.

Table 49: Foetal findings in the study by Suresh et al. (1993, TOX9551106)

Dose group (mg/kg bw/day)	0	20	100	500
Percentage of foetuses with 'dilated heart'	0.0	5.1*	5.2*	17.9*
No. affected/total number of foetuses examined	-	4/78	4/77	5/28
Litters affected/no. of litters	-	3/13	2/12	2/6
Foetuses with major visceral malformations	4/133	6/78	6/77	8/28
Percentage of foetuses with extra 13th rib	0.0	1.3	2.6	3.6*

* statistically significant, p≤0.05

- The study by Brooker et al. (1991, TOX9552391) was of particular relevance since evaluation of developmental effects was feasible also at the top dose level of 450 mg/kg bw/day since the number of foetuses and litters was sufficient. The maternal NOAEL is based on clinical signs and decreased food consumption at 150 and 450 mg/kg bw/day. At the high dose level, one dam died following occurrence of clinical signs and abortion. The developmental NOAEL was established because of a higher frequency of late embryonic death at the highest dose level that was significantly elevated over the control value and was just at the upper edge of the historical control range. Furthermore, total embryonic losses were increased in all treated groups. However, this data is difficult to interpret since a comparison with historical control data from the performing laboratory proved a remarkably low percentage of post-implantation loss in the control group (5.7 %) that was below the historical control range (6.5-17.5 %). In contrast, the percentages for the low and high dose groups (19.5 and 21 %) were above its upper edge, but the 15.3% in the mid dose group was well within and there was no clear dose response. In this study, there was also an increase in cardiac malformations, mainly interventricular septal defects, at 450 mg/kg bw/day. This finding was observed in four foetuses from 4 litters as compared to one foetus showing this defect in each the control, low and mid dose groups. It must be emphasised that these malformations are apparently different from what is presumably defined by Suresh et al. (1993, TOX9551106) as 'dilated heart'.

Maternal and litter parameters from this study as well as an overview on foetal anomalies are given in Table 50 and Table 51.

Table 50: Summary of the maternal and litter parameters (group mean values) in the study by Brooker et al. (1991, TOX9552391)

Parameter	Dose Group (mg/kg bw/day)				Historical control range (mean value)
	0 (Control)	50	150	450	
No. of mated females	19	19	16	20	--
No. not pregnant	0	6	1	5	--
No. of premature deaths	0	0	0	1§	
No. of does with live young or	18	12	15	13	--

Parameter	Dose Group (mg/kg bw/day)				Historical control range (mean value)
	0 (Control)	50	150	450	
litters at Day 29					
Corpora lutea	11.5	12.4	11.7	11.3	9.0 – 12.9 (11.2)
Implantations	9.7	10.5	9.0	9.2	7.0 – 11.1 (9.5)
Pre-implantation loss	14.6	15.4	23.4	18.8	2.3 – 26.1 (15.1)
Early embryonic deaths	0.4	0.9	0.9	0.5	0.3 – 1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1 – 1.3 (0.7)
Abortions	0.0	0.0	0.1	0.0[#]	0.0 – 0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8**	0.6 – 2.0 (1.2)
Post-implantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5 – 17.5 (12.9)
Live young	9.1	8.7	7.5	7.3	6.1 – 9.5 (8.3)
Litter weight (g)	389.5	370.6	320.5	315.0	281.9 – 402.2 (352.9)
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--

§ Day 20, following abortion on the day before
* Statistically significant by Kruskal –Wallis 'H' test P < 0.05
** Statistically significant by Kruskal –Wallis 'H' test P < 0.01
[#] Fisher exact test follow-up by intergroup comparison with control was not statistically significant P > 0.05

Table 51: Summary of foetal parameters in the study by Brooker et al. (1991, TOX9552391)

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y ◊ (mean)
	0(control)	50	150	450	
Number of does with live young or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--
Malformations					--
Total number of foetuses examined	163	104	112	95	1511
No. of malformed foetuses	3	3	5	6	51
%	1.9	5.8	4.3	5.9 (F)	0.7 – 5.9 (3.8)
Number of Affected Litters	3	3	3	5	43/188
%	16.67	25	20	38.5	22.9
Thoracic region malformations					--
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	4	10/188
%	5.56	8.3	6.67	30.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y ◊ (mean)
	0(control)	50	150	450	
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1.0	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25
Anomalies					--
Total number of foetuses examined[#]	160	101	107	89	--
No. of foetuses with gross/visceral anomalies	9	14	14	6	--
%	6.4	19.5	12.9	9.6 (K)	--
No. of foetuses with skeletal anomalies	21	13	14	11	--
%	11.7	17.7	12.5	10.1 (K)	--
No. of foetuses with reduced ossification	7	4	5	4	--
%	4.4	4.0	4.7	4.5	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

◊ number affected / total number examined
[#] Malformed foetuses are excluded
(F) Fisher's exact test applied, not statistically significant (P > 0.05)
(K) Kruskal-Wallis 'H' statistic, not significant (P > 0.05)
-- no data

- The study of Bhide and Patil (1989, TOX9551960) was seriously flawed by serious deficiencies. Thus, no individual data is given and it is not clear whether statistical analysis of data has been performed and, if so, which statistical tests had been applied. Uterine weights and the results of maternal necropsy have not been reported. It is surprising that no maternal deaths have occurred even though the mid and high dose levels of 250 or 500 mg/kg bw/day had proven clearly toxic in other studies. It seems that the total number of foetuses and litters with malformations was higher in the groups receiving the mid and high doses of glyphosate but it is not clear whether they were found in different foetuses or if some foetuses had multiple malformations. The rather high number of visceral malformations at the top dose level was mainly due to absent kidneys or lung lobes, i.e., findings that can hardly be attributed to test substance administration. However, ventricular septal defects as in the study by Brooker et al. (1991, TOX9552391) were also noted but only in 2 out of 78 foetuses in the high dose group as compared to a control incidence of 0/109.

From all these studies, when taken together, the overall conclusion may be drawn that in rabbits, in

contrast to rats, some developmental effects and, in addition, post-implantation losses have been observed which can be allocated to glyphosate administration to the does. However, these findings were confined to dose levels at which severe maternal toxicity was apparent.

4.10.2.2 Human information

The same general constraints on the use of epidemiological data as discussed with regard to carcinogenicity and reproductive toxicity above (such as the lack of reliable exposure data, the impact of co-formulants or parallel exposure to other chemicals) apply also to developmental toxicity and teratogenicity. So far, there is no convincing evidence that exposure to glyphosate formulations will increase the risk for an adverse developmental outcome in humans.

Two studies on residential proximity to agricultural pesticide applications in California by and examined whether early gestational exposure to pesticides was associated with an increased risk of hypospadia (Carmichael et al., 2013, ASB2014-9307) or neural tube defects and orofacial clefts (Yang et al., 2013, ASB2014-9644) in offspring. In both studies formulated glyphosate (mentioned as ”phosphonoglycine”) was included in the analyses and exposure was frequent but no positive correlation was found.

In a study from Ontario (Canada), Arbuckle et al. (2001, ASB2012-11545) reported a slight increase in the pre-conception glyphosate exposure odds ratio for spontaneous abortion of borderline significance (OR = 1.4). Due to strong limitations in this study, no firm conclusion is possible. Thus, 395 spontaneous abortions were reported out of 3936 pregnancies giving a rate of spontaneous aborting of 10% that is below the baseline rate in the general population of 12 to 25 %. Recall bias is reflected in the recall of spontaneous abortion over the previous 5 years (64 % of all spontaneous abortions reported) being much higher than the recall of those greater than 10 years prior to the survey (34 % of all spontaneous abortions reported).

There are some reports from South America claiming an increasing frequency of birth defects in rural areas where the population is heavily exposed to agrochemicals (e.g., Campana et al., 2010, ASB2013-10559). Lopez et al. (2012, ASB2013-10534) also reported an increase in malformations but also in cancer incidence from certain regions but these increases were more general without clear-cut evidence of a distinct anomaly or a certain cancer type. The general weaknesses of such data collected in so-called ”ecological” (“correlational”) studies are the unknown exposure level and the impossibility to attribute a certain outcome to exposure to a single substance (Paumgartten et al., 2012, ASB2013-10538). There is no evidence so far that the reported increases might be related to glyphosate. Thus, Benitez-Leite et al. (2009, ASB2012-11563) reported the incidence of anomalies in newborn babies in a hospital in Paraguay but from this data it cannot be concluded if there was in fact an increase. Many of the reported anomalies were variations rather than malformations and, according to inquiries by the RMS, a similar incidence might be expected in an average German birth clinic. Furthermore, a single “hospital-based” analysis is not sufficient to prove changes in the prevalence of malformations in a region. The authors themselves reported a (not specified) “high” exposure of the parents to agrochemicals and pesticides in general but glyphosate or glyphosate-containing herbicides were not explicitly mentioned. In everyday life, people in these rural areas were exposed to a great number of agrochemicals that, taken together, might result in a higher risk for adverse outcomes such as malformations or cancer, in particular if exposure is high and appropriate safety measures are not taken. However, this assumption is of not much use neither for risk assessment for a single substance nor for its classification and labelling. Even if the claimed increases could be substantiated in future, it is unlikely that they were due to glyphosate, taking into account the extensive toxicological database and the long history of its worldwide safe use.

The absence of reproductive and developmental effects in humans is not surprising since human *in utero* exposures would be very limited. On one hand, the perfusion rate of glyphosate across the placenta is low (Mose et al., 2008, ASB2012-11914). On the other hand, systemic intake of glyphosate in the general population is low. McQueen et al. (2012, ASB2012-11898) calculated a very low dietary exposures of pregnant women in Australia ranging from 0.005 to 2 % of the ADI of 0.3 mg/kg bw for glyphosate as established by the Australian authorities. In combination, both facts will contribute to a nearly negligible *in utero* exposure.

4.10.3 Other relevant information

There are a large amount of *in vitro* and a few *in vivo* studies on different aspects of reproductive and developmental toxicity of glyphosate and its formulations for which the reader is referred to the attached Vol. 3 of the RAR. For purposes of classification and labelling, this often contradictory information is not that useful since there is a sufficient and adequate database of higher tier animal studies that have been performed in compliance to current guidelines employing very high doses.

However, it should be highlighted that glyphosate was found to be devoid of a potential for endocrine disruption in recent testing on request of U.S. EPA. Glyphosate was included into the U.S. EPA Endocrine Disruptor Screening Program's (EDSP) first list of 67 compounds that were foreseen to Tier 1 Screening. The compounds were selected on their potential for exposure rather than suspected interference with the endocrine system and tested for their potential to interact with the oestrogen, androgen and thyroid endocrine pathways. Levine et al. (2012, ASB2014-9609) published a short summary of the results. According to this, very brief information, glyphosate was tested in Tier 1 assays for (anti-)estrogenic and (anti-)androgenic properties and an impact on steroidogenesis *in vitro*. *In vivo* testing comprised the uterotrophic, Hershberger and male and female pubertal assays. These tests were performed at different laboratories. Bailey et al. (2013, ASB2013-3464) summarized the first results of the male and female pubertal assays in which glyphosate did not exhibit evidence of endocrine disruption.

Based on this new data and on the outcome of the reproductive and developmental studies in animals, the DS does not consider glyphosate to be a substance with endocrine disrupting properties.

In the past, two reports on a teratogenic potential of glyphosate gained notable public attention and are discussed here briefly.

Paganelli et al. (2010, ASB2012-11986) exposed embryos of the clawed frog Xaenopus laevis to a glyphosate formulation via the water or via injection of the test substance directly into frog embryos. In another experiment and, chicken embryos were exposed directly to a glyphosate formulation through a hole cut in the egg shell. The authors claimed to have found evidence of teratogenicity, in particular of neural crest lesions that might progress to craniofacial malformations. A mechanism similar to that of excess retinoic acid was suspected. However, the relevance of these findings must be questioned because of highly artificial routes of exposure as well as the application of excessive doses. Craniofacial malformations were not noted in developmental studies in rats or rabbits. Decisions on classification and labelling are mainly based on effects in adequate studies in mammals and not on mechanistic considerations.

Krüger et al. (2014, ASB2014-8935) reported glyphosate residues in different organs/tissues (brain, gut wall, heart, kidneys, liver, lungs, and muscle tissue) from a total of 38 malformed one-day old piglets (breed not specified) which had been brought in by a Danish farmer. Various, very different malformations were seen, including craniofacial but also visceral and leg anomalies. For determination of glyphosate, apparently the same ELISA as for urine measurements (Abraxis, USA) was used after mincing and diluting tissue samples from the various organs. Its previous validation

for the new matrix was not reported and no LOD or LOQ were mentioned. Mean glyphosate concentrations between 2.1 ppm (liver) and 12.9 ppm (heart) were found. For most organs, the standard deviation was extremely large and individual values in single animals ranged from 0 (liver) and 0.1 ppm (kidney) to occasional findings as high as 80 ppm in lung and heart. The authors speculated if there was a correlation between the malformations and intake of glyphosate residues to which the piglets might have become exposed via the placenta. The farmer claimed that the rate of malformed piglets had increased from 1:1432 when the sows had been fed a diet containing 0.25 ppm glyphosate to 1:260 when the sows received a diet with a glyphosate content of 0.87-1.13 ppm during the first 40 days of pregnancy. This publication cannot be considered as describing a reliable scientific study. Apart from the analytical uncertainties, the main weakness of the study is that only malformed piglets had been investigated for glyphosate concentrations in their organs. Thus, there was no control group to prove the hypothesis of a potential correlation.

Such a correlation is unlikely because of the following considerations:

- In a multitude of developmental studies and multi-generation studies in rats, no evidence of teratogenicity was obtained. Even in rabbits which proved more vulnerable, developmental effects were confined to exaggerated dose levels which also caused clear maternal toxicity. It is very unlikely that pigs, receiving much lower amounts of glyphosate by ingestion of residues in the diet, should be that much more sensitive and, if so, it is hardly conceivable that such effects would not have become apparent earlier and also in other countries and on other farms.
- Many different malformations were reported. However, most chemical teratogens produce a specific teratogenic effect or a certain pattern of findings. Moreover, teratogenic effects usually follow a dose response relationship. In this case, the glyphosate concentrations in the organs and tissues were so variable that such a dose response relationship may be excluded.
- Malformations in piglets are quiet frequent and often have a genetic background. Infectious diseases may also play a role. There is no indication in the paper that an alternative diagnosis had been considered.

4.10.4 Summary and discussion of reproductive toxicity

There was a very large database submitted by different applicants and from published scientific literature to evaluate reproductive and developmental toxicity of glyphosate. At least six valid multi-generation studies in rats, six developmental toxicity studies in rats and seven developmental toxicity studies in rabbits have been evaluated. All available data were considered together using a weight of evidence approach with consideration of the biological significance, maternal toxicity and the consistency of the reproductive and developmental findings.

In the rat, there was no evidence of specific reproductive toxicity or of a teratogenic potential since effects, if observed at all, were very weak and confined to very high dose levels causing already some parental or maternal toxicity.

In the developmental studies in rabbits some adverse developmental effects have occurred only in the presence of maternal toxic effects for which a comparison with criteria is needed (see below).

No convincing evidence of reproductive or developmental effects of glyphosate may be derived from epidemiological studies or from *in vitro* or *in vivo* studies on different aspects of reproduction.

4.10.5 Comparison with criteria

4.10.5.1 Effects on fertility

The following criteria for classification for adverse effects on sexual function and fertility are given in CLP regulation:

CLP criteria
Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies — clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant — some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and — where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

Reproductive studies in rats have clearly shown that these criteria were not met.

4.10.5.2 Developmental toxicity

The following criteria for classification for adverse effects on development are given in CLP regulation:

CLP criteria
Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies — clear evidence of an adverse effect on development in the absence of other toxic effects, or — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant — some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and — the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). — the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

General remark: For the majority of chemical substances evaluated under the CLP-Regulation, normally one study addressing developmental toxicity in the rats and rabbits, respectively is required and therefore available for classification and labelling purposes. In contrast, for

glyphosate, a large quantity of animal data regarding developmental toxicity is available, and six developmental toxicity studies in rats and seven developmental toxicity studies in rabbits have been evaluated. Therefore, all available data from all studies were considered together using a weight of evidence approach. Basing any conclusion only on the statistical significance of an increased incidence of a finding identified in a single study without consideration of the biological significance, the influence of maternal toxicity and the consistency of the developmental findings should be avoided.

Category 1A does not apply since there are no reliable human data and epidemiological studies that would provide convincing evidence of teratogenicity to humans.

Whereas the results of the studies in rats were not of concern, the cardiac malformations (i.e., interventricular septal defects) in rabbit foetuses have provoked a lot of controversial discussions (e.g., Antoniou et al., ASB2012-15927; Kimmel et al., 2013, ASB2013-3462). They are discussed in the following in greater detail and compared with the criteria for categories 1B and 2.

These findings were observed in few foetuses at various dose levels including the control. An increase was confined to the very high dose levels of 450 mg/kg bw/day (Brooker et al., 1991, TOX9552391) and 500 mg/kg bw/day (Bhide and Patil, 1989, TOX9551960), with the latter being a study of questionable reliability. The effect dose of 450 mg/kg bw/day was clearly in a dose range that is toxic to pregnant rabbits. In the Guideline-compliant study of Brooker et al. (1991, TOX9552391), a higher frequency of interventricular septal defects was indeed associated with some maternal toxicity including one death following abortion, gastrointestinal signs and slightly lower food consumption and body weight gain. When all the rabbit studies are taken together, first deaths were observed at a dose level of 100 mg/kg bw/day or 175 mg/kg bw/day and excessive toxicity resulting in the loss of nearly one half of the does was observed from 350 mg/kg bw/day onwards (Suresh et al., 1993, TOX9551106; Tasker et al., 1980, TOX9552390). Mortality was also seen at high dose levels in the studies by Coleman and Doles (1996, ASB2012-11499), Hojo (1995, ASB2012-11498) and Brooker et al. (1991, TOX9552391) even though the number of affected does was lower. Gastrointestinal signs, abortion and post-implantation losses also suggest severe maternal toxicity. As shown above, it is proposed to classify glyphosate as STOT RE for the maternal deaths in pregnant rabbits.

Despite administration of high doses, interventricular septal defects were not observed in two further studies in NZW rabbits from the mid-90s (Coleman and Doles, 1996, ASB2012-11499; Moxon, 1996, TOX2000-2002). Moreover, such findings were not reported in another rabbit strain (Hojo, 1995, ASB2012-11498). In fact, the top dose levels in these studies were lower (300 or 400 mg/kg bw/day) but, on the other hand, it would have been hardly possible to increase the maximum doses without causing excessive maternal toxicity.

The study by Suresh et al. (1993, TOX9551106) cannot not be taken as supportive evidence for cardiac malformations because the heart findings there (‘dilated heart’) were of a completely different nature. Dose response for this ‘dilatation’ was questionable, description of the findings was poor and a similar effect was not reported in other studies. Thus, it seems reasonable to disregard this equivocal finding with regard to classification and labelling.

Category 1B is not applicable because the higher incidence of interventricular septal defects at 450 mg/kg bw/day was associated with marked maternal toxicity in the same study (Brooker et al., 1991, TOX9552391) and even more pronounced maternal effects at lower doses in other rabbit studies. Thus, adverse developmental effects have occurred only in the presence of other toxic effects. It may be concluded that an increased risk for foetal heart effects in rabbit foetuses was confined to levels of exposure that also caused severe maternal toxicity. Therefore, and taking into consideration the rather low foetal incidence of interventricular septal defects at 450 mg/kg bw/day and their complete absence at 400 mg/kg bw/day in another study in the same strain (Coleman and

Doles, 1996, ASB2012-11499), it may be assumed that this finding is a non-specific secondary consequence of marked maternal toxicity. Accordingly, category 2 would be also not appropriate.

4.10.6 Conclusions on classification and labelling

No classification and labelling of glyphosate for reproductive or developmental effects is proposed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 52: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolyses determination of glyphosate at different pH values US EPA 540/9-85-013, Series 161-1	Glyphosate, purity 96.6% In range of pH 5-9 stable, no hydrolysis products were detected	Accepted during EU review (2001)	Burgener (1990)
Photodegradation study of glyphosate in water at pH 5,7 and 9 US EPA 540/9-82-021, Series 161-2	Glyphosate, purity 96.6% DT_{50} = 33 d (pH 5) DT_{50} = 69 d (pH 7) DT_{50} = 77 d (pH 9)	Accepted during EU review (2001)	Van Dijk (1992)
Biodegradation OECD 302 B, 1981	Glyphosate, purity 96.6% 0 % after 28 days	Accepted during EU review (2001)	Wüthrich (1990)
Biodegradation OECD 302 B, 1981	Glyphosate, purity 96.6% 2 % after 28 days	Accepted during EU review (2001)	Carrick (1991)
Biodegradation OECD 301 F	< 60 % after 28 days	Study report not available	Feil (2009)

5.1.1 Stability

The hydrolysis study with glyphosate (Burgener (1990, BVL no 2442046) was assessed as acceptable during the EU review of glyphosate (2001). The results are summarised in the monograph of glyphosate:

Solutions of ^{14}C-1-methane glyphosate (purity 96.6 %) in water at pH 5, 7 and 9 were reacted in the dark under sterile conditions at 25 °C for 30 days. After an incubation time of 30 days, no hydrolysis products were detected in the test solution and no significant amount of volatile products were observed in the absorption traps (<0.1 %). In the pH range 5 to 9 tested glyphosate is stable towards hydrolysis.

The photochemical degradation of glyphosate was investigated during the 2001 EU approval of glyphosate. The results of the acceptable study with glyphosate (van Dijk, 1992, BVL no 2252558) are summarized in the Monograph of glyphosate:

The rate of photolysis of ^{14}C-1-methane glyphosate was determined in distilled and sterile water solutions after 0,1,4,7 and 16 days at pH of 5.1, 7.3 and 9.2 at 25 °C in a suntest irradiation apparatus simulating natural sunlight. At every pH, the parent compound was not significantly degraded in the dark, i.e. the amount of parent compound from day 0 to day 15 did not decrease more than 3.5 %. The half-lives of glyphosate are a function of solution pH: at pH 5 (DT_{50} of 33 days), at pH 7 (DT_{50} of 69 days) and at pH 9 (DT_{50} of 77 days).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

In the 2001 EU evaluation of glyphosate, several studies assessing glyphosate's ready biodegradability have been reviewed. Two out of these reviewed studies were conducted according to the OECD guideline 302 for test on inherent biodegradability (Wüthrich, 1990, BVL no 1934369; Carrick, 1991, BVL no 2325628). An additional study according to OECD guideline 301 F (Mamometric Respirometry Test) was prepared by a Glyphosate Task Force (GTF) member (Feil, 2009).

In all studies, glyphosate did not show mineralisation of more than 60 % within 28 days. Therefore, the active substance is classified as not ready biodegradable. Table 47 summarizes all the available compliant studies mentioned above.

The study of Feil (2009) was not presented to the RMS and therefore could not be checked. However, the results presented in the dossier of the notifier are in line with the available studies and therefore are plausible.

Table 53: Overview of the glyphosate biodegradability studies

Reference		Guideline	Inocolum	Conc. (g dry material/L)	Test Conc. (mg/L)	Fraction of CO_2 produced from parent	
						Functional control	Glyphos-ate
Studies from the 2001 Evaluation	Wüthrich, 1990, BVL no 1934369	OECD 302 B, 1981	1. Sludge from domestic WTP (CH) 2. Sludge from WTP of Cheminova (DK)	0.2	620	88 % and 89 % within 7 days	0 % after 28 days for both systems
	Carrick, 1991, BVL no 2325628	OECD 302 B, 1981	Activated sludge from Kendal WTP	0.2	250	100 % within 2 days	2 % after 28 days
New study	Feil, 2009	OECD 301 F	Activated sludge from Darmstadt (Germany) WTP	1.5	103	98 % after 28 days	< 60 % after 28 days

Conc. = concentration; WTP = waste water treatment plant

5.1.2.3 Simulation tests

5.1.3 Summary and discussion of degradation

The study on ready biodegradability according to OECD 301 F (Manometric Respirometry Test) shows that glyphosat is not readily degradable (< 60 % degradation at 28 days).

The study on inherently biodegradability according to OECD 302 B (Modified Zahn Wellens Test) shows that glyphosat is not rapidly degradable (0-2 % degradation at 28 days).

Glyphosat is hydrolytically stable under acidic and neutral conditions. Aquatic photolysis is not considered as an important transformation route for glyphosate in the environment with DT_{50} of 33 – 77 days.

The results of the tests on the biodegradation of glyphosat show that glyphosate is not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

5.2 Environmental distribution

Not relevant for this dossier.

5.3 Aquatic Bioaccumulation

Table 54: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water EEC A 8 shake flask	log $P_{o/w}$ < - 1.3 (measured)	accumulation potential in aquatic non-target organisms is hence considered to be low	Wollerton and Husband (1997)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Glyphosate acid has a log P_{OW} value of < -1.3. Therefore, based on the low log P_{ow}-values the potential for bioconcentration is considered negligible. The octanol/water partition coefficient of glyphosate acid, expressed as log P_{ow}, is < -1.3. Values less than 3 indicate a low potential for bioaccumulation, therefore no further assessment is necessary.

5.3.1.2 Measured bioaccumulation data

No data available.

5.4 Aquatic toxicity

Table 55: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Acute toxicity of Glyphosate acid to Bluegill Sunfish (*Lepomis macrochirus*) OECD 203/FIFRA 72-1 Static exposure	96 hour LC_{50} = 47 mg/L (nominal) with a 95 % confidence interval of 35 to 66 mg/L	--	Kent, S.J., Caunter, J.E., Morris, D.S., Johnson,P.A. (1995)
Chronic Toxicity of Glyphosate acid to zebra fish larvae (*Brachydanio rerio*) OECD 212 semi-static exposure	NOEC (168 h) = 1.0 mg/L (nominal)	recalculated value key study	Dias Correa Tavares, C.M. (2000)
Acute toxicity of Glyphosate acid to *Daphnia magna* OECD 202 Static exposure	LC_{50} (48 h) = 84 mg/L (nominal) with a 95 % confidence interval of 73.3 to 101 mg/L	--	Wüthrich, V. (1990)
Glyphosate acid: Chronic toxicity to *Daphnia magna* OECD 202, part II semi-static exposure	NOEC (21 d) = 12.5 mg/L (nominal) for reproduction	--	Magor, S.E., Shillabeer, N. (1999)
Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum* OECD 201 Static exposure	E_rC_{50} (72 h) = 18 mg/L (nominal) with a 95 % confidence interval of 10 to 42 mg/L NOE_rC (72 h) = 1.82 mg/L (nominal)	--	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N. (1996)
Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae* OECD 201 Static exposure	E_rC_{50} (72 h) = 22 mg/L (nominal) with a 95 % confidence interval of 8.8 to >96 mg/L NOE_rC (72 h) = 12 mg/L (nominal)	--	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J. (1996)
Glyphosate acid: Toxicity to duckweed (*Lemna gibba*) EPA FIFRA Guideline 123-2 semi-static exposure	EC_{50} (14 d) = 12 mg/L (nominal) with a 95 % confidence interval of 11 to 14 mg/L for inhibition of frond number NOEC (14 d) = 3 mg/L (nominal) for inhibition of frond number	--	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N. (1996)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1

Author:	Kent, S.J.,Caunter, J.E.,Morris, D.S., Johnson,P.A.
Title:	Glyphosate acid: Acute toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
Date:	21.12.1995
Doc ID:	2310926 /BL5553/B
Guidelines:	OECD 203/FIFRA Guideline 72-1
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 % a.s.
Control:	Filtered and dechlorinated tap water
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Juvenile
Size:	30 mm (mean)
Body weight:	0.54 g (mean)
Loading:	10 test individuals for 20 L test solution
Source:	Aquatic Research Organisms, Hampton, New Hampshire, USA
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	19 days at 22 °C prior to the test initiation
Temperature:	22 ± 1 °C
Photoperiod:	16 hours with 20 min transition period
pH:	Control (start – 96 h): 7.3–6.8 10 mg/L (start – 96 h): 5.9 – 6.4 18 mg/L (start – 96 h): 5.2 – 5.8 32 mg/L (start – 96 h): 4.6 – 4.8 56 mg/L(start – 96 h): 3.8 – 3.9 100 mg/L (start – 24 h): 3.4 180 mg/L (start – 24 h): 3.1
Dissolved oxygen:	6.2 – 9.0 mg/L
Conductivity:	100 µS/cm
Hardness:	16.0 mg $CaCO_3$/L.
Methods:	The acute toxicity test was performed at nominal concentrations of 10, 18, 32, 56, 100 and 180 mg test item/L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions (no media renewal). A

	negative control group (dilution water only) was also prepared. A single vessel was prepared for the control and each test media group, each containing ten fish (27.5 L borosilicate glass vessels containing 20 L test medium). Observations: All fish were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. Samples of test media were analysed for glyphosate acid content using HPLC analysis at test initiation and after 48 and 96 hours. Statistical calculations: The 96 hour LC50 values and 95 % confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

Results

The measured concentrations of glyphosate acid in fresh media at test initiation ranged between 96.9 and 110 % of nominal. In aged test media at 96 hours, mean measured glyphosate acid concentrations ranged between 94.4 and 97.0 % of nominal. At 100 and 180 mg/L, no chemical analysis was performed at 48 and 96 hours, as all fish died within the first 24 hours following addition. As measured concentrations of glyphosate acid were between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

There were no mortalities in the control or the 10, 18 and 32 mg /L treatments. At 56 mg test item/L, there was 90 % mortality. There was 100 % mortality at 100 mg/L and higher test concentrations that occurred after 24 hours. There was a strong negative correlation between pH value and test item concentrations observed. At 56 mg test item/L, the pH was reduced to 3.8 and lower.

Table 56: Effects of glyphosate acid on Bluegill sunfish (Lepomis macrochirus)

Glyphosate acid (mg/L)	**% of dead fish and observed symptoms**			
	24 h	**48 h**	**72 h**	**96 h**
Control	< 10	< 10	< 10	< 10
10	< 10	< 10	< 10	< 10
18	< 10	< 10	< 10	< 10
32	< 10	< 10	< 10	< 10
56	40	80	90	90
100	100	100	100	100
180	100	100	100	100

RMS Conclusions

The 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg glyphosate acid/L (nominal) with a 95 % confidence interval of 35 to 66 mg/L, with a 96 hour NOEC values of 32 mg glyphosate acid/L. The study is considered to be acceptable and valid.

5.4.1.2 Long-term toxicity to fish

Study 1

Author:	Dias Correa Tavares, C.M.
Title:	Chronic Toxicity of Glifosate Técnico Nufarm to zebra fish larvae (*Brachydanio rerio*)
Date:	13.01.2000
Doc ID:	2310938 /RF-D62.16/99
Guidelines:	OECD 212/ IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg acid equivalent
2. Vehicle and/or positive control:	Tap water; Potassium dichromate ($K_2Cr_2O_7$)
Species:	Zebra fish (*Danio rerio*) larvae
Age:	Larvae, approx. 48 hours old
Size:	Not stated
Loading:	1 L for 10 larvae
Source:	Eggs: in-house. Matrix fish: Peixe Vivo Aquicultura Ltda, Muriae, Brasil
Acclimation period:	48 hours prior to testing during embryo incubation and hatching
Temperature:	23.8-24.3 °C
Photoperiod:	16 hours light / 8 hours dark
Dissolved oxygen:	60-100%
Conductivity:	168 µS/cm
Hardness of test medium:	44.1 mg/L $CaCO_3$
Methods:	The fish early life-stage toxicity test was performed under semi-static exposure conditions renewing the test solution every 48 hours. Following a range finding test, the freshly hatched fry of *Danio rerio* was exposed to test concentrations of 0.32, 0.56, 1.0, 3.2, 5.6, 10 and 32 mg glyphosate acid/L for 168 hours. A control consisting of reconstituted water and five toxic reference concentrations (32, 56, 100, 140 and 180 mg $K_2Cr_2O_7$/L were maintained concurrently. Observations for mortality and sublethal responses were made every 24 hours. Dead individuals were removed at each observation. Temperature, dissolved oxygen, pH and conductivity were measured daily. The active ingredient analysis of stock solutions was performed by liquid chromatography. LC_{50} and its confidence limits were determined using trimmed Spearman-Karber method. Fisher's Exact test was used for determination of significant differences in survival between control and exposure.

Results

The active ingredient concentration in each stock solution was at least 80 % of the nominal concentration. For the reference compound potassium dichromate ($K_2Cr_2O_7$) a 168 hour LC_{50} value of 124.66 mg a.s./L (95 % C.I. 112.08 – 138.67 mg a.s./L) was determined.

With regard to the validity criteria of the pertaining OECD guideline 212 survival of fertilised eggs on successive days was 100 %. Analysis of test item treatments was performed for the stock solutions, the test was carried out in a semi-static system, with renewal of the test solution each 48 h. The water temperature did not differ more than +/- 1.5 °C between test chambers on successive days at any time during the test at the recommended temperature, as well as pH remained constant. Mortality in control group did not exceed 10 %, dissolved oxygen concentration was between 60 and 100 % of air saturation. The present study is considered valid according to OECD guideline 212.

A significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L, behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. The following observations for mortality were made every 24 h during the 168 h test period:

Table 57: Lethal effects of glyphosate acid for zebra fish

	Glyphosate acid (mg a.s./L)							
	0 (Control)	**0.32**	**0.56**	**1.0**	**3.2**	**5.6**	**10**	**32**
Introduced	30	30	30	30	30	30	30	30
Survided (168 h)	30	30	30	30	27	25	22	13
Mortality (168 h) (%)	0	0	0	0	10	16.7*	26.7*	56.7*

*statistically significant different from control

RMS Conclusions

In the guideline OECD 212 it is recommended that the duration of the test should be 30 days post hatch. By contrast, the present study was performed for 168 h. It is also stated that the test is to be continued at least until all the fish in control treatment are free feeding. Moreover, the time of first feeding should start 6-7 days after spawning. In the current test it is not clear, if fish in the control treatment are free feeding totally. Nevertheless, significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L. Despite these deficiencies, the study is considered to be valid and acceptable.

In the short term toxicity test on fish larvae, the LC_{50} after 168 hours was determined to be 24.71 mg a.s./L. The No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) for zebra fish (Danio rerio) exposed to glyphosate acid were determined by the author to be 3.2 mg a.s./L and 5.6 mg a.s./L, respectively, based on nominal concentrations. Nevertheless, the mortality effect in the study with *Danio rerio* followed a dose response relationship and in the treatment level at 3.2 mg/L a mortality of 10% was observed. Considering these biological effects as relevant, although not statistically significant, results in a NOEC of 1.0 mg/L.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Author:	Wüthrich, V.
Title:	48-Hour Acute toxicity of Glyphosate techn. to *Daphnia magna* (OECD-Immobilisation Test)
Date:	09.11.1990
Doc ID:	2310947 /272968
Guidelines:	OECD 202 (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %
Positive control:	Reconstituted water (EEC), Potassium dichromate ($K_2Cr_2O_7$)
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 20 mL test medium
Source:	In-house culture
Diet/Food:	Not fed during test or during the 24 hours preceding test initiation.
Acclimation period:	Approximately 24 hours
Temperature:	21.0 ± 0.5 °C
Photoperiod:	16 hours light
pH:	Control:8.4 – 7.9 62.5 mg test item/L:6.3 – 7.6 125 mg test item/L:4.8 – 5.2 250 mg test item/L:3.2 – 3.4 500 mg test item/L: 2.7 – 2.9 1000 mg test item/L:2.3 – 2.6
Dissolved oxygen:	8.3 – 8.1 mg O_2/L (mean)
Conductivity:	Not stated
Hardness:	250 mg $CaC0_3$/L(reconstituted water)
Methods:	The toxicity test was performed with five test nominal glyphosate acid concentrations of 62.5, 125, 250, 500 and 1000 mg glyphosate acid/L, prepared using reconstituted water (EEC). The test was conducted using a static test design (without media renewal) over 48 hours, in duplicate 50 mL beakers each containing 20 mL of the appropriate test or control (reconstituted water only) solution. Juvenile Daphnid (<24 hours old) were added impartially to the test vessels until all contained 10 daphnia. In addition, a test item stability control without daphnids was also prepared at 1000 mg glyphosate acid/L.

	The number of immobile *Daphnia magna* in each vessel was recorded at 24 h and 48 h after test initiation. The pH-values and oxygen saturation were measured in each test vessel at test initiation and termination. Samples of control and test media were taken at the start – 0 hours (freshly prepared – before animal addition) and end – 48 hours (pooled replicates according to treatment) and analysed for glyphosate content using an HPLC method of analysis. The EC_{50} (immobilisation) was estimated by the authores using the Logit-model, NOEC, EC_{50} and EC_{100} values were determined by linear regression. EC_{50} values were recalculate by RMS via ToXRatPro Version 2.10 using Probit analysis using linear max. likelihood regression and Multiple testing to find the NOEC (Bonferroni-Fisher Test).

Results

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L. Measured concentrations of glyphosate acid in the test media at 62.5, 125, 250 and 500 mg glyphosate acid/L were in the range of 69.7 – 95.2 % of nominal. Authors reported results based on nominal glyphosate acid concentrations. According to the actual criteria in this case results should be based on measured concentrations. Therefore endpoints were recalculated by RMS. Results of the probit analysis using linear max. likelihood regression proposed an EC_{50} value of 74.0 (95 % CL: 16.96 - 130.34). A NOEC of 53.2 mg glyphosate/L is calculated.

The pH in test medium was decreasing due to increasing test concentrations, as the test item is an acid.

Immobilisation of daphnids was observed beginning with 62.5 mg/L test item and all daphnids were immobilised after 48 h at the next higher concentration of 125 mg/L test item.

Table 58: Effects of glyphosate on *Daphnia magna*

		Glyphosate acid (mg/L)									
	Control	**62.5**		**125**		**250**		**500**		**1000**	
Mean measured concentrations (mg/L) (% nominal)	**-**	**53.2 (85)**		**97.6 (78)**		**232.3 (93)**		**475.1 (95)**		**775.2 (78)**	
% immobile daphnids after 24 h	0	10	0	30	60	100	100	100	100	100	100
% immobile daphnids after 48 h	0	10	0	100	100	100	100	100	100	100	100
pH after 24 h	8.4	6.3		4.8		3.2		2.7		2.3	
pH after 48 h	7.9	7.6		5.2		3.4		2.9		2.6	

RMS Conclusions

The authors concluded that the 48 hour EC_{50} (immobilisation) value for *Daphnia magna* exposed to glyphosate acid was 84.0 mg glyphosate/L with a 95 % CL of 73.3 to 110.1 mg/L. The 48 hour NOEC value was 60.3 mg glyphosate /L based on nominal concentrations.

These values were recalculated by the RMS. Results of the probit analysis using linear max. likelihood regression proposed and EC_{50} value of 74 mg/L (95 % CL: 16.966 - 130.338). A NOEC of 53 mg glyphosate/L is suggested by the program.

The study is considered to be acceptable and valid. Nevertheless to address actual criteria recalculation of the endpoints was necessary.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Author:	Magor, S.E., Shillabeer, N.
Title:	Glyphosate acid: Chronic toxicity to *Daphnia magna*
Date:	29.06.1999
Doc ID:	2310962 /BL6535/B
Guidelines:	OECD 202, Part II, Reproduction Test (1984)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P30
Purity:	97.6 %
2. Vehicle and/or positive control:	Elendt M4
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	1 organism per vessel (glass beakers containing 80 mL test solution)
Source:	Continuous laboratory cultures
Temperature:	19.4 to 20.2 °C
pH:	3.67-8.02 (new solutions) ; 3.46-8.00 (old solutions)
Dissolved oxygen:	9.2-9.2 mg O_2/L (dilution water, new); 8.8-9.2 mg O_2/L (test solutions, old)
Conductivity:	572-617 mg/L µS/cm (test solutions)
Hardness:	202.7-218.3 mg $CaCO_3$
Photoperiod:	16 hours light /8 hours dark, 20 minute dawn and dusk transition period; 480 lux
Methods:	The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one daphnia per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 daphnia were exposed to test medium without test substance (blank control). The daphnia were randomly placed into the test beaker and exposed to the test item for 21 days. The test daphnia were fed daily with cultured algae (*Chlorella vulgaris*). A primary stock solution of 200 mg a.s./L was prepared on day 0 by dissolving 400 mg test item in 2000 mL of dilution water. On days 2, 4, 7, 9, 11, 14, 16, and 18 a primary stock solution of 100 mg a.s./L was prepared by dissolving 200 mg test item in 2000 mL dilution water. The test solutions were prepared by the addition of appropriate aliquots of the stock solutions to dilution water. At each renewal of the test solutions, the surviving P0 generation of daphnia were transferred to the new solutions. The F1 generation of daphnia were removed from each vessel and counted. The numbers of alive and dead F1daphnia were recorded. Mortality of P0 generation of daphnia and observation for the presence of alive and dead offspring (termed F1 generation) were recorded daily in each test vessel. At the end of the test, the length of each surviving P0 daphnia was measured. The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer of

	the P0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21. The reproduction and length data for each individual P0 generation daphnid were entered into electronic data files and analysed using statistical procedures contained in the Brixham Environmental Laboratory computer programs 'STATS' (version 4.10) and 'EPA' (version1.04).

Results

The validity criteria according to OECD 202 were fulfilled, as immobility of daphnids was < 20 % in control groups and mean offspring number at day 21 was > 60.

The effects of glyphosate acid on *Daphnia magna* mortality and reproduction are shown in the following table.

Table 59: Offspring per day and female of *Daphnia magna*

Nominal concentration (mg a.s./L)	Mean adult mortality (%)	Total offsprings per parent (No.)	Total offsprings (No.)
Control	10	108± 20	1028
12.5	0	100±21	1003
25	0	84±12*	840
50	0	91±18	912
100	50	105±23	763

* Statistically significant difference

At the nominal concentration of 25 mg/L the total number of offspring per parent was significantly lower when compared to control. Even though the results of this study do not show a classical dose response relation, significant effects were observed and it is proposed to consider these effects. The relevant and accepted long term endpoint for invertebrates established in the EU evaluation of glyphosate in 2001 is in the same order of magnitude.

RMS Conclusions

The study was performed according to OECD 202, Part II. According to current criteria, the OECD 211 would be the relevant directive. Since daphnids were held individually in the test vessel, it is possible to determine the exact number of offspring per parent and therefore a statistical evaluation according to the criteria of OECD 211 is possible. RMS proposes to consider significant effects at 25 mg/L and recommends an NOEC for reproduction 12.5 mg a.s./L based on nominal concentration.

The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid is 12.5 mg a.s./L based on nominal concentration.

5.4.3 Algae and aquatic plants

Study 1

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N.
Title:	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Date:	08.11.1996
Doc ID:	2310972 /BL5684/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item::	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Cell growth medium	Cell growth medium (Walsh & Alexander 1980)
Species:	Marine alga *Skeletonema costatum*, strain CCAP 1077/1C
Source:	Culture centre of algae and protozoa, Dunstaffnage Marine Laboratory, Oban, Argyll, UK
Initial cell concentration	1.00 x 10^4 cells/mL
Temperature:	20.0-20.1°C (measured by thermometer). The hourly temperature measured automatically remained within 20±1 °C.
Photoperiod:	16 h light
Light intensity:	4340 lux
pH:	7.1 – 8.1 at the start of the test, 8.1 – 8.8 at the end of the test
Methods:	The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A stock solution of nominal concentration of 56 mg a.s./L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, and 32 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test was performed in 6 replicates cultures for control and 3 replicates for each concentration of glyphosate acid. Each replicate was inoculated with 1.250 mL of the inoculum culture to give a nominal cell density of 1.00 x 10^4 cells/mL. The culture vessels were incubated at 20±1°C for 120 h. During incubation, the cells were kept in suspension by continuous shaking. The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The

	concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. One-way analysis of variance, and Dunnett's procedure. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 were therefore fulfilled.

The mean measured concentrations of glyphosate acid ranged from 94 to 106 % of the nominal values. On the basis of the analytical results being with 80 and 120 % of the nominal test concentration, ecotoxicological endpoints were evaluated using the nominal concentrations.

Table 60: Mean cell densities and percentage of inhibition of cell growth of *Skeletonema costatum* exposed for 72 and 96 hours to glyphosate

Nominal concentration (mg a.s./L)	Mean growth rates 72h		Mean areas under the growth curve 72h		Mean growth rates 96h		Mean areas under the growth curve 96h	
	Mean growth rate	% of control	Mean areas under the growth curve	% of control	Mean growth rate	% of control	Mean areas under the growth curve	% of control
Control	1.423		37.4		1.113		97.6	
1.0	1.423	101	38.0	102	1.112	100	99.0	101
1.8	1.433	101	38.9	104	1.113	100	100.8	103
3.2	1.443	93	29.5*	79	1.128	101	84.5	87
5.6	1.322*	97	34.2	92	1.121	101	92.6	95
10.0	1.387	78	17.9*	48	1.122	101	62.6	64
18.0	1.111*	25	2.8*	8	0.317*	28	4.6	5
32.0	0.362*	21	2.3*	6	0.190*	17	3.3	3
56.0	0.295*	13	1.5*	4	0.087*	8	1.9	2

* Significant difference from the culture control (α =0.05)

RMS Conclusions

The 72 h E_bC_{50} for *Skeletonema costatum* exposed to glyphosate acid was 11 mg/L (95 % C.I. 7.1 to 20 mg a.s./L) and the 96 h E_bC_{50} was 11 mg/L (95 % C.I. 7.2 to 19 mg a.s./L); the 72 h E_rC_{50} was 18 mg/L (95 % C.I. 10 to 42 mg a.s./L) and the 96 h E_rC_{50} was 29 mg/L (95 % C.I. 16 to > 56 mg a.s./L) (nominal). The 72-hour NOE_bC and NOE_rC values were 1.82 mg/L (nominal), respectively.

The study is considered to be valid and acceptable.

Study 2

Author:	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J.
Title:	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae*
Date:	08.11.1996
Doc ID:	2310970 /BL5698/B
Guidelines:	OECD 201 (1984), US EPA Guideline 540/09-82-020 (1982)
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6 %
Medium	acc. to Miller et al. (1978)
Species:	Blue-green alga *Anabaena flos-aquae*
Source:	Brixham Environmental Laboratory culture from strain CCAP 1403/13A, Culture Centre of Algae and Protozoa, Institute of Freshwater Ecology. Windermere Laboratory, Far Sawrey, Ambleside, Cumbria, UK
Initial cell concentration	2.05 x 10^4 cells/mL
Temperature:	24.1-24.2 °C (measured by thermometer) The hourly temperature measured automatically remained within 24±1°C
Photoperiod:	Continuous illumination
Light intensity:	3600 lux
pH:	3.5 – 7.2 at the start of the test, 3.6 – 8.2at the end of the test
Methods:	The toxicity of glyphosate acid to Anabaena flos-aquae was determined in a 120-hour, static toxicity test. The test incorporated 8 nominal concentrations of glyphosate acid (0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg a.s./L) and a negative control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. A stock solution at a nominal concentration of 96 mg glyphosate/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, and 48 mg a.s./L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test was performed in 6 replicates for the control group and 3 replicates for each concentration of glyphosate acid. Each replicate was inoculated with 1.120 mL of the inoculum culture to give a nominal cell density of 2.05 x 104 cells/mL. Single blank vessels were prepared for the control and each test concentration without algal cells. The culture vessels were incubated at 24±1°C under continuous illumination for 120 h. During incubation, the algal cells were kept in suspension by continuous shaking. The algal cell densities were determined by spectrophotometric absorbance, using a Uvikon 860 UV/visible spectrophotometer. After 1, 2, 3, 4, and 5 days, samples were removed from each control, test and blank vessel. The appropriate blank solution absorbance was subtracted from that of the test culture to obtain the algal absorbance reading. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily and hourly. The

	concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test. One-way analysis of variance, and Dunnett's procedure. Median effective concentrations and its 95% confidence limits were determined by linear regression against log concentration.

Results

The biomass in the control cultures increased by a factor of > 16, the coefficient of variance for section specific growth rates was ≤ 35 %, for the whole test period it was ≤ 7 %. The validity criteria according to guideline OECD 201 are therefore fulfilled.

The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values. On the basis of the analytical results being with 80 and 120 % of the nominal test concentration, ecotoxicological endpoints were evaluated using the nominal concentrations.

Table 61: Mean growth rates and mean areas under the growth curve of *Anabaena flos-aquae* exposed for 72 and 96 hours to glyphosate acid

Nominal concentration (mg a.s./L)	**Mean growth rates 72h**		**Mean areas under the growth curve 72h**		**Mean growth rates 96h**		**Mean areas under the growth curve 96h**	
	Mean growth rate	**% of control**	**Mean areas under the growth curve**	**% of control**	**Mean growth rate**	**% of control**	**Mean areas under the growth curve**	**% of control**
Control	1.392	-	1.331	-	1.331		1.5	-
0.75	1.365	91	1.357	98	1.357	102	1.5	103
1.5	1.336	85	1.355	96	1.355	102	1.5	99
3.0	1.328	80	1.344	95	1.344	101	1.4	94
6.0	1.321	82	1.342	95	1.342	101	1.4	94
12	1.299	76	1.321	93	1.321	99	1.3	87
24	1.231*	6	0.216*	17	0.216*	16	0.0*	2
48	0.231*	5	0.173*	17	0.173*	13	0.0*	2
96	0.231*	5	0.173*	17	0.173*	13	0.0*	2

* Significant difference from the culture control (α =0.05)

RMS Conclusions

The 72 h E_bC_{50} for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg a.s./L (95 % CL 2.6 to 28 mg a.s./L), the 72 h E_rC_{50} was 22 mg/L (95 % CL 8.8 to >96 mg a.s./L) and the 72-hour NOE_bC and NOE_rC values were 12 mg/L (nominal), respectively.

The study is considered to be valid and acceptable.

Study 3

Author:	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N
Title:	GLYPHOSATE ACID: Toxicity to duckweed (*Lemna gibba*)
Date:	31.01.1996
Doc ID:	2310988 /BL5662/B
Guidelines:	EPA FIFRA Subdivision J Guideline 123-2
GLP:	YES
Validity:	YES

Materials and Methods

Test item:	Glyphosate acid	
Description:	White solid	
Lot/Batch #:	P24	
Purity:	95.6 %	
2. Vehicle and/or positive control:	Hoaglands M medium	
Species:	*Lemna gibba*, Strain G3	
Source:	In-house culture originally obtained from University of Waterloo, Canada	
Temperature:	24.6 – 25.0 °C	
Photoperiod:	24 h illumination	
Light intensity	5000 lux	
pH:	Freshly prepared test media: Control:4.7 – 4.9 0.75 mg/L: 4.7 – 4.8 1.5 mg/: 4.6 – 4.7 3.0 mg/L: 4.6 6.0 mg/L: 4.5 12 mg/L: 4.4 24 mg/L: 4.2 – 4.3 48 mg/L: 3.9 – 4.0 96 mg/: 3.5 – 3.6	Old test media: Control:5.3 – 5.7 0.75 mg/L: 5.3 – 5.8 1.5 mg/: 5.2 – 5.8 3.0 mg/L: 5.2 – 5.8 6.0 mg/L: 5.1 – 5.7 12 mg/L: 4.8 – 5.6 24 mg/L: 4.6 – 5.0 48 mg/L: 4.0 – 4.2 96 mg/: 3.6 – 3.7
Methods	The toxicity test on *Lemna gibba* was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg glyphosate acid/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions as the test groups. The plants were placed in 400 mL beakers (test vessels), containing 160 mL of Hoagland's M-medium prepared according to Hillman (1961). The test was conducted under semi-static conditions with renewal of the test medium after 5 and 9 days. Three uniform healthy-looking plants with 4 fronds each were added to each control and test vessel. The number of plants and fronds were counted after 2, 5, 7, 9, 12 and 14 days. Also symptoms of toxicity were recorded on these dates. At test end the weight of the dried plant tissue (at 60 °C) was recorded. The pH was measured in the old and the new test medium (new= day 0, 5 and 9, old = day 5, 9 and 14). Temperature in the test chamber was recorded daily and light intensity was recorded once a week. Analytical measurements of glyphosate acid were performed by means of HPLC analysis at test start and after 5 and 9 d (after test medium renewal). Fresh media was analysed on days 0, 5 and 9. Old media were analysed on days 5, 9 and 14. The EC_{50} and its 95% confidence interval were calculated by moving average angle method. The	

	NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at p = 0.05.

Results

Analytical measurements were performed in the freshly prepared (day 0, 5 and 9) and the old (day 5, 9 and 14) test media. The measured concentrations in the fresh media ranged from 90 – 108 % of nominal and in the old media from 87 – 102 % of nominal (overall mean measured: 93 – 100 % of nominal).

All validity criteria according to OECD 221 were fulfilled, as the doubling time of frond numbers in the control were less than 2.4/d. According to EPA FIFRA Subdivision J Guideline 123-2, endpoints were determined after 14 days.

The increase in frond number was significantly inhibited at nominal test concentration of 6.0 mg test item/L and higher, when compared to the control. The growth of the plant in terms of tissue dry weight was significantly reduced at 12 mg test item/L and higher. At 24, 48 and 96 mg test item/L dose related symptoms like pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface were observed from day 2 onwards. Visually observed effects were apparent at concentrations of 3.0 mg/L and above.

Table 62: Frond numbers, increase in frond numbers and inhibition compared to the control

Test item rate (mg a.s./L)	Number of fronds						Increase in frond numbers	Inhibition (%)
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14	(Day 0 – 14)	
Control	21	48	85	134	222	327	315	-
0.75	23	47	79	125	232	343	331	0
1.5	23	45	78	113	220	323	311	1
3.0	21	48	78	120	206	300	288	9
6.0	21	49	81	116	198	269	257	18*
12	20	44	74	105	148	173	161	49*
24	16	28	44	59	82	91	79	75*
48	15	21	24	28	28	30	18	94*
96	13	14	15	16	18	17	5	98*

* significant at p = 0.05

Table 63: Mean dry weight of plant tissue after 14 d, main increase in dry weight and inhibition compared to the control

Test item rate (mg a.s./L)	Mean tissue dry weight after 14 day (mg)	Mean increase (mg)	Inhibition (%)
Control	40.7	39.2	-
0.75	51.3	49.8	0
1.5	49.8	48.3	0
3.0	44.0	42.5	0
6.0	40.3	38.8	1
12	29.8	28.3	28*
24	16.5	15.0	62*
48	6.0	4.5	89*
96	1.4	> 0.1	100*

* significant at p = 0.05

RMS Conclusions

Glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.s./L. The 14-d EC_{50} value for inhibition of frond number was 12 mg a.s./L (95% CL = 11 - 14 mg a.s./L) and for tissue dry weight 20 mg a.s./L (95% CL = 18 – 22 mg a.s./L).The NOEC was determined to be 3.0 and 6.0 mg a.s./L for frond number and weight increase, respectively.

The study is considered to be valid and acceptable

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Glyphosat produces acute $L(E)C_{50}$ values in concentrations 18 - 22 mg/L for algae, 12 mg/L for aquatic plants, 84 mg/L for crustaceans and 47 mg/L for fish. Chronic NOEC values in concentrations of > 1 mg/L for algae and aquatic plants, > 10 mg/L for invertebrates and 1 mg/L for fish were determined.

The results of the test on the biodegradation of glyphosat in the water/sediment system show that glyphosat is considered not rapidly degradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

Glyphosat has a log $K_{o/w}$ of – 3.2. The experimentally derived kinetic BCF of 1.1 for glyphosat related to total radioactivity, whole fish is lower than the trigger of 500 (criterion for bioaccumulation potential conform Regulation EC 1272/2008).

CLP- Acute aquatic hazards

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of ≤ 1 mg/l is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

The lowest $L(E)C_{50}$ obtained for glyphosat are 18, 12, 84 and 47 mg/L in algae, aquatic plants, invertebrates and fish, respectively. Glyphosat therefore do not fulfil the criteria for classification as Aquatic Acute Cat. 1.

CLP - Aquatic chronic hazards

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 1 mg/L is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

Glyphosat is considered not rapidly degradable (see section 5.1.3). NOEC values for glyphosat are available for all trophic levels. The lowest NOEC is 1 mg/L obtained for fish. Glyphosat therefore fulfils criteria for classification as Aquatic Chronic Cat. 2.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Glyphosat fulfils the criteria for classification as Aquatic Chronic 2.

6 OTHER INFORMATION

None

7 REFERENCES

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
1	Acquavella, J. F.; Weber, J. A.; Cullen, M. R.; Cruz, O.A. et al.	1999	Human ocular effects from self-reported exposures to Roundup herbicides Human & Experimental Toxicology (paper) vol.18 (1999) 479-486 BVL-2309482, TOX2002-699	No	CAD DOW LIT MOT
2	Akanuma, M.	1995	HR-001: DNA Repair Test (Rec-Assay) IET 94-0141 GLP: Yes Published: No BVL-2309325, ASB2012-11477	No	ALS
3	Akanuma, M.	1995	HR-001: Reverse Mutation Test IET 94-0142 GLP: Yes Published: No BVL-2309291, ASB2012-11462	No	ALS
4	Alavanja, M. C. R.; Bonner, M. R.	2012	Occupational pesticide exposures and cancer risk: a review page 238-263 Journal of Toxicology and Environmental Health, Part B, 15: 238–263, 2012 GLP: No Published: Yes BVL-2716359, ASB2014-9173	No	LIT
5	Alavanja, M. C. R.; Ross, M. K.; Bonner, M. R.	2013	Increased cancer burden among pesticide applicators and others due to pesticide exposure page 120-142 CA Cancer J Clin 2013; 63: 120–142 GLP: No Published: Yes BVL-2716403, ASB2014-9174	No	LIT
6	Alavanja, M.C., Samanic, C., Dosemeci, M., Lubin, J., Tarone, R., Lynch, C.F., Knott, C., Thomas, K., Hoppin, J.A., Barker, J., Coble, J., Sandler, D.P., Blair, A.	2003	Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort Am J Epidemiol vol.157, 9 (2003) 800-814 GLP: No Published: Yes BVL-2309554, ASB2012-11535	No	LIT
7	Alvarez-Moya, C.; Reynoso Silva, M.; Valdez Ramírez, C.; et al.;	2014	Comparison of the in vivo and in vitro genotoxicity of Glyphosate Isopropylamine salt in three different organisms page 105-110 Genetics and Molecular Biology, 37, 1, 105-110 (2014) GLP: No Published: Yes BVL-2716311, ASB2014-6902	No	LIT
8	Anadon, A., Martinez-Larranaga, M.R.,	2009	Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats Toxicol Lett Vol.190, 1 (2009) 91-95	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
	Martinez, M.A., Castellano, V.J., Martinez, M., Martin, M.T., Nozal, M.J., Bernal, J.L.		GLP: No Published: Yes BVL-2309568, ASB2012-11542		
9	Andreotti, G., Freeman, L.E.B., Hou, L., Coble, J., Rusiecki, J., Hoppin, J.A., Silverman, D.T., Alavanja, M.C.R.	2009	Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort International Journal of Cancer vol.124, 10 (2009) 2495-2500 GLP: No Published: Yes BVL-2309572, ASB2012-11544	No	LIT
10	Anon.	2015	Lesion-related incidence data. RITA database RITA database tools ASB2015-2532		
11	Antal, A.	1981	Teratological investigation of Glyphosate in rats and rabbits GLP: No (5) Open (7) Published: No (6) Open (6) BVL-2331368, TOX9650160	Yes	ALK
12	Antoniou, M.; Habib, M.E.M; Howard, C.V.; Jennings, R.C.; Leifert, C.; Nodari, R.O.; Robinson, C.J.; Fagan, J.	2012	Teratogenic effects of Glyphosate-Based herbicides: Divergence of regulatory decisions from scientific evidence Journal of Environmental and Analytical Toxicology, 2012; S4:006. GLP: No Published: Yes BVL-2716227, ASB2012-15927	No	LIT
13	Arbuckle, T.E., Lin, Z.Q., Mery, L.S.	2001	An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population Environmental Health Perspectives vol.109, 8 (2001) 851-857 GLP: No Published: Yes BVL-2309574, ASB2012-11545	No	LIT
14	Arcelin, G.	2007	Glyphosate Technical material: Acute oral toxicity study in rats (Up and Down procedure) B02755; T007035-05 GLP: Yes Published: No BVL-2309111, ASB2012-11391	Yes	SYN
15	Arcelin, G.	2007	Glyphosate Technical material: Acute dermal toxicity study in rats B02766 (T007036-05) GLP: Yes Published: No BVL-2309141, ASB2012-11404	Yes	SYN
16	Arcelin, G.	2007	Glyphosate Technical material: Primary skin irritation study in rabbits (4-hour semi-occlusive application) B02777 (T007037-05) GLP: Yes Published: No BVL-2309193, ASB2012-11426	Yes	SYN
17	Arcelin, G.	2007	Glyphosate Technical material: Primary eye irritation study in rabbits B02788 (T007038-05) GLP: Yes Published: No	Yes	SYN

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309219, ASB2012-11437		
18	Atkinson, C.; Martin, T.; Hudson, P.; Robb, D.	1993	Glyphosate: 104 week dietary carcinogenicity study in mice 7793 ! IRI 438618 BVL-1345023, TOX9552382	Yes	BAY CAD CHE DOW MOD MOT NUD
19	Atkinson, C.; Perry, C. J.; Hudson, P.; Snodgrass, E.	1989	Glyphosate: 4 week dietary toxicity study in rats 5626 ! IRI 437462 BVL-1344983, TOX9552351	Yes	BAY CAD CHE DOW MOD MOT NUD
20	Atkinson, C.; Strutt, A. V.; Henderson, W.; Finch, J.; Hudson, P.	1993	Glyphosate: 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks) IRI 438623 ! IRI 7867 ! Page: 1-1510 BVL-1345018, TOX9750499	Yes	BAY CAD CHE DOW MOD MOT NUD
21	Bailey, J.; Hauswirth, J.; Stump, D.;	2013	No evidence of endocrine disruption by Glyphosate in male and female pubertal assays. Abstract The Toxicologist. 52nd Annual Meeting and ToxExpo, March 10-14, 2013, Texas, USA. GLP: No Published: Yes BVL-2716229, ASB2013-3464	No	LIT
22	Band, P.R., Abanto, Z., Bert, J., Lang, B., Fang, R., Gallagher, R.P., Le, N.D.	2011	Prostate Cancer Risk and Exposure to Pesticides in British Columbia Farmers Prostate vol.71, 2 (2011) 168-183 GLP: No Published: Yes BVL-2309594, ASB2012-11555	No	LIT
23	Benitez-Leite, S., Macchi, M., Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos Archives of Pediatrics 80 (3):377-378. vol.80, 3 (2009) 377-378 GLP: No Published: Yes BVL-2309612, ASB2012-11563	No	LIT
24	Beswick, E.; Millo, J.	2011	Fatal poisoning with Glyphosate - surfactant herbicide page 37-39 JICS Volume 12, Number 1, January 2011 GLP: No Published: Yes BVL-2716366, ASB2014-9283	No	LIT
25	Betts, C.J.	2007	Glyphosate Technical Material - Skin Sensitisation (Local Lymph Node Assay in the Mouse) GM8048-REG GLP: Yes Published: No BVL-2309245, ASB2012-11449	Yes	SYN
26	Bhide, M. B.	1988	Carcinogenicity and chronic toxicity study of Glyphosate (technical) of Excel Industries Ltd., Bombay BVL-2327344, TOX9551831	Yes	BCL LUX
27	Bhide, M. B.	1988	Report on effect of Glyphosate technical of Excel Industries Ltd., Bombay, on fertility and general reproductive performance (Segment I) BVL-2331649, TOX9551832	Yes	BCL LIT
28	Bhide, M. B.	1988	Report on effect of Glyphosate technical of Excel Industries Ltd., Bombay - on reproductive process segment II teratological study BVL-2328487, TOX9551834	Yes	BCL LUX

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29	Bhide, M. B.	1988	Report on effect of pesticides on reproductive process - Segment IV - three generation reproduction study with albino rats using Glyphosate technical of Excel Industries Ltd., Bombay BVL-2328485, TOX9551965	Yes	LIT LUX
30	Bhide, M. B.; Patil, U. M.; Vikrant, B.	1989	Rabbit teratology study with Glyphosate technical IIT 1086 BVL-2309462, TOX9551960	Yes	BCL EXC LUX
31	Bhide, R.M.	1997	Combined chronic toxicity / carcinogenicity of Glyphosate technical in Sprague Dawley rat 1231 GLP: No Published: No BVL-2309388, ASB2012-11489	Yes	EXC
32	Blagden, S. M.	1995	Glyphosate: Acute inhalation toxicity study four-hour exposure (nose only) in the rat 710/16 BVL-2332787, TOX9500247	Yes	HPQ
33	Blair, A., Freeman, L.B.	2009	Epidemiologic Studies in Agricultural Populations: Observations and Future Directions Journal of Agromedicine vol.14, 2 (2009) 125-131 GLP: No Published: Yes BVL-2309618, ASB2012-11566	No	LIT
34	Blech, S.; Stratmann, A.	1995	Glyphosate: ADME-study in rats - Final report A&M 038/94 BVL-2323314, TOX9552251	Yes	FSG
35	Bolognesi, C.; Bonatti, S.; Degan, P. et al.	1997	Genotoxic activity of Glyphosate and its technical formulation Roundup page 1957-1962 J. Agric. Food Chem. 1997, 45, 1957-1962 GLP: No (2) Open (1) Published: Open (1) Yes (2) BVL-2309628, BVL-2716350, Z59299	No	LIT
36	Botham, P. A.	1996	First revision to Glyphosate acid: 90 day feeding study in rats - incl. Individual animal data CTL/P/1599 ! PR 0663 BVL-2154311, TOX2000-1990	Yes	SYD SYN
37	Bradberry, S. M.; Proudfoot, A. T.; Vale, J. A.	2004	Glyphosate poisoning page 159-167 Toxicol Rev 2004, 23 (3), 159-167 GLP: No Published: Yes BVL-2309642, ASB2012-11576	No	LIT
38	Brammer, A.	1996	Glyphosate acid: 1 year dietary toxicity study in dogs CTL/P/5079 ! PD 1006 BVL-2154313, TOX2000-1992	Yes	SYD SYN
39	Brammer, A.	2001	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats CTL/PR1111 GLP: Yes Published: No BVL-2309368, ASB2012-11488	Yes	SYN
40	Brett, M. G	1990	Acute oral toxicity in the rat: Glyphosate technical	Yes	AGC EBR GTT SNC

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			R231 ! AGC-900823B ! AGC-101 BVL-1226624, TOX9500261		
41	Brett, M. G.	1990	Acute dermal toxicity study in the rat: Glyphosate technical AGC-900823A ! AGC-301 ! R232 BVL-2146638, TOX9551793	Yes	AGC GTT
42	Brewster, D. W.; Warren, J.; Hopkins, W. E.	1991	Metabolism of glyphosate in Sprague-Dawley rats: Tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose page 43-51 BVL-2146633, TOX9551791	Yes	DOE EGT FSG GTT LIT SIN
43	Brooker, A. J.; Brennan, C.; John, D. M.; Anderson, A.; Dawe, I. S.	1991	The effect of Glyphosate on pregnancy of the rabbit (incorporates preliminary investigations) CHV 45 u. 39 u. 40/901303 BVL-1345032, TOX9552391	Yes	BAY CAD CHE DOW MOD MOT NUD
44	Brooker, A. J.; Homan, B. A.; Hadley, J. C.; Offer, J. M.	1991	Dietary range finding study of glyphosate in pregnant rats and their juvenile offspring CHV 42/90619 BVL-1345026, TOX9552388	Yes	BAY CAD CHE DOW MOD MOT NUD
45	Brooker, A. J.; John, D. M.; Anderson, A.; Dawe, I. S.	1991	The effect of Glyphosate on pregnancy of the rat (incorporates preliminary investigation) CHV 43 u. 41/90716 BVL-1345030, TOX9552393	Yes	BAY CAD CHE DOW MOD MOT NUD
46	Brooker, A. J.; Myers, D. P.; Parker, C. A.; Offer, J. M.; Singh, H.; Anderson, A.; Dawe, I. S.	1992	The effect of dietary administration of Glyphosate on reproductive function of two generations in the rat CHV 47/911129 BVL-1345025, TOX9552389	Yes	BAY CAD CHE DOW MOD MOT NUD
47	Brown, J. C.; Ogilvie, S. W.	1995	Glyphosate technical 95%: Acute oral toxicity (LD50) test in rat 10670 ! IRI 556073 BVL-2332613, TOX9500377	Yes	MAR SIN
48	Burger, R.; Begemann, K.; Meyer, H.; Hahn, A.;	2009	Severe dyspnoea after spraying of a pesticide containing glyphosate. Lung damage histologically confirmed Clinical Toxicology (2009) 47, 506 ASB2013-11831		
49	Calandra, J. C.	1974	2-year chronic oral toxicity study with CP 67573 in albino rats B564 ! BTL-71-32 GLP: Open Published: No Z35230	Yes	
50	Callander, R.D.	1996	Glyphosate acid: An evaluation of mutagenic potential using S. typhimurium and E. coli CTL/P/4874 GLP: Yes Published: No BVL-2309313, ASB2012-11473	No	SYN
51	Campaña, H.; Pawluk, M. S.; López Camelo, J.	2010	Prevalencia al nacimiento de 27 anomalías congénitas seleccionadas, en 7 regiones geográficas de la Argentina. Births prevalence of 27 selected congenital anomalies in 7	No	LIT

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	S.; Grupo de Estudio del ECLAMC		geographic regions of Argentina page 409-417 Archivos Argentinos de Pediatría, 2010; 108(5): 409-417. GLP: No Published: Yes BVL-2716285, ASB2013-10559		
52	Canabrava Frossard de Faria, B.C.F.	2008	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical RF-3996.311.476.07 GLP: Yes Published: No BVL-2309185, ASB2012-11425	Yes	HAG
53	Canabrava Frossard de Faria, B.C.F.	2008	Acute Eye Irritation/Corrosion Study in Rabbits with Glyphosate Technical RF-3996.312.599.07 GLP: Yes Published: No BVL-2309213, ASB2012-11436	Yes	HAG
54	Carmichael, S. L.; Yang, W.; Roberts, E. M. et al.	2013	Hypospadias and residential proximity to pesticide applications page 216-1226 PEDIATRICS Volume 132, Number 5, November 2013 GLP: No Published: Yes BVL-2716407, ASB2014-9307	Yes	LIT
55	Carreon, T., Butler, M.A., Ruder, A.M., Waters, M.A., Davis-King, K.E., Calvert, G.M., Schulte, P.A., Connally, B., Ward, E.M., Sanderson, W.T., Heinemann, E.F., Mandel, J.S., Morten, R.F., Reding, D.J., Rosenmann, K.D., Talaska, G.	2005	Gliomas and farm pesticide exposure in women: The Upper Midwest Health Study Environmental Health Perspectives vol.113, 5 (2005) 546-551 GLP: No Published: Yes BVL-2309660, ASB2012-11585	No	LIT
56	Carter, L.	2009	Glyphosate - Acute Inhalation Toxicity Study in Rats 12107-08 GLP: Yes Published: No BVL-2309155, ASB2012-11411	Yes	HAG
57	Carvalho Marques, M.F.	1999	A micronucleus study in mice for glifosate técnico Nufarm RF-G12.79/99 GLP: Yes Published: No BVL-2309335, ASB2012-11482	Yes	NUF
58	Chan, P. C.; Mahler, J. F.	1992	NTP technical report on toxicity studies of Glyphosate administered in dosed feed to F344/N rats and B6C3F1 mice 92-3135 BVL-1344981, TOX9551954	Yes	BAY CAD CHE DOW EGT LIT LUX MOD MOT NUD
59	Chruscielska, K.; Brzezinski, J.; Grafstein, B. et al.	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 2. Studies on mutagenic activity	No	EGT LIT

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			Page: 21-25 Pestycydy, 2000, (3-4), 21-25. GLP: No Published: Yes BVL-2716167, ASB2013-9830		
60	Chruscielska, K.; Brzezinski, J.; Kahlhorn, D. et al.	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 3. Prenatal toxicity Page; 37-31 Pestycydy, 2000, (3-4), 27-31. GLP: No Published: Yes BVL-2716168, ASB2013-9831	No	EGT LIT
61	Chruscielska, K.; Brzezinski, J.; Kita, K. et al.	2000	Glyphosate: Evaluation of chronic activity and possible far - reaching effects - Part 1. Studies on chronic toxicity Page: 11-19 Pestycydy, 2000, (3-4), 11-20. GLP: No Published: Yes BVL-2716174, ASB2013-9829	No	LIT
62	Clay, P.	1996	Glyphosate acid: L5178Y TK+/- mouse lymphoma mutation assay CTL/P/4991 ! VV 0123 BVL-2154316, TOX2000-1994		SYD SYN
63	Cocco, P.; Satta, G.; Dubois, S.; Pili, C.; Pilleri, M.; Zucca, M.; Martine 't Mannetje, A.; Becker, N.; Benavente, Y.; de Sanjosé, S.; Foretova, L.; Staines, A.; Maynadié, M.; Nieters, A.; Brennan, P.; Miligi, L.; Ennas, M. G.; Boffetta, P.;	2012	Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study page 91-98 Occup Environ Med 2012;0:1–7 GLP: No Published: Yes BVL-2716321, ASB2014-7523	No	LIT
64	Coles, L.J., Thomas, O.N., Bartlett, A.J., Brooks, P.N	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat 434/016 GLP: Yes Published: No BVL-2309256, ASB2012-11451	Yes	NUF
65	Coles, R.J., Doleman, N.	1996	Glyphosate technical: Oral gavage teratology study in the rabbit 434/020 GLP: Yes Published: No BVL-2309448, ASB2012-11499	Yes	NUF
66	Colvin, L. B.; Miller, J. A.	1973	Final report on CP 67573 residue and metabolism. Part 9: The gross distribution of n-phosphonomethylglycine-14C in the rabbit 298 ! 9-23-760.06-7863 BVL-1345067, TOX9552353	Yes	BAY CAD CHE DOW MOD MON MOT NUD
67	Colvin, L. B.; Miller, J. A.	1973	CP 67573 residue and metabolism. Part 13: The dynamics of accumulation and depletion of orally ingested N-phosphonomethylglycine-14C	Yes	BAY CAD CHE DOW MOD

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			309 BVL-1345065, TOX9552355		MON MOT NUD
68	Costa, K. C.	2010	Amendment No. 1 to report: Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice 3996.402.395.07 BioagriI Laboratorios Ltda. GLP: Yes Published: No BVL-2715988, ASB2014-9284	Yes	Helm
69	Costa, K.C.	2008	Evaluation of the mutagenic potential of Glyphosate technical by micronucleus assay in mice RF - 3996.402.395.07 GLP: Yes Published: No BVL-2309333, ASB2012-11481	Yes	HAG
70	Cuthbert, J. A.; Jackson, D.	1989	Glyphosate technical: Acute dermal toxicity (limit) test in rats 243268/5884 BVL-2309119, TOX9300328	Yes	CHE DOW
71	Cuthbert, J. A.; Jackson, D.	1989	Glyphosate technical: Acute oral toxicity (limit) test in rats 5883 ! IRI 243268 BVL-1344956, TOX9552319	Yes	BAY CAD CHE DOW MOD MOT NUD
72	Cuthbert, J. A.; Jackson, D.	1989	Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs 5887 ! IRI 243268 BVL-1344980, TOX9552343	Yes	BAY CAD CHE DOW MOD MOT NUD
73	Dallegrave, E., Mantese, F.D., Coelho, R.S., Pereira, J.D., Dalsenter, P.R., Langeloh, A.	2003	The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats page 45-52 Toxicology Letters 142 (2003) 45-52 GLP: No Published: Yes BVL-2309692, ASB2012-11600		LIT
74	Dallegrave, E.; Mantese, F.D.; Oliveira, R.T.; Andrade A.J.; Dalsenter, P.R.; Langeloh, A.	2007	Glyphosat: Pre-and postnatal toxicity of the commercial glyphosate formulation in Wistar rats page 665-673 Arch Toxicol (2007) 81:665–673 GLP: No Published: Yes BVL-2309694, ASB2012-2721		LIT
75	Davies, D. J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat CTL/P/4940 GLP: Open (1) Yes (3) Published: No BVL-2154302, TOX2000-1977	Yes	SYD SYN
76	Davies, D. J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat CTL/P/4942 BVL-2154303, TOX2000-1978	Yes	SYD SYN
77	Davies, D. J.	1996	Glyphosat acid: Whole body autoradiography in the rat (10 mg/kg) CTL/P/4943 ! UR 0509 BVL-2154300, TOX2000-1980	Yes	SYD SYN

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78	Davies, D. J.	1996	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat following repeat dosing CTL/P/4944 BVL-2154304, TOX2000-1979	Yes	SYD SYN
79	De Roos, A.J., Blair, A., Rusiecki, J.A., et al.	2005	Cancer incidence among glyphosate-exposed pesticide applicators in the agricultural health study page 49-54 Environmental Health Perspectives, VOLUME 113, NUMBER 1 GLP: No Published: Yes BVL-2309704, ASB2012-11605	No	LIT
80	De Roos, A.J., Zahm, S.H., Cantor, K.P., Weisenburger, D.D., Holmes, F.F., Burmeister, L.F., Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men Occupational and Environmental Medicine vol.60, 9 (2003) GLP: No Published: Yes BVL-2309706, ASB2012-11606	No	LIT
81	Decker, U.	2007	Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats B02327 GLP: Yes Published: No BVL-2309161, ASB2012-11414	Yes	NUF
82	Dhinsa, N.K., Watson, P., Brooks, P.N	2007	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat 2060/0013 GLP: Yes Published: No BVL-2309418, ASB2012-11494	Yes	NUF
83	Dideriksen, L. H.; Skydsgaard, K.	1991	Assessment of acute oral toxicity of "Glyphosate technical" to mice - incl. Addendum 12321 BVL-1344955, TOX9552320	Yes	BAY CAD CHE DOW MOD MOT NUD
84	Do Amaral Guimaraes, S. P.	2008	Acute oral toxicity study in Wistar Hannover rats for Glyphosate technical RF-3996.305.475.07 GLP: Yes Published: No BVL-2309100, ASB2012-11389	Yes	HAG
85	Do Amaral Guimaraes, S.P.	2008	Acute Dermal Toxicity in Wistar Hannover Rats for Glyphosate Technical RF-3996.310.456.07 GLP: Yes Published: No BVL-2309135, ASB2012-11402	Yes	HAG
86	Doyle, C. E.	1996	Glyphosate acid: Acute oral toxicity study in rats CTL/P/4660 ! AR 5959 BVL-2154305, TOX2000-1982	Yes	SYD SYN
87	Doyle, C. E.	1996	Glyphosate acid: Acute dermale toxicity study in the rats CTL/P/4664 ! CR 3236 BVL-2154306, TOX2000-1983	Yes	SYD SYN
88	Doyle, C. E.	1996	Glyphosate acid: Skin irritation to the rabbit CTL/P/4695 ! EB 4365 BVL-2154308, TOX2000-1985	Yes	SYD SYN

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89	Doyle, C. E.	1996	Glyphosate acid: Skin sensitisation to the guinea pig CTL/P/4699 ! GG 6427 BVL-2154310, TOX2000-1987	Yes	SYD SYN
90	Durward, R.	2006	Glyphosate Technical: Micronucleus Test In The Mouse 2060/014 GLP: Yes Published: No BVL-2309327, ASB2012-11478	Yes	NUF
91	Eadie, A.; Barrins, C.; Cleere, W. F. et al.	1989	Glyphosate technical: 90 day oral toxicity study in the rats - incl. Amendment to Protocol BY-401 BY-891002 ! BY-401 BVL-2331648, TOX9551821	Yes	BCL
92	EFSA	2012	Final review of the Séralini et al. (2012a) publication on a 2-year rodent feeding study with Glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology EFSA Journal 2012;10(11):2986 ! EFSA-Q-2012-00842 EFSA Journal 2012; 10(11): 2986. vol.10, 11 (2012) 2986-2996 GLP: No Published: Yes BVL-2716077, ASB2012-15513	Yes	LIT
93	EFSA	2015	Peer Review Report on Glyphosate ASB2015-12200		
94	El-Zaemey, S.; Heyworth, J.	2013	Noticing pesticide spray drift from agricultural pesticide application areas and breast cancer: a case-control study Aust NZ J Public Health. 2013 GLP: No Published: Yes BVL-2716417, ASB2014-9473	Yes	LIT
95	Engel, L.S., Hill, D.A., Hoppin, J.A., Lubin, J.H., Lynch, C.F., Pierce, J., Samanic, C., Sandler, D.P., Blair, A., Alavanja, M.C.	2005	Pesticide use and breast cancer risk among farmers' wives in the agricultural health study American Journal of Epidemiology vol.161, 2 (2005) 121-135 GLP: No Published: Yes BVL-2309720, ASB2012-11613	No	MOD
96	Enomoto, A.	1997	HR-001: 24-Month Oral Chronic Toxicity and Oncogenicity Study in Rats, Vol. 1 (Seite 1-500) IET 94-0150 Vol.1 GLP: Yes Published: No BVL-2309360, ASB2012-11484	Yes	ALS
97	Eriksson, M., Hardell, L., Carlberg, M., Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis Int J Cancer vol.123, 7 (2008) 1657-1663 GLP: No Published: Yes BVL-2309722, ASB2012-11614	No	LIT
98	Flower, K.B., Hoppin, J.A., Lynch, C.F., Blair, A., Knott, C., Shore, D.L., Sandler, D.P.	2004	Cancer risk and parental pesticide application in children of agricultural health study participants Environmental Health Perspectives vol.112, 5 (2004) 361-635 GLP: No Published: Yes BVL-2309734, ASB2012-11620	No	LIT

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99	Flügge, C.	2009	Mutagenicity study of glyphosate TC in the salmonella typhimurium reverse mutation assay (in vitro) LPT 23916 GLP: Yes Published: No BVL-2309303, ASB2012-11468	No	HAG
100	Flügge, C.	2009	Micronucleus Test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration LPT 23917 GLP: Yes Published: No BVL-2309329, ASB2012-11479	Yes	HAG
101	Flügge, C.	2010	Mutagenicity study of Glyphosate TC in the salmonella typhimurium reverse mutation assay (in vitro) LPT 24880 GLP: Yes Published: No BVL-2309305, ASB2012-11469	No	HAG
102	Fox, V.	1998	Glyphosate acid: In vitro cytogenetic assay in human lymphocytes CTL/P/6050 ! SV 0777 BVL-2154314, TOX2000-1995	No	SYD SYN
103	Fox, V.; Mackay, J. M.	1996	Glyphosate acid: Mouse bone marrow micronucleus test CTL/P/4954 ! SM 0796 BVL-2154317, TOX2000-1996	Yes	SYD SYN
104	Freeman,L.B.	2009	Evaluation of agricultural exposures: the agricultural health study and the agricultural cohort consortium Reviews on Environmental Health vol.24, 4 (2009) 311-318 GLP: No Published: Yes BVL-2309740, ASB2012-11623	No	MOD
105	Fritschi, L., Benke, G., Hughes, A.M., Kricker, A..,Turner, J., Vajdic, C.M., Grulich, A., Milliken, S., Kaldor, J., Armstrong, B.K.	2005	Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma American Journal of Epidemiology vol.162, 9 (2005) 849-857 GLP: No Published: Yes BVL-2309746, ASB2012-11624	No	LIT
106	Gaou, I.	2007	Glyphosate Technical: 13-Week Toxicity Study By Oral Route (Capsule) In Beagle Dogs 29646 TCC GLP: Yes Published: No BVL-2309262, ASB2012-11454	Yes	NUF
107	Garry, V.F., Harkins, M.E., Erickson, L.L., Long-Simpson, L.K., Holland, S.E., Burroughs, B.L.	2002	Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA Environmental Health Perspectives 110:441-449 vol.110 (2002) 441-449 GLP: No Published: Yes BVL-2309750, ASB2012-11626	No	LIT
108	George, J., Prasad, S., Mahmood, Z.,	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach J Proteomics vol.73, 5 (2010) 951-964	No	LIT

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	Shukla, Y.		GLP: No Published: Yes BVL-2309766, ASB2012-11829		
109	Germany	1998	glyphosate (Monograph) 11 Dezember 1998 GLP: Open Published: Yes ASB2010-10302	Open	
110	Giknis, M. L. A.; Clifford, C. B.;	2005	Spontaneous neoplastic lesions in the Crl:CD1 (ICR) mouse in control groups from 18 month to 2 year studies ASB2007-5200	Yes	DOW
111	Goburdhun, R.	1990	Glyphosate: 52 week oral toxicity study in dogs 7502 ! IRI 642675 BVL-1344992, TOX9552384	Yes	BAY CAD CHE DOW MOD MOT NUD
112	Goburdhun, R.; Oshodi, R. O.	1989	Glyphosate: Oral maximum tolerated dose study in dogs 5660 ! IRI 640683 BVL-1344982, TOX9552352	Yes	BAY CAD CHE DOW MOD MOT NUD
113	Griffith, D.R.	2009	Glyphosate Tech: Acute Inhalation Toxicity (Nose only) Study in the Rat 2743/0001 GLP: Yes Published: No BVL-2309149, ASB2012-11408	Yes	EXC
114	Haag, V.	2007	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs 29647 TCC GLP: Yes Published: No BVL-2309274, ASB2012-11457	Yes	NUF
115	Hadfield, N.	2012	Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [14C]-glyphosate JV2182-REG GLP: Yes Published: No BVL-2309282, ASB2012-11459	No	EGT
116	Haferkorn, J.	2009	Acute oral toxicity study of Glyphosate TC in rats 23910 GLP: Yes Published: No BVL-2309092, ASB2012-11385	Yes	HAG
117	Haferkorn, J.	2009	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 23911 GLP: Yes Published: No BVL-2309151, ASB2012-11409	Yes	HAG
118	Haferkorn, J.	2009	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 23912 GLP: Yes Published: No BVL-2309127, ASB2012-11398	Yes	HAG
119	Haferkorn, J.	2009	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 23915	Yes	HAG

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			GLP: Yes Published: No BVL-2309231, ASB2012-11443		
120	Haferkorn, J.	2010	Acute oral toxicity study of Glyphosate TC in rats 24602 GLP: Yes Published: No BVL-2309096, ASB2012-11387	Yes	HAG
121	Haferkorn, J.	2010	Acute Inhalation Toxicity Study of Glyphosate TC In Rats 24603 GLP: Yes Published: No BVL-2309145, ASB2012-11406	No	HAG
122	Haferkorn, J.	2010	Acute oral toxicity study of Glyphosate TC in rats 24874 GLP: Yes Published: No BVL-2309094, ASB2012-11386	Yes	HAG
123	Haferkorn, J.	2010	Examination Of Glyphosate TC In The Skin Sensitisation Test In Guinea Pigs According To Magnusson And Kligman (Maximisation Test) 24879 GLP: Yes Published: No BVL-2309225, ASB2012-11440	Yes	HAG
124	Haferkorn, J.	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24604 GLP: Yes Published: No BVL-2309131, ASB2012-11400	Yes	HAG
125	Haferkorn, J.	2010	Examination of Glyphosate TC in Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test) LPT 24607 GLP: Yes Published: No BVL-2309233, ASB2012-11444	Yes	HAG
126	Haferkorn, J.	2010	Acute Inhalation Toxicity Study of Glyphosate TC in Rats LPT 24875 GLP: Yes Published: No BVL-2309153, ASB2012-11410	Yes	HAG
127	Haferkorn, J.	2010	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats LPT 24876 GLP: Yes Published: No BVL-2309129, ASB2012-11399	Yes	HAG
128	Hardell, L., Eriksson, M.	1999	A case-control study of non-Hodgkin lymphoma and exposure to pesticides Cancer vol.85, 6 (1999) 1353-1360 GLP: No Published: Yes BVL-2309788, ASB2012-11838	No	MOD
129	Hardell, L., Eriksson, M., Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies page 1043-1049	No	LIT

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			Leukemia and Lymphoma, 2002 VoI. 43 5), pp. 1043-1049 GLP: No Published: Yes BVL-2309790, ASB2012-11839		
130	Hatakenaka	1995	HR-001: Teratogenicity Study in Rats IET 94-0152 GLP: Yes Published: No BVL-2309444, ASB2012-11497	Yes	ALS
131	Heath, J.; Strutt, A.; Hudson, P.; Iswariah, V.	1993	Glyphosate: 3 week toxicity study in rats with dermal administration 7839 ! IRI 450881 BVL-1344993, TOX9552367	Yes	BAY CAD CHE DOW MOD MOT NUD
132	Heenehan, P. R.; Braun, W. G.; Rinehart, W. E.; Oleson, F. B.	1978	Acute oral LD50 of Glyphosate in rats 4-5438 ! 4880-77 ! BDN-77-428 BVL-2309107, Z35541	Yes	MON
133	Hideo, U.	1995	HR-001: Primary Eye Irritation study in rabbits IET 95-0034 GLP: Yes Published: No BVL-2309201, ASB2012-11430	Yes	ALS
134	Hideo, U.	1995	HR-001: Primary Dermal irritation study in rabbits IET 95-0035 GLP: Yes Published: No BVL-2309175, ASB2012-11420	Yes	ALS
135	Hideo, U.	1995	HR-001: Dermal sensitisation study in Guinea pigs IET 95-0036 GLP: Yes Published: No BVL-2309227, ASB2012-11441	Yes	ALS
136	Hodge, M. C. E.	1996	First revision to Glyphosate acid: 90 day feeding study in dogs CTL/P/1802 ! PD 0674 BVL-2154312, TOX2000-1991	Yes	SYD SYN
137	Hojo, H.	1995	HR-001: A Teratogenicity Study in Rabbits IET 94-0153 GLP: Yes Published: No BVL-2309446, ASB2012-11498	Yes	ALS
138	Honarvar, N.	2008	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse 1158500 GLP: Yes Published: No BVL-2309339, ASB2012-11483	Yes	SYN
139	Horner, S.A	1996	Glyphosate acid: Acute neurotoxicity study in rats CTL/P/4866 GLP: Yes Published: No BVL-2309464, ASB2012-11500	Yes	SYN
140	Howe, R. K.; Chott, R. C.; McClanahan, R. H.	1988	The metabolism of glyphosate in Sprague/Dawley rats. Part II. Identification, characterization, and quantitation of Glyphosate and its metabolites after intravenous and oral administration MSL-7206 ! 206300	Yes	BAY CAD CHE DOW MOD MON MOT NUD

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			BVL-1344949, TOX9552357		
141	IARC	2015	Glyphosate. IARC Monographs - 112 ASB2015-8421		
142	Jensen, J. C.	1991	Mutagenicity test: Ames salmonella assay with Glyphosate, batch 206-JaK-25-1 12323 BVL-1345005, TOX9552371	No	BAY CAD CHE DOW MOD MOT NUD
143	Jensen, J. C.	1991	Mutagenicity test: Micronucleus test with Glyphosate, batch 206-JaK-25-1 12324 BVL-1345016, TOX9552374	Yes	BAY CAD CHE DOW EGT MOD MOT NUD
144	Jensen, J. C.	1991	Mutagenicity test: In vitro mammalian cell gene mutation test with Glyphosate, batch 206-JaK-25-1 12325 BVL-1345007, TOX9552372	No	BAY CAD CHE DOW MOD MOT NUD
145	JMPR;	2004	WORLD HEALTH ORGANIZATION and FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Rome: Pesticide residues in food – 2004; Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues Rome, Italy, 20–29 September 2004 ASB2008-6266		
146	Johnson, D. E.	1982	21-day dermal toxicity study in rabbits IR-81-195 ! 401-168 BVL-1344994, TOX9552366	Yes	BAY CAD CHE DOW MOD MON MOT NUD
147	Johnson, I. R.	1997	Glyphosate acid: Eye irritation to the rabbit CTL/P/5138 ! FB 5378 BVL-2154309, TOX2000-1986	Yes	SYD SYN
148	Kachuri, L.; Demers, P. A.; Blair, A. et al.	2013	Multiple pesticide exposures and the risk of multiple myeloma in Canadian men DOI: 10.1002/ijc.28191 ! page 1846-1858 Int. J. Cancer: 133, 1846–1858 (2013) GLP: No Published: Yes BVL-2716322, ASB2014-8030	Yes	LIT
149	Karunanayake, C.P., Spinelli, J.J., McLaughlin, J.R., Dosman, J.A., Pahwa, P., McDuffie, H.H.	2011	Hodgkin Lymphoma and Pesticides Exposure in Men: A Canadian Case-Control Study Journal of Agromedicine vol.17, 1 (2011) 30-39 GLP: No Published: Yes BVL-2309844, ASB2012-11865	No	LIT
150	Kimmel, G.L.; Kimmel, C.A.; Williams, A.L.; DeSesso, J.M.;	2013	Evaluation of developmental toxicity studies of Glyphosate with attention to cardiovascular development page 79-95 Critical Reviews in Toxicology 2013; 43(2): 79-95. GLP: No Published: Yes BVL-2716230, ASB2013-3462	Yes	LIT
151	Kinoshita, M.	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Rats IET 94-0138 GLP: Yes Published: No	Yes	ALS

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			BVL-2309258, ASB2012-11452		
152	Kitazawa, T.	2013	IET historical control data on milignant lymphoma incidence in control ICR (Crj:CD-1) mice HR-001: Carcinogenicity study in mice (IET 94-0151) 13-C015 Institute of Environmental Toxicology GLP: No Published: No BVL-2716297, ASB2014-9146	No	EGT
153	Knezevich, A. L.; Hogan, G. K.	1983	A chronic feeding study of Glyphosate (Roundup technical) in mice 77-2061 ! (BDN-77-420) BVL-1345024, TOX9552381	Yes	BAY CAD CHE DOW MOD MON MOT NUD
154	Knowles, S. L.; Mookherjee, C. R.	1996	[14C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat 1413/2-1011 GLP: Yes Published: No BVL-2309072, ASB2012-11380	Yes	NUF
155	Koichi, E.	1995	HR-001: Acute inhalation toxicity study in rats IET 94-0155 GLP: Yes Published: No BVL-2309147, ASB2012-11407	Yes	ALS
156	Koller, V. J.; Fürhacker, M.; Nersesyan, A. et al.	2012	Cytotoxic and DNA-damaging properties of Glyphosate and Roundup in human-derived buccal epithelial cells DOI 10.1007/s00204-012-0804-8 Arch Toxicol (2012) 86: 805–813 GLP: No Published: Yes BVL-2716316, ASB2014-7618	Yes	LIT
157	Komura, H.	1995	HR-001: Acute oral toxicity study in mice IET 94-0133 GLP: Yes Published: No BVL-2309088, ASB2012-11383	Yes	ALS
158	Komura, H.	1995	HR-001: Acute oral toxicity study in rats IET 94-0134 GLP: Yes Published: No BVL-2309086, ASB2012-11382	Yes	ALS
159	Komura, Hitoshi	1995	HR-001: Acute dermal toxicity study in rats IET 94-0154 GLP: Yes Published: No BVL-2309123, ASB2012-11396	Yes	ALS
160	Koutros, S.; Andreotti, G.; Berndt, S. I. et al.	2011	Xenobiotic-metabolizing gene variants, pesticide use, and the risk of prostate cancer page 615-623 Pharmacogenetics and Genomics 2011, Vol 21 No 10 GLP: No Published: Yes BVL-2716382, ASB2014-9594	No	LIT
161	Krüger, M.; Schrödl, W.; Pedersen, I; Shehata, A. A.	2014	Detection of Glyphosate in malformed piglets 10.4172/2161-0525.1000230 ! ISSN: 2161-0525 JEAT Environmental & Analytical Toxicology vol.Volume 4, Issue 5 (2014) ASB2014-8935		

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162	Kuhn, J. O.; Harrison, L. V.	1996	CHA 440: Primary eye irritation study in rabbits 2981-96 ! S9-FF81-4.C41 STILLMEADOW, Inc. BVL-1344970, TOX1999-881	Yes	BAY CAD CHE DOW MOD MOT NUD
163	Kumar, D.P.S.	2001	Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice Toxi: 1559.CARCI-M GLP: Yes Published: No BVL-2309396, ASB2012-11491	Yes	FSG
164	Kuwahara	1995	HR-001: 13-week Oral Subchronic Toxicity Study in Mice IET 94-0136 GLP: Yes Published: No BVL-2309260, ASB2012-11453	Yes	ALS
165	Kyomu, M.	1995	HR-001: In vitro cytogenetics test IET 94-0143 GLP: Yes Published: No BVL-2309317, ASB2012-11475	No	ALS
166	Landgren, O., Kyle, R.A., Hoppin, J.A., Freeman, L.E.B., Cerhan, J.R., Katzmann, J.A., Rajkumar, S.V., Alavanja, M.C.	2009	Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study DOI 10.1182/blood-2009-02-203471 GLP: No Published: Yes BVL-2309874, ASB2012-11875	No	LIT
167	Lankas, G. P.	1981	A lifetime feeding study of Glyphosate in rats - Data evaluation report 77-2062 BVL-2154319, TOX2000-1997		SYD
168	Lankas, G. R.	1981	Lifetime feeding study of Glyphosate (Roundup technical) in rats 77-2062 ! BDN-77-416 BVL-2309378, TOX2000-595		CAD DOW MON MOT
169	Lee, H-L., Chen, K.-W., Chi, C.-H., Huang, J.-J., Tsai, L.-M.	2000	Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: a review of 131 cases Academic Emergency Medicine (paper) vol.7, 8 (2000) 906-910 GLP: No Published: Yes BVL-2309492, ASB2012-11512	No	LIT
170	Lee, W.J., Colt, J.S., Heineman, E.F., McComb, R., Weisenburger, D.D., Lijinsky, W., Ward, M.H.	2005	Agricultural pesticide use and risk of glioma in Nebraska, United States Occupational and Environmental Medicine vol.62 (2005) 786-792 GLP: No Published: Yes BVL-2309886, ASB2012-11882	No	LIT
171	Lee, W.J., Lijinsky, W., Heineman, E.F., Markin, R.S., Weisenburger, D.D., Ward, M.H.	2004	Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus Occupational and Environmental Medicine 61 (9):743-749 vol.61, 9 (2004) 743-749 GLP: No Published: Yes	No	LIT

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			BVL-2309888, ASB2012-11883		
172	Leuschner, J.	1995	Metabolism study of 14C-labelled glyphosate after single oral and intravenous administration to Sprague-Dawley rats 9202/95 BVL-2332809, TOX9650071	Yes	FSG
173	Leuschner, J.	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits 24877 GLP: Yes Published: No BVL-2309173, ASB2012-11419	Yes	HAG
174	Leuschner, J.	2009	Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits 24878 GLP: Yes Published: No BVL-2309199, ASB2012-11429	Yes	HAG
175	Leuschner, J.	2009	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 23913 GLP: Yes Published: No BVL-2309177, ASB2012-11421	Yes	HAG
176	Leuschner, J.	2009	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 23914 GLP: Yes Published: No BVL-2309205, ASB2012-11432	Yes	HAG
177	Leuschner, J.	2010	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits LPT 24605 GLP: Yes Published: No BVL-2309179, ASB2012-11422	Yes	HAG
178	Leuschner, J.	2010	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits LPT 24606 GLP: Yes Published: No BVL-2309207, ASB2012-11433	Yes	HAG
179	Levine, S.	2012	EDSP assays and regulatory safety studies provide a weight of evidence that Glyphosate is not an endocrine disruptor page 128 ASB2014-9609		
180	Li, A. P.	1983	CHO/HGPRT gene mutation assay with Glyphosate ML-83-155 ! 830079 BVL-1345008, TOX9552369	No	BAY CAD CHE DOW MOD MON MOT NUD
181	Li, A. P.	1983	In vivo bone marrow cytogenetics study of Glyphosate in Sprague-Dawley rats ML-83-236 ! 830083 BVL-1345015, TOX9552375	Yes	BAY CAD CHE DOW MOD MON MOT NUD

Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
182	Li, A. P.; Long, T. J.	1988	An evaluation of the genotoxic potential of Glyphosate Page: 537-546 ! L 361 BVL-2146649, TOX9500253	Yes	BCL GTT LIT
183	Lioi, M. B.; Scarfi, M. R.; Santoro, A. et al.	1998	Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro Page: 13-20 Mutation Research 403 1998. 13–20. GLP: No Published: Yes BVL-2716170, ASB2013-9836	No	LIT
184	Lioi, M. B.; Scarfi, M. R.; Santoro, A. et al.	1998	Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to Glyphosate, Vinclozolin, Atrazine and DPX-E9636 Page: 39-46 Environmental and Molecular Mutagenesis 32: 39-46 (1998). GLP: No Published: Yes BVL-2716169, ASB2013-9837	No	LIT
185	Lopez, S. L.; Aiassa, D.; Benitez-Leite, S.; Lajmanovich, R.; Manas, F.; Poletta, G.; Sanchez, N.; Simoniello, M. F.; Carrasco, A. E.;	2012	Pesticides used in South American GMO-based agriculture: A review of their effects on humans and animal models doi.org/10.1016/B978-0-444-59389-4.00002-1 ! page 41-75 Advances in Molecular Toxicology Volume 6. GLP: No Published: Yes BVL-2716286, ASB2013-10534	Yes	LIT
186	Macpherson, D.	1996	Glyphosat acid: Biotransformation in the rat CTL/P/5058 GLP: Open (1) Yes (3) Published: No BVL-2154301, TOX2000-1981	Yes	SYD SYN
187	Manas, F.; Peralta, L.; Raviolo, J.; Ovando, H. G.; Weyers, A.; Ugnia, L.; Gonzalez Cid, M.; Larripa, I.; Gorla, N.	2009	Genotoxicity of Glyphosate assessed by the comet assay and cytogenetic tests page 37-41 Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests GLP: No Published: Yes BVL-2309908, ASB2012-11892	No	LIT
188	Mañas, F.; Peralta, L.; Ugnia, L. et al.	2013	Oxidative stress and comet assay in tissues of mice administered Glyphosate and Ampa in drinking water for 14 days page 67-75 Journal of Basic & Applied Genetics GLP: No Published: Yes BVL-2716300, ASB2014-6909	No	LIT
189	McDonald, P.; Anderson, B. T.	1989	Glyphosate technical: Acute inhalation toxicity study in rats (limit test) 5993 ! IRI 642062 BVL-1344964, TOX9552329	Yes	BAY CAD CHE DOW MOD MOT NUD
190	McDuffie, H.H., Pahwa, P., McLaughlin, J.R., Spinelli, J.J., Fincham, S.,	2001	Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross Canada study of pesticides and health CanEpi 10:1155-1163 Cancer Epidemiol Biomarkers Prev vol.10, 11 (2001)	No	LIT

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	Dosman, J.A., Robson, D., Skinnider, L.F., Ch		1155-1163 GLP: No Published: Yes BVL-2009742, ASB2011-364		
191	McEwen, A. B.	1995	HR-001: Metabolism in the rat SNY 332/951256 GLP: Yes Published: No BVL-2309070, ASB2012-11379	Yes	ALS
192	McQueen, H., Callan, A.C., Hinwood, A.L.	2012	Estimating maternal and prenatal exposure to glyphosate in the community setting. International Journal of Hygiene and Environmental Health (2012) GLP: No Published: Yes BVL-2309926, ASB2012-11898	No	LIT
193	Merkel, D.	2005	Glyphosate Acid Technical: Acute oral toxicity up and down procedure in rats PSL 15274 GLP: Yes Published: No BVL-2309098, ASB2012-11388	Yes	HAG
194	Merkel, D.	2005	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats - Limit Test PSL 15275 GLP: Yes Published: No BVL-2309133, ASB2012-11401	Yes	HAG
195	Merkel, D.	2005	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats - Limit Test PSL 15276 GLP: Yes Published: No BVL-2309157, ASB2012-11412	Yes	HAG
196	Merkel, D.	2005	Eye Irritation/Corrosion Effects in rabbits (Oryctolagus cuniculus) of Glyphosate 95 TC PSL 15277 GLP: Yes Published: No BVL-2309211, ASB2012-11435	Yes	HAG
197	Merkel, D.	2005	Glyphosate Acid Technical - Primary Skin Irritation Study in Rabbits PSL 15278 GLP: Yes Published: No BVL-2309183, ASB2012-11424	Yes	HAG
198	Meyer-Carrive, I.; Bolt, A. G.	1994	Acute dermal toxicity of Glyphosate technical in the rat T1586.3.A BVL-2332616TOX9500378	Yes	MAR SIN
199	Milburn, G. M.	1996	Glyphosate acid: One year dietary toxicity study in rats CTL/P/5143 ! PR 1012 BVL-2154318, TOX2000-1998	Yes	SYD SYN
200	Mink, P. J.; Mandel, J. S.; Sceurman, B. K. et al.	2012	Epidemiologic studies of Gyphosate and cancer: A review page 440-452 Regulatory Toxicology and Pharmacology 63 (2012) 440–452 GLP: No Published: Yes BVL-2716296, ASB2014-9617	No	LIT

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201	Mink, P.J., Mandel, J.S., Lundin, J.I., Sceurman, B.K.	2011	Epidemiologic studies of glyphosate and non-cancer health outcomes: A review Regulatory Toxicology and Pharmacology vol.61, 2 (2011) 172-184 GLP: No Published: Yes BVL-2309938, ASB2012-11904	No	LIT
202	Mladinic, M., Berend, S., Vrdoljak, A.L., Kopjar, N., Radic, B., Zeljezic, D.	2009	Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro Environmental and Molecular Mutagenesis vol.50, 9 (2009) 800-807 GLP: No Published: Yes BVL-2309942, ASB2012-11906	No	LIT
203	Mladinic, M., Perkovic, P., Zeljezic, D.	2009	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay Toxicol Lett vol.189, 2 (2009) 130-137 GLP: No Published: Yes BVL-2309944, ASB2012-11907	No	LIT
204	Monge, P., Wesseling, C., Guardado, J., Lundberg, I., Ahlbom, A., Cantor, K.P., Weideroass, E., Partanen, T.	2007	Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica Scandinavian Journal of Work Environment & Health vol.33, 4 (2007) 293-303 GLP: No Published: Yes BVL-2309948, ASB2012-11909	No	LIT
205	Monroy, C.; Cortes, A.; Sicard, D. et al.	2005	Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate page 335-345 GLP: No Published: Yes BVL-2309950, ASB2012-11910		LIT
206	Mose, T.; Kjaerstad, M. B.; Mathiesen, L. et al.	2008	Placental passage of benzoic acid, caffeine, and glyphosate in an ex vivo human perfusion system page 984-991 GLP: No Published: Yes BVL-2309958, ASB2012-11914		LIT
207	Moxon, M. E.	1996	Glyphosate acid: Developmental toxicity study in the rabbits CTL/P/5009 ! RB 0709 BVL-2154323, TOX2000-2002	Yes	SYD SYN
208	Moxon, M. E.	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats CTL/P/6332 ! RR 0784 BVL-2154321, TOX2000-2000	Yes	SYD SYN
209	Moxon, M. E.	2002	Glyphosate acid: Developmental toxicity study in the rat - Amendment - 001 CTL/P/4819 ! RR0690 Central Toxicology Laboratory GLP: Yes Published: No BVL-2154322, ASB2012-10080	Yes	EGT SYD SYN Syngenta Agro
210	Multigner, L., Ndong, J.R.,	2008	Environmental pollutants and prostate cancer: epidemiological data	No	LIT

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	Oliva, A., Blanchet, P.		Gynecol Obstet Fertil vol.36, 9 (2008) 848-856 GLP: No Published: Yes BVL-2309964, ASB2012-11917		
211	Nagy, K.	2011	Glyphosate Technical - Acute inhalation Toxicity Study (Nose-only) in the Rat 11/054-004P GLP: Yes Published: No BVL-2309165, ASB2012-11415	Yes	SYN
212	Nakashima, N.	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs IET 94-0157 GLP: Yes Published: No BVL-2309276, ASB2012-11458	Yes	ALS
213	Ndong, J.R., Blanchet, P., Multigner, L.	2009	Pesticides and prostate cancer: epidemiological data Bulletin Du Cancer vol.96, 2 (2011) 171-180 GLP: No Published: Yes BVL-2309974, ASB2012-11922	No	LIT
214	Nordström, M.; Hardell, L.; Magnuson, A.; Hagberg, H.; Rask-Andersen, A.	1998	Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study Page: 2048-2052 British Journal of Cancer (1998) 77(11), 2048-2052. GLP: No Published: Yes BVL-2716207, TOX1999-687		BVL DOW LIT
215	Pahwa, P. P.; Karunanayak, C. P.; Dosman, J. A. et al.	2011	Soft-tissue sarcoma and pesticides exposure in men results of a canadian case-control study page 1279-1286 JOEM, Volume 53, Number 11, November 2011 GLP: No Published: Yes BVL-2716393, ASB2014-9625	Yes	LIT
216	Pahwa, P., Karunanayake, C.P., Dosman, J.A., Spinelli, J.J., McDuffie, H.H., McLaughlin, J.R.	2011	Multiple Myeloma and Exposure to Pesticides: A Canadian Case-Control Study Journal of Agromedicine vol.17, 1 (2012) 40-50 GLP: No Published: Yes BVL-2309996, ASB2012-11987	No	LIT
217	Parker, R. M.	1993	90 day range finding study of glyphosate in rats TSI 011-0001 BVL-2309252, TOX9650149	Yes	ALK
218	Patel, N. N.	2012	Micronucleus test of Glyphosate TGAI in mice 120709 ! 485-1-06-4696 ! DR-0112-6927-003 ! 10001701-27-1 JAI Research Foundation (JRF) GLP: Yes Published: No BVL-2715972, ASB2014-9277	Yes	DOW
219	Paumgartten, F. J. R.	2012	Pesticide exposure and poor pregnancy outcomes: weaknesses of the evidence // Exposição a agrotóxicos e resultados adversos da gravidez: a fragilidade da evidência Cad. Saúde Pública, Rio de Janeiro, 28(10):2009-2012. GLP: No Published: Yes BVL-2716287, ASB2013-10538	No	LIT

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220	Peluso, M.; Munnia, A.; Bolognesi, C.; Parodi, S.	1997	32P-Postlabeling detection of DNA adducts in mice treated with the herbicide Roundup page 55-59 Environmental and Molecular Mutagenesis 31:55±59 (1998) BVL-2310014, TOX1999-318		BVL DOW LIT
221	Perry, C. J.; Atkinson, C.; Strutt, A.; Henderson, W.; Hudson, P.	1991	Glyphosate: 13 week dietary toxicity study in rats 7136 ! IRI 437876 BVL-1344987, TOX9552364	Yes	BAY CAD CHE DOW MOD MOT NUD
222	Perry, C. J.; Atkinson, C.; Strutt, A.; Hudson, P.; Jones, M.	1991	Glyphosate: 13 week dietary toxicity study in mice 7024 ! IRI 437918 BVL-1344988, TOX9552363	Yes	BAY CAD CHE DOW MOD MOT NUD
223	Pinto, P.J.	1996	Glyphosate acid: 21-day dermal toxicity study in rats CTL/P/4985 GLP: Yes Published: No BVL-2309288, ASB2012-11461	Yes	SYN
224	Pooles, A.	2014	Glyphosate: Acute oral toxicity in the rat - fixed dose method 41401853 GLP: Yes Published: No BVL-2715934, ASB2014-9147	Yes	Albaugh
225	Pore, M. P.; Bhide, M. B.; Naik, P. Y.	1993	Skin sensitisation test in guinea-pigs with Glyphosate technical 95% min of Excel Industries Ltd., Bombay. IIT 1230 TOX9650652	Yes	LUX
226	Powles, P.; Hopkins, R.	1992	(14C)-glyphosate: Absorption and distribution in the rat - preliminary study 6365-676/1 BVL-1344948, TOX9552358	Yes	BAY CAD CHE DOW MOD MOT NUD
227	Powles, P.; Hopkins, R.	1992	(14C)-glyphosate: Absorption, distribution, metabolism and excretion in the rat 7006-676/2 BVL-2005461, TOX9300343	Yes	CHE DOW GTT MOD
228	Prakash, P.J.	1999	Subchronic (90 Day) Oral Toxicity Study With Glyphosate Technical In Beagle Dogs AND Test compound stability in experimental diet (dog feed) 1816 / 1817-R.FST GLP: Yes Published: No BVL-2309264, ASB2012-11455	Yes	FSG
229	Rank, J.; Jensen, A. G.; Skov, B. et al.	1992	Genotoxicity testing of the herbicide roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telephase test Mutat. Res. (1992) 29-36 GLP: Open Published: Open Z82234	Yes	
230	Rattray, N. J.	1996	Glyphosate acid: 4-hour acute inhalation toxicity study in rats	Yes	SYD SYN

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			CTL/P/4882 ! HR 2284 BVL-2154307, TOX2000-1984		
231	Reagan, E. L.; Laveglia, J.	1988	Acute oral toxicity of Glyphosate Batch/lot/nbr no. XLI-55 in Sprague/Dawley rats 88.2053.007 ! FD-88-29 BVL-2309105, Z35389	Yes	MON
232	Reagan, E. L.; Laveglia, J.	1988	Acute dermal toxicity of Glyphosate Batch/lot/nbr no. XLI-55 in new zealand white rabbits 88.2053.008 ! FD-88-29 BVL-1344960, TOX9552325	Yes	BAY CAD CHE DOW MOD MON MOT NUD
233	Reagan, E. L.; Laveglia, J.	1988	Primary eye irritation study of Glyphosate Batch/lot/nbr no. XLI-55 in new zealand white rabbits 88.2053.009 ! FD-88-29 BVL-2309215, Z35395	Yes	MON
234	Reyna, M. S.	1990	Two generation reproduction feeding study with Glyphosate in sprague-dawley rats + Appendices 1-6 MSL-10387 BVL-1345027, TOX9552387	Yes	BAY CAD CHE DOW MOD MOT NUD
235	Riberri do Val, R.	2007	Bacterial reverse mutation test (Ames Test) for Glifosato Técnico Helm 3393/2007-2.0AM-B GLP: Yes Published: No BVL-2309299, ASB2012-11466	No	HAG
236	Richeux, F.	2006	Glyphosate Technical: Skin Sensitisation in the Guinea Pig - Magnusson and Kligman Maximisation method 2060/009 (SMK-PH-05- GLP: Yes Published: No BVL-2309241, ASB2012-11448	Yes	NUF
237	Ridley, W.P.; Mirly, K.	1988	The metabolism of Glyphosate in Sprague/Dawley rats. I. Excretion and tissue distribution of Glyphosate and its metabolites following intravenous and oral administration MSL-7215 ! EHL 86139 ! ML-86-438 BVL-1344950, TOX9552356	Yes	BAY CAD CHE DOW MOD MON MOT NUD
238	Roe, F. J. C.; Tucker, M. J.;	1974	Recent developments in the design of carcinogenicity tests on laboratory animals Proc. Europ. Soc. Stud. Drug Tox., 15:171-177 (1974) ASB2015-2534		
239	Rossberger, St.	1994	Glyphosat: DNA repair test with primary rat hepatocytes 931564 ! 94-03-28 ro GLP: Open (4) Yes (7) Published: No (6) Open (5) BVL-2327069, TOX9400697		FSG
240	Roth, M.	2012	Glyphosate technical - Micronucleus assay in bone marrow cells of the mouse 1479200 ! TK0112981 Harlan Cytotest Cell Research GmbH (Harlan-CCR) GLP: Yes Published: No BVL-2716029, ASB2014-9333	Yes	Syngenta Agro
241	Schinasi, L.; Leon, M. E.;	2014	Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: A systematic review and meta-analysis		

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			doi:10.3390/ijerph110404449 ASB2014-4819		
242	Schreib, G.	2012	Reverse mutation assay using Bacteria (Salmonella typhimurium) with Glyphosate tech. 126159 BSL Bioservice Scientific Laboratories GmbH GLP: Yes Published: No BVL-2715924, ASB2014-9133	No	INA
243	Schroeder, R. E.; Hogan, G. K.	1981	Three generation reproduction study in rats with Glyphosate 77-2063 ! (BDN 77-417) BVL-1345029, TOX9552385	Yes	BAY CAD CHE DOW MOD MON MOT NUD
244	Séralini, G. E.; Clair, E.; Mesnage, R.; Gress, S.; Defarge, N.; Malatesta, M.; Hennequin, D.; Spiroux de Vendomois, J.	2012	Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize Page: 4221-4231 Food and Chemical Toxicology 50 (2012) 4221–4231 GLP: No Published: Yes BVL-2716397, ASB2012-15514	No	LIT
245	Sharp, V. M.	1995	Final report for oral and dermal LD 50 tests with Sanachem Glyphosate acid technical in rats, limit test 00917 BVL-2333109, TOX9650909	Yes	DOE SLE
246	Sharp, V. M.	1995	Final report for oral and dermal LD 50 tests with Sanachem Glyphosate 62 % IPA in rats, limit test 00926 BVL-2333108, TOX9650910	Yes	DOE SLE
247	Sher, S. P.	1974	Review article - Tumors in control mice: Literature tabulation Toxicol. Appl. Pharmacol. 30(1974)337-359 GLP: Open Published: Open Z22020	Yes	
248	Shirasu, Y.; Moriya, M.; Ota, T.; Ohta, T.	1978	Glyphosate: The report of mutagenic study with bacteria for CP 67573 - Microbial mutagenicity testing on CP67573 ET-78-241 BVL-1345064, TOX9552368	No	BAY CAD CHE DOW MOD MON MOT NUD
249	Simon, C.	2009	Glyphosate Technical: Acute oral toxicity study in rat C22864 GLP: Yes Published: No BVL-2309090, ASB2012-11384	Yes	EXC
250	Simon, C.	2009	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs - Maximization-Test C22908 GLP: Yes Published: No BVL-2309229, ASB2012-11442	Yes	EXC
251	Snell, K.	1994	Glyphosate: Acute oral toxicity (limit test) in the rat 710/14 BVL-2332785, TOX9500245	Yes	HPQ

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252	Snell, K.	1994	Glyphosate: Acute dermal toxicity (limit test) in the rat 710/15 BVL-2332786, TOX9500246	Yes	HPQ
253	Snell, K.	1994	Glyphosate: Magnusson & Kligman maximisation study in the guinea pig 710/19 BVL-2332789, TOX9500250	Yes	HPQ
254	Sokolowski, A.	2007	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05068) 1061401 GLP: Yes Published: No BVL-2309293, ASB2012-11463	No	NUF
255	Sokolowski, A.	2007	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05070) 1061402 GLP: Yes Published: No BVL-2309295, ASB2012-11464	No	NUF
256	Sokolowski, A.	2007	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05067) 1061403 GLP: Yes Published: No BVL-2309297, ASB2012-11465	No	NUF
257	Sokolowski, A.	2009	Glyphosate technical - Salmonella typhimurium and Escherichia coli Reverse Mutation Assay 1264500 GLP: Yes Published: No BVL-2309315, ASB2012-11474	No	SYN
258	Sokolowski, A.	2010	Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine 1332300 GLP: Yes Published: No BVL-2309307, ASB2012-11470	No	HAG
259	Son, W.-C.; Gopinath, C.;	2004	Early occurrence of spontaneous tumors in CD-1 mice and Sprague–Dawley rats DOI: 10.1080/01926230490440871 Toxicologic Pathology, 32:371–374, 2004 ASB2015-2533		
260	Sribanditmongkol, P.; Jutavijittum, P.; Pongraveevongsa, P.; Wunnapuk, K.; Durongkadech, P.	2012	Pathological and toxicological findings in Glyphosate-surfactant herbicide fatality Page: 234-237 Am J Forensic Med Pathol 2012;33: 234Y237 GLP: No Published: Yes BVL-2716398, ASB2014-9731	No	LIT
261	Stout, L. D.; Johnson, C. W.	1987	90 day study of Glyphosate administered in feed to Sprague-Dawley rats MSL-7375 ! ML-86-351 ! EHL 86128 BVL-1344989, TOX9552362	Yes	BAY CAD CHE DOW MOD MON MOT NUD
262	Stout, L. D.; Ruecker, F. A.	1990	Chronic study of Glyphosate administered in feed to albino rats - Appendix 1-6	Yes	BAY CAD CHE DOW

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			MSL 10495 ! ML-87-148 BVL-1345021, TOX9300244		MOD MON MOT NUD
263	Sugimoto, K.	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice IET 940151 GLP: Yes Published: No BVL-2309415, ASB2012-11493	Yes	ALS
264	Suresh, T. P.	1991	Acute oral toxicity study with Glyphosate technical (FSG 03090 H/05 march 90) in Wistar rats ES.874.AOR ! ES-GPT-AOR ! TOXI-874/1990 BVL-2323967, TOX9551088	Yes	FSG
265	Suresh, T. P.	1991	Acute oral toxicity study with Glyphosate technical (FSG 03090 H/05 march 90) in swiss albino mice ES.875.AOM ! ES-GPT-AOM ! TOXI-875/1990 BVL-2324773, TOX9551089	Yes	FSG
266	Suresh, T. P.	1991	Acute dermal toxicity study with Glyphosate technical (FSG 03090 H/05 march 90) in Wistar rats ES.876.ADR ! ES-GPT-ARD ! TOXI-876/1990 BVL-2332810, TOX9551090	Yes	FSG
267	Suresh, T. P.	1991	Glyphosat techn. (FSG 03090 H/05 March 1990): Teratogenicity study in Wistar rats ES.883.TER-R ! TOXI-883/1991 ! ES-GPT-TER-R BVL-2328595, TOX9551105	Yes	FSG
268	Suresh, T. P.	1992	Glyphosat techn. (FSG 03090 H/05 March 1990): 90 day oral toxicity study in wistar rats TOXI-882/1991 ! ES-GPT-90 OR ! ES-882 90 OR BVL-2326328, TOX9551096	Yes	FSG
269	Suresh, T. P.	1996	Combined chronic toxicity and carcinogenicity study with Glyphosate technical in Wistar rats TOXI-886/1996 ! ES-GPT-C.C-R ! TOXI 886.C.C-R BVL-2309343, TOX9651587	Yes	FSG
270	Suresh, T. P. et al.	1991	28-day dietary study in rats on Glyphosate technical ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR BVL-2326272, TOX9551095	Yes	FSG MOD
271	Suresh, T. P. et al.	1992	Glyphosate technical (FSG 03090 H/05, March 1990): Dominant lethal test in wistar rats 888-DLT ! TOXI-888/1992 ! ES-GPT-DLT BVL-2327264, TOX9551102	Yes	FSG
272	Suresh, T. P. et al.	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Teratogenicity study in rabbits 884-TER-RB ! TOXI-884/1992 ! ES-GPT-TER-RB BVL-2309457, TOX9551106	Yes	FSG
273	Suresh, T. P. et al.	1994	28-day dietary study in rats on glyphosate technical - Amendment ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR GLP: Open Published: No Z102035	Yes	
274	Suresh, T. P. et al.	1994	28-day dietary study in rats on glyphosate technical - Second amendment	Yes	

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			ES.881.28 DDR ! TOXI-881/1991 ! ES-GPT-28 DDR GLP: Open Published: No Z102043		
275	Suresh, T. P.; Ponnanna, D.; Asha, M. et al.	1994	Glyphosate technical (FSG 03090 H/05 March 1990): Genetic toxicology - In vivo mammalian bone marrow cytogenetic test 890-MUT-CH.AB ! TOXI-890/1993 ! ES-GPT-MUT-CH.AB BVL-2327261, TOX9400323	Yes	FSG
276	Suresh, T. P.; Rajendran, S.; Shivakumar S.Hosamath et al.	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Two generation reproduction study in wistar rats 885-RP-G2 ! TOXI-885/1993 ! ES-GPT-RP-G2 BVL-2309427, TOX9300009	Yes	FSG
277	Suresh, T.P.	1993	Glyphosate technical (FSG 03090 H/05 March 1990): Mutagenicity-micronucleus test in swiss albino mice 889-MUT.MN ! TOXI-889/1993 ! ES-GPT-MUT-MN BVL-2327258, TOX9551100	Yes	FSG
278	Taddesse-Heath, L.; Chattopadhyay, S. K.; Dillehay, D. L.; et al.;	2000	Lymphomas and high-level expression of murine leukemia viruses in CFW mice J. Virol. 74(2000)15:6832-6837 ASB2015-2535		
279	Takahashi, K.	1997	HR-001: A two-generation reproduction study in rats IET 96-0031 GLP: Yes Published: No BVL-2309425, ASB2012-11495	Yes	ALS
280	Talvioja, K.	2007	GLYPHOSATE TECHNICAL (NUP05068): Acute dermal toxicity study in rats B02283 GLP: Yes Published: No BVL-2309137, ASB2012-11403	Yes	NUF
281	Talvioja, K.	2007	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) B02294 GLP: Yes Published: No BVL-2309171, ASB2012-11418	Yes	NUF
282	Talvioja, K.	2007	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits B02305 GLP: Yes Published: No BVL-2309197, ASB2012-11428	Yes	NUF
283	Talvioja, K.	2007	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test B02316 GLP: Yes Published: No BVL-2309223, ASB2012-11439	Yes	NUF
284	Talvioja, K.	2007	GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats BO2272 GLP: Yes Published: No	Yes	NUF

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			BVL-2309103, ASB2012-11390		
285	Tasker, E. J.; Rodwell, D. E.; Jessup, D. C.	1980	Glyphosate: Teratology study in rats 401-054 ! IR-79-016 BVL-1345031, TOX9552392	Yes	BAY CAD CHE DOW MOD MON MOT NUD
286	Tasker, E. J.; Rodwell, D. E.; Jessup, D. C.	1980	Glyphosate: Teratology study in rabbits 401-056 ! IR-79-018 BVL-1345033, TOX9552390	Yes	BAY CAD CHE DOW MOD MON MOT NUD
287	Tavaszi, J.	2011	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) 10/218-001P GLP: Yes Published: No BVL-2309113, ASB2012-11392	Yes	SYN
288	Tavaszi, J.	2011	Glyphosate Technical: Acute eye irritation study in rabbits 10/218-005N GLP: Yes Published: No BVL-2309221, ASB2012-11438	Yes	SYN
289	Thompson, P.	2014	Glyphosate: Reverse mutation assay 'Ames test' using Salmonella typhimurium and Escherichia coli 41401854 GLP: Yes Published: No BVL-2715935, ASB2014-9148	Yes	Albaugh
290	Thompson, P.W.	1996	Technical glyphosate: Reverse mutation assay "Ames test" using Salmonella typhimurium and Escherichia coli 434/014 GLP: Yes Published: No BVL-2309311, ASB2012-11472	No	NUF
291	Tornai, A.	1994	Repeated dose 28-day dermal toxicity study with Glyphosate in rabbits GLY-94-410/N ! MÜF 214/94 BVL-2309284, TOX9650151	Yes	ALK MON
292	Tornai, A.; Kovacs, C.; Rozsnyoi, F. et al.	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute inhalation toxicity in rats GHA-94-403/R BVL-2331355, TOX9650144	Yes	ALK
293	Tornai, A.; Rozsnyoi, F. Turczer, K. et al.	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute oral toxicity in rats GHA-94-401/R BVL-2331353, TOX9650142	Yes	ALK
294	Tornai, A.; Rozsnyoi, F. Turczer, K. et al.	1994	Glyphosate (Alkaloida, Tiszavasvari): Acute dermal toxicity in rats GHA-94-402/R BVL-2331354, TOX9650143	Yes	ALK
295	Török-Bathó, M.	2011	Glyphosate technical - Local lymph node assay in the mouse - Final report amendment 2 10/218-037E GLP: Yes Published: No	Yes	SYN

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			BVL-2309247, ASB2012-11450		
296	Tos, E. G.; Maraschin, R.; Orlando, L.	1994	Glyphosate technical: Acute oral toxicity study in mice 940020 ! PRO629 BVL-2331271, TOX9551624	Yes	IPC
297	Tucker, M. J.	1979	The effect of long-term food restriction on tumours in rodents Int. J. Cancer: 23, 803-807 (1979) GLP: Open Published: Open Z83266	Yes	
298	van de Waart, E. J.	1995	Evaluation of the ability of Glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) 141918 BVL-2146653, TOX9651525	No	GTT
299	Vereczkey,L.; Csanyi, E.	1992	18 month carcinogenicity study of Glyphosate in mice 24 151/92 ! 8010 BVL-2331365, TOX9650154	Yes	ALK
300	Walker, D. J.; Jones, J. R.	1992	Glyphosate technical: Acute oral toxicity (limit test) in the rat 134/37 BVL-2331643, TOX9551810	Yes	BCL
301	Walker, D. J.; Jones, J. R.	1992	Glyphosate technical: Acute dermal toxicity (limit test) in the rat 134/38 BVL-2331645, TOX9551813	Yes	BCL
302	Walker, D. J.; Pateman, J. R.; Jones, J. R.	1991	Luxan Glyphosate techn.: Magnusson & Kligman maximisation study in the guinea pig 349/11 BVL-2142260, TOX9551796	Yes	AGC GTT LUX UPL
303	Wallner, B.	2010	Reverse Mutation Assay using Bacteria (Salmonella typhimurium) with Glyphosate TC BSL 101268 GLP: Yes Published: No BVL-2309309, ASB2012-11471	No	HAG HEL
304	Weichenthal, S., Moase, C., Chan, P.	2010	A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort Environ Health Perspect vol.118, 8 (2010) 1117-1125 GLP: No Published: Yes BVL-2310122, ASB2012-12048	No	LIT
305	Wood, E., Dunster, J., Watson, P. Brooks, P.	2009	Glyphosate Technical: Dietary combined chronic toxicity / carcinogenicity study in the rat SPL2060-0012 GLP: Yes Published: No BVL-2309391, ASB2012-11490	Yes	NUF
306	Wood, E., Dunster, J., Watson, P., Brooks, P.	2009	Glyphosate Technical: Dietary carcinogenicity study in the mouse SPL 2060-0011 GLP: Yes Published: No BVL-2309412, ASB2012-11492	Yes	NUF

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307	Wood, E.;	2010	Historical Incidence of Malignant lymphoma in CD-1 Mouse ASB2015-2531		
308	Wrenn, J. M.; Rodwell, D. E.; Jessup, D. C.	1980	Dominant lethal mutagenicity assay with technical Glyphosate in mice 401-064 ! IR-79-014 BVL-1345017, TOX9552377	Yes	BAY CAD CHE DOW MOD MON MOT NUD
309	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells in vitro 434/015 GLP: Yes Published: No BVL-2309319, ASB2012-11476	No	NUF
310	Yang, W.; Carmichael, S. L.; Roberts, E. M. et al.	2013	Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California page 1-9 American Journal of Epidemiology Advance Access published February 18, 2014 GLP: No Published: Yes BVL-2716461, ASB2014-9644	No	LIT
311	Yoshida, A.	1996	HR-001: 13-week Oral Subchronic Toxicity Study in Dogs IET 94-0158 GLP: Yes Published: No BVL-2309269, ASB2012-11456	Yes	ALS
312	You, J.	2009	Glyphosate: Acute oral toxicity study (UDP) in rats 12170-08 GLP: Yes Published: No BVL-2309084, ASB2012-11381	Yes	HAG
313	You, J.	2009	Glyphosate - Acute Dermal Toxicity Study in Rats 12171-08 GLP: Yes Published: No BVL-2309121, ASB2012-11395	Yes	HAG
314	You, J.	2009	Glyphosate - Acute Eye Irritation Study in Rabbits 12172-08 GLP: Yes Published: No BVL-2309209, ASB2012-11434	Yes	HAG
315	You, J.	2009	Glyphosate - Acute Dermal Irritation Study in Rabbits 12173-08 GLP: Yes Published: No BVL-2309181, ASB2012-11423	Yes	HAG
316	Zelenak	2011	Glyphosate Technical - Acute Dermal Toxicity Study in Rats - Final Report Amendmend 1 10/218-002P GLP: Yes Published: No BVL-2309143, ASB2012-11405	Yes	SYN
317	Zelenák, V.	2011	Glyphosate Technical - Primary skin irritation study in rabbits - Final report Amendment 1 10/218-006N GLP: Yes Published: No	Yes	SYN

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			BVL-2309195, ASB2012-11427		
318	Zoriki Hosomi, R.	2007	Mammalian Erythrocyte Micronucleus Test for Glifosato Técnico Helm 3393/2007-3.0MN-B GLP: Yes Published: No BVL-2309331, ASB2012-11480	Yes	HAG
319	Zouaoui, K.; Dulaurent, S.; Gaulier, J. M. et al.	2012	Determination of Glyphosate and AMPA in blood and urine from humans: About 13 cases of acute intoxication page e1-e6 Forensic Science International xxx (2012) xxx–xxx GLP: No Published: Yes BVL-2716400, ASB2014-9734	Yes	LIT
320	Alavanja, M.C.R.; Sandler, D.P.; McMaster, S.B. et al.	1996	The agricultural health study page 362-369 Environmental Health Perspectives, Vol. 104, No 4 Published: Yes ASB2015-7849		
321	Blair, A.; Thomas, K.; Coble, J. et al.	2011	Impact of pesticide exposure misclassification on estimates of relative risks in the agricultural health study page 537-541 Occup. Environ. Med. 68(7) doi:10.1136/oem.2010.059469 Published: Yes ASB2015-7868		
322	Dennis, L.K.; Lynch, C.F.; Sandler, D.P. et al.	2010	Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study page 812-817 Environmental Health Perspectives, Vol. 118, No 6 doi:10.1289/ehp.0901518 ! PMID:20164001 Published: Yes ASB2015-8439		
323	De Roos, A.J.; Svec, M.A.; Blair, A. et al.	2005	Glyphosate results revisited: De Roos et al. respond page A366-A367 Environmental Health Perspectives, Vol. 113, No 6 doi:10.1289/ehp.113-a366 Published: Yes ASB2015-8437		
324	Lee, W.J.; Sandler, D.P.; Blair, A. et al.	2007	Pesticide use and colorectal cancer risk in the Agricultural Health Study page 339-346 Int. J. Cancer. 121(2) doi:10.1002/ijc.22635 Published: Yes ASB2015-8228		
325	Sorahan, T.	2015	Multiple myeloma and Glyphosate use: A re-analysis of US Agricultural Health Study (AHS) data page 1548-1559 Int. J. Environ. Res. Public Health, Vol. 12 doi:10.3390/ijerph120201548 ASB2015-2284		
326	Brown, L.M.;	1990	Pesticide exposures and other agricultural risk factors for		

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	Blair, A.; Gibson, R. et al.		leukemia among men in Iowa and Minnesota Page 6585-6591 Cancer Res. 50(20) PMID: 2208120 Published: Yes TOX2003-999		
327	Brown, L. M.; Burmeister, L. F.; Everett, G. D. et al.	1993	Pesticide exposures and multiple myeloma in Iowa men Page 153-156 Cancer Causes and Control, Vol. 4 Published: Yes BVL-1968123, TOX2002-1000		
328	Cantor, K.P.; Blair, A.; Everett, G. et al.	1992	Pesticides and Other Agricultural Risk Factors for Non-Hodgkin's Lymphoma among Men in Iowa and Minnesota Page 2447-2455 Cancer Research, Vol. 52 Published: Yes ASB2015-7885		
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Number	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Published: Yes ASB2015-8078		
334	JMPR	2016	Joint FAO/WHO Meeting on Pesticide Residues, Geneva, 9–13 May 2016, Summary Report pages: 6 http://www.who.int/foodsafety/jmprsummary2016.pdf?ua=1 Published: Yes ASB2016-4292		
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			GLP: Y, published: N BVL-2310947		
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345	Smyth, D.V., Shillabeer, N., Morris, D.S., Wallace, S.J.	1996	Glyphosate acid: Toxicity to blue-green alga Anabaena flos-aquae BL5698/B SYN GLP: Yes, published: No BVL-2310970	No	SYN
346	Smyth, D.V., Kent, S.J., Morris, D.S., Shearing, J.M., Shillabeer, N.	1996	Glyphosate acid: Toxicity to the marine alga Skeletonema costatum BL5684/B SYN GLP: Yes, published: No BVL-2310972	No	SYN
347	Smyth, D.V., Kent, S.J., Morris, D.S., Cornish, S.K., Shillabeer, N	1996	GLYPHOSATE ACID: Toxicity to duckweed (Lemna gibba) BL5662/B SYN GLP: Yes, published: No BVL-2310988	No	SYN

8 ANNEXES

- Final Addendum to the Renewal Assessment Report on Glyphosate (containing the public version of the RAR on glyphosate, Addendum 1 to RAR on glyphosate ("Assessment of IARC Monographs Volume 112 (2015): Glyphosate") and Addendum 1 to RAR on glyphosate, Part Ecotoxicology ("Assessment of IARC Monographs Volume 112 (2015): Glyphosate)
- EFSA Conclusion on pesticide peer review, EFSA Journal 2015;13(11):4302
- Confidential Annex

Health Canada Pest Management Regulatory Agency (PMRA)

Glyphosate Re-evaluation Decision

April 2017

Frequently Asked Questions on the Re-evaluation of Glyphosate

28 April 2017

Health Canada's Pest Management Regulatory Agency (PMRA) conducted a rigorous scientific re-evaluation for glyphosate based on relevant data and information from registrants, published scientific reports, federal and provincial governments, and other regulatory agencies. The proposed re-evaluation decision document was published in April 2015 for consultation (PRVD2015-01). Heath Canada considered all comments received and determined that it will continue the registration of products that contain glyphosate with changes to product labels that will provide Canadians with additional information on how to use these products safely.

The following are some commonly asked questions on the use of glyphosate in Canada. If you have any further questions regarding the glyphosate re-evaluation decision, or about any other pesticide issue, please contact Health Canada's Pest Management Information Service.

Q1. What is glyphosate used for in Canada?

Glyphosate, marketed under brand names such as Roundup™ and Vision™, is the most widely used herbicide in Canada. It plays an important role in weed management for both agricultural production and non-agricultural land management. Glyphosate products are used to control many weeds, including many invasive weeds and toxic plants, such as poison ivy.

Q2. What were the main findings of Health Canada's re-evaluation?

Health Canada has carried out a rigorous science-based re-evaluation for pesticides containing glyphosate to ensure that they continue to meet modern standards for human health and environmental protection and provide value. The findings

are that, when used according to the label instructions, products containing glyphosate are not expected to pose risks of concern to human health or the environment.

Label directions such as those described later in this document are intended to further reduce exposure. For example, as glyphosate is a herbicide, it may harm non-target terrestrial and aquatic plants. Therefore, spray buffer zones are being required to protect sensitive plants from spray drift.

Q3. What are the new label changes?

Manufacturers are required to make label changes on product labels no later than 24 months after the publication of the re-evaluation decision on glyphosate.

As a result of the re-evaluation, the Department is requiring the following information to be conveyed through statements on labels:

Human Health

- To protect commercial and residential applicators: glyphosate is not to be applied using hand-wicking or hand-daubing methods, which involve applying the herbicide directly by hand, or with a hand-held tool, on individual plants.
- To protect workers entering treated sites: a restricted-entry interval of 12 hours is required for agricultural uses.

- To protect bystanders: a statement is required indicating that the product is to be applied only when the potential for drift to areas of human habitation or areas of human activity, such as houses, cottages, schools and recreational areas, is minimal.

Environment

- Environmental hazard statements will be added to inform users of toxicity to non-target species.
- Spray buffer zones are required, to protect non-target terrestrial and aquatic habitats.
- To reduce the potential for runoff of glyphosate to adjacent aquatic habitats, precautionary statements are required (for sites with characteristics that may be conducive to runoff and when heavy rain is forecasted). In addition, a vegetative strip between the treatment area and the edge of a water body is recommended to reduce runoff of glyphosate to aquatic areas.

Q4. What information did PMRA consider during the re-evaluation?

PMRA considered relevant data and information from registrants, published scientific reports, federal and provincial governments, and other regulatory agencies. Rigorous scientific evaluations were conducted to determine whether

glyphosate would cause any negative effects to people, animals, birds, insects, plants as well as on soil and water, when used according to label directions.

Q5. What other ingredients are in pesticides that contain glyphosate?

Pesticides, including glyphosate products, are marketed in different formulations, such as solutions and granules. Other substances called formulants are intentionally added to pesticides to improve how they work, such as making them more soluble or spreadable so they can be more effective in destroying weeds. Both the active ingredient glyphosate and its formulated products were considered during the re-evaluation.

Certain glyphosate products also contain polyethoxylated tallow amines (POEA), which function as surfactants. No risk of concerns to human health or the environment were identified provided that products contained no more than 20% POEA by weight and proposed label directions (including larger spay buffer zones for products that contain POEA) are followed. All currently registered glyphosate end-use products in Canada meet the 20% limit.

Q6. Was the public consulted on the glyphosate re-evaluation decision? What did Health Canada do with the comments received?

Health Canada’s re-evaluation program ensures that registered pesticides regularly undergo re- evaluation, using internationally accepted assessment techniques and current scientific information. This is a legal requirement under the *Pest Control Products Act*.

As part of this process, Health Canada published the proposed re-evaluation decision on glyphosate for public consultation in 2015. Comments were received from various stakeholders including registrants, growers, and the public. All comments received during the consultation period were taken into consideration. These comments and new information resulted in only minor revisions, which are reflected in the final re-evaluation decision.

Q7. Why does Health Canada consider glyphosate as unlikely to be a cancer risk while the World Health Organization's International Agency

of Research on Cancer has deemed glyphosate as "possibly carcinogenic to humans?"

Hazard classifications are not the same as health risk assessments. Hazard classifications established by the World Health Organization do not take into account the levels of human exposure, which determines the actual risk. Pesticides are registered for use in Canada only if the level of exposure to Canadians does not cause any harmful effects, including cancer.

To reach its decision, the PMRA applies risk assessment methods that consider sensitive population subgroups in both humans (for example, children) and organisms in the environment (for example, those most sensitive to environmental contaminants).

As part of the re-evaluation decision for glyphosate, Health Canada reviewed the dietary exposure to glyphosate and found that the levels found in food would not be a health risk to Canadians.

Q8. What are the findings of other jurisdictions on glyphosate?

In November 2015, the European Union Member States finalized their re-assessment of glyphosate, finding that glyphosate is unlikely to pose a carcinogenic hazard to humans. In May 2016, the United Nation's Food and Agriculture Organization and World Health Organization Joint Meeting on Pesticide Residues concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet. In March 2017, the European Chemical Agency released their determination that glyphosate is not classified as a carcinogen, which will be forwarded to the European Commission for final decision.

Currently, no pesticide regulatory authority in the world, including Health Canada, considers glyphosate to be a carcinogenic risk of concern to humans.

In December 2016, the United States Environmental Protection Agency Scientific Advisory Panel discussed the cancer potential of glyphosate. The final meeting report of the panel, Meeting Materials for the December 13-16, 2016, Scientific Advisory Panel, was posted on March 17, 2017. The PMRA is continuing to monitor activities of regulatory organizations, including the

United States Environmental Protection Agency review of the panel recommendations and final determination regarding the potential carcinogenicity of glyphosate.

Health Canada will take appropriate action if human health or environmental risks of concern are identified.

Q9. Why has Canada come out with a decision to continue registration of glyphosate products ahead of Europe and the United States? Aren't you working together?

Canada works closely with its international counterparts to ensure that regulations for pesticides are aligned internationally. During the re-evaluation of glyphosate, Health Canada worked cooperatively with the United States Environmental Protection Agency by sharing study reviews, as well as reviews of relevant published literature. However, consistent with other joint activities, each country conducts their own risk assessments, taking into consideration country-specific legislation and policies. This is why decisions are not always published at the same time.

Health Canada is aware of the recent United States Environmental Protection Agency's Scientific Advisory Panel report on glyphosate. The Department will continue to monitor regulatory activities from the United States, including the Environmental Protection Agency's review of the Scientific Advisory Panel recommendations, and their final determination regarding the potential carcinogenicity of glyphosate.

Health Canada will take appropriate action if human health or environmental risks of concern are identified.

Q10. Does glyphosate affect the traditional diet of First Nations communities?

The First Nations traditional diet may consist of vegetation foraged from the land and forest instead of farmed vegetation. Depending on the province, each provincial jurisdiction may use pesticides, including glyphosate, to treat invasive weeds on the land. Forestry management falls under provincial jurisdiction.

Based on the dietary risk assessment conducted by Health Canada, the Department can extrapolate that the anticipated residues of glyphosate in edible forest vegetation would not be of concern when ingested as part of the traditional diet.

Q11. There are so many published reports regarding the safety of genetically modified foods in relation to the use of glyphosate products. What is Health Canada's position on this topic?

Health Canada conducts a rigorous and thorough science-based assessment of all genetically modified food products before they are allowed to enter the Canadian marketplace. The assessments are conducted under the Food and Drug Regulations, which prohibit manufacturers of these products from selling them in Canada until Health Canada has completed a full safety assessment and has found them to be as safe and nutritious as conventional foods.

Q12. What are the Maximum Residue Limits for glyphosate?

Health Canada establishes Maximum Residue Limits (MRLs) for pesticide residues in all foods, including genetically modified foods, regardless of whether they are grown in Canada or imported. Canadian MRLs are set only after an extensive review of the scientific information and after a thorough risk assessment confirms that there are no health concerns to all

segments of the population (including pregnant and nursing women, infants, children and seniors), when all possible food sources are eaten every day, over a lifetime. MRLs are set for each pesticide-crop combination and are well below levels that could pose a health concern. For more information, visit the Health Canada website on Maximum Residue Limits for Pesticides.

Q13. Are the levels of glyphosate found on food in Canada considered safe? How are the Maximum Residue Limits enforced?

Yes, based on the data and information Health Canada reviewed, the Department has assessed dietary risks and found that the levels present are not a risk of concern for human.

The Canadian Food Inspection Agency (CFIA) is responsible for monitoring pesticide residues in food. The CFIA works closely with Health Canada to ensure that foods available on the Canadian market comply with the MRLs. Activities include testing of fresh fruits, vegetables, grains, pulses, and oil seeds that are domestically produced, as well as monitoring of

imported foods. To date, the results from monitoring pesticide residues in food show a high degree of compliance with the MRLs.

In 2015, the CFIA tested a large number of samples for glyphosate, consisting of a wide variety of food commodities. The CFIA anticipates having its full analysis completed by spring 2017, and the summary of their report will be available on the CFIA website.

Q14. In the United States, a non-governmental organization (Moms Across America) claimed that glyphosate was detected in breast milk. How was this viewed by Health Canada?

Trace levels of pesticide residues can occur on food including breast milk. However, these are at extremely low levels, and well below the amount that would pose a health concern. (Trace levels are in the parts per billion or parts per trillion range, well below most glyphosate Maximum Residue Limits (MRLs) which are in parts per million).

Glyphosate MRLs for various food commodities range from 0.08 ppm to 35 ppm, depending on the commodity. MRLs for pesticides can be found by searching the Canadian Pesticide MRL Database on the Pesticides and Pest Management portion of Health Canada's website.

The Moms Across America article cited health effects in rats exposed to low levels of glyphosate. These findings were from a study that was internationally discredited by various international organizations and regulatory authorities, including Health Canada, and later retracted by the original publishing journal. For more information, you can read the Health Canada and Canadian Food Inspection Agency statement on the Séralini et al. (2012) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603.

Q15. There are reports claiming that use of glyphosate may affect human health by affecting gastrointestinal tract and its microbiome. What is Health Canada's view on this claim?

Glyphosate targets an amino acid synthesis pathway in plants that is shared by certain types of bacteria, but not humans. There is not much scientific evidence to support the claim that

glyphosate has any direct impact on human gut microflora, or has any subsequent health effect. Several reports postulate that environmental chemicals may potentially lead to changes in normal gut microbiota. However, information to date is based on studies done in cell cultures, with animal evidence being limited and inconclusive.

The risk assessment conducted by the PMRA includes consideration of clinical signs of toxicity on the gastrointestinal tract and is protective of potential effects on the gastrointestinal tract.

Q16 What is the impact of glyphosate use on beneficial insects and bees as well as amphibians?

Health Canada has conducted a detailed analysis of relevant studies to determine the impact of glyphosate use on pollinators, beneficial insects and amphibians. It was determined that, when used according to label directions, glyphosate is not expected to pose a risk of concern. Buffer zones calculated for the protection of more sensitive aquatic organisms provide additional protection for amphibians.

Date modified:
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Re-evaluation Decision RVD2017-01

Glyphosate

(publié aussi en français)

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Executive Summary

Health Canada's primary objective in regulating pesticides is to protect Canadians' health and their environment. Pesticides must be registered by Health Canada's Pest Management Regulatory Agency (PMRA) before they can be imported, sold, or used in Canada. Pesticides must go through rigorous science-based assessments before being approved for sale in Canada.

All registered pesticides must be re-evaluated by the PMRA on a cyclical basis to make sure they continue to meet modern health and environment safety standards and continue to have value. In 2015, the PMRA published the outcome of its extensive re-examination of glyphosate for public comment (PRVD2015-01), which concluded that the products containing glyphosate do not present unacceptable risks to human health or the environment when used according to the revised product label directions.

During this re-examination, the PMRA assessed the potential human health risk of glyphosate from drinking water, food, occupational and bystander exposure, as well as the environmental risk to non-target organisms. Both the active ingredient and formulated products were included in the re-evaluation. The assessment was carried out based on available information provided by the manufacturer of the pesticide, as well as a large volume of published scientific literature, monitoring information (for example, ground water and surface water) and reviews conducted by other regulatory authorities.

The overall finding from the re-examination of glyphosate is highlighted as follows:

- Glyphosate is not genotoxic and is unlikely to pose a human cancer risk.
- Dietary (food and drinking water) exposure associated with the use of glyphosate is not expected to pose a risk of concern to human health.
- Occupational and residential risks associated with the use of glyphosate are not of concern, provided that updated label instructions are followed.
- The environmental assessment concluded that spray buffer zones are necessary to mitigate potential risks to non-target species (for example, vegetation near treated areas, aquatic invertebrates and fish) from spray drift.
- When used according to revised label directions, glyphosate products are not expected to pose risks of concern to the environment.
- All registered glyphosate uses have value for weed control in agriculture and non-agricultural land management.

All comments received during the consultation process were taken into consideration. These comments and new data/information resulted in only minor revisions to the proposed regulatory decision described in PRVD2015-01. Therefore, the PMRA is granting continued registration of products containing glyphosate with requirements of additional label updates to further protect human health and the environment.

To comply with this decision, the required label changes must be implemented on all product labels sold by registrants no later than 24 months after the publication date of this document.

Re-evaluation Decision for Glyphosate

After a re-evaluation of the herbicide glyphosate, Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is granting continued registration of products containing glyphosate for sale and use in Canada.

An evaluation of available scientific information found that products containing glyphosate do not present risks of concern to human health or the environment when used according to the revised label directions. As a requirement for the continued registration of glyphosate uses, new risk reduction measures are required for the end-use products registered in Canada. No additional data are being requested at this time.

Findings of the re-evaluation of glyphosate were first presented for public consultation in the Proposed Re-evaluation Decision PRVD2015-01, *Glyphosate*,[1] whereas this Re-evaluation Decision (RVD2017-01)[2] summarizes the Agency's final decision on the re-evaluation of glyphosate and the reasons for it.

Comments received during the consultation period were taken into consideration. These comments and new data/information resulted in revisions to some parts of the risk assessments, however, they did not result in substantial changes to the proposed regulatory decision as described in PRVD2015-01. Appendix I of this document summarizes the comments received and provides the PMRA's response.

To comply with this decision, the required mitigation measures must be implemented on all product labels sold by registrants no later than 24 months after the publication date of this document. Registrants of the products containing glyphosate will be informed of the specific requirements affecting their product registration(s) and of the regulatory options available to them.

What Does Health Canada Consider When Making a Re-evaluation Decision?

Health Canada's pesticide re-evaluation program considers potential risks[3] as well as the value[4] of pesticide products to ensure they meet modern standards established to protect human health and the environment. Re-evaluation draws on data from registrants, published scientific reports, information from other regulatory agencies and any other relevant information.

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1 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act.*

2 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act.*

3 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

4 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "…the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact".

In 2010, Health Canada published a re-evaluation work plan for glyphosate (REV2010-02) outlining the focus of this re-evaluation and indicating that the PMRA is working cooperatively with the United States Environmental Protection Agency. As part of this re-evaluation, the effect of Polyethoxylated Tallow Amines (POEA) and the metabolite and transformation product Aminomethylphosphonic acid (AMPA) are also included.

What Is Glyphosate?

Glyphosate is a broad-spectrum, non-selective herbicide. It controls many annual weeds, perennial weeds, woody brush and weedy trees. It is registered for use on a wide variety of sites including terrestrial feed and food crops, terrestrial non-food, non-feed and fibre crops, and for non-agricultural, industrial and residential weed management for non-food sites, forests and woodlots, outdoor ornamentals and turf.

Glyphosate is present as the free acid or as a salt in formulated end use products. Glyphosate products are formulated as solutions, pastes or tablets and can be applied using ground or aerial application equipment. Other application techniques are also used to apply glyphosate, such as with a wiper or wick applicator, cut stump or stem injection treatment. The rate of application ranges from 0.25 to 4.32 kg a.e./ha, depending on weed species (for example, annual vs. perennial) and use site. All products containing glyphosate currently registered under the authority of the *Pest Control Products Act* are listed in Appendix II.

Health Considerations

Can Approved Uses of Glyphosate Affect Human Health?

Products containing glyphosate are unlikely to affect your health when used according to label directions.

Potential exposure to glyphosate may occur through diet (food and water), or when handling and applying the product, or by entering treated sites. When assessing health risks, two key factors are considered: the levels at which no health effects occur in animal testing and the levels to which people may be exposed. The dose levels used to assess risks are established to protect the most sensitive human population (for example, children and nursing mothers). Only those uses where exposure is well below levels that cause no effects in animal testing are considered acceptable for registration.

Glyphosate is of low acute oral, dermal and inhalation toxicity. It is severely irritating to the eyes, non-irritating to skin and does not cause an allergic skin reaction.

Registrant-supplied short and long term (lifetime) animal toxicity tests, as well as numerous peer-reviewed studies from the published scientific literature were assessed for the potential of glyphosate to cause neurotoxicity, immunotoxicity, chronic toxicity, cancer, reproductive and developmental toxicity, and various other effects.

The most sensitive endpoints for risk assessment were clinical signs of toxicity, developmental effects, and changes in body weight. The young were more sensitive than the adult animals. However, the risk assessment approach ensures that the level of exposure to humans is well below the lowest dose at which these effects occurred in animal tests.

Residues in Food and Water

Dietary risks from food and water are not of concern.

Reference doses define levels to which an individual can be exposed over a single day (acute) or lifetime (chronic) and expect no adverse health effects. Generally, dietary exposure from food and water is acceptable if it is less than 100% of the acute reference dose or chronic reference dose (acceptable daily intake). An acceptable daily intake is an estimate of the level of daily exposure to a pesticide residue that, over a lifetime, is believed to have no significant harmful effects.

Potential acute and chronic dietary exposures to glyphosate were estimated from residues of glyphosate and relevant metabolites in both treated crops and drinking water. Exposure to different subpopulations, including children and women of reproductive age, were considered. The acute dietary exposure estimate from food and drinking water at the 95th percentile represents 31% of the acute reference dose (ARfD) for females 13-49 years of age, and ranges from 12% to 45% of the ARfD for all other population subgroups. The chronic dietary exposure estimate for the general population represents 30% of the acceptable daily intake (ADI). Exposure estimates for population subgroups range from 20% of the ADI (for adults aged 50 years or older) to 70% of the ADI (for children 1-2 years old). Thus, acute and chronic dietary risks are not of concern.

The *Food and Drugs Act* prohibits the sale of adulterated food; that is, food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act.* Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose a health risk concern.

Canadian MRLs for glyphosate are currently specified for a wide range of commodities (MRL database http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php). Residues in all other agricultural commodities, including those approved for treatment in Canada but without a specific MRL, are regulated under Subsection B.15.002(1) of the Food and Drug Regulations, which requires that residues do not exceed 0.1 ppm. Separate MRLs have been established for the trimethylsulfonium (TMS) cation, the major metabolite of the glyphosate-TMS salt, in/on a variety of commodities. Given that all glyphosate-TMS-containing products have been discontinued in Canada, all MRLs for the TMS cation will be revoked.

Risks in Residential and Other Non-Occupational Environments

Non-occupational risks are not of concern when used according to label directions.

Residential exposure may occur from the application of products containing glyphosate to residential lawns, and turf (including golf courses), gardens and trees. Residential handler exposure could occur from mixing, loading and applying domestic-class glyphosate products. These products can be applied as a liquid by a manually pressurized handwand, backpack, sprinkler can and ready-to-use sprayer.

Residential postapplication exposure may occur for persons performing activities on treated areas. This includes areas treated by residential handlers as well as residential areas treated by commercial applicators. Exposure is predominantly dermal. Incidental oral exposure may also occur for children (1 to <2 years old) playing in treated areas.

For all domestic class products, the target dermal and inhalation margins of exposure (MOE) were met for adults applying glyphosate and are not of concern. Residential postapplication activities also met the target dermal MOE for all populations (including golfers) and are not of concern. For incidental oral exposure, the target oral MOEs were met for children (1 to <2 years old) and are not of concern.

Non-occupational scenarios were aggregated with background (chronic) dietary exposure (food and drinking water). The resulting aggregate risk estimates reached the target MOE for all uses and are not of concern.

Non-occupational risks from bystander dermal exposure are not of concern.

Bystander exposure may occur when the general public enter non-cropland areas (for example, hiking through forests or parks) that have recently been treated with glyphosate. The resulting risk estimates associated with bystander dermal exposure met the target MOE for all populations and are not of concern.

Occupational Risks from Handling Glyphosate

Occupational risks to handlers are not of concern when used according to label directions.

Risks to handlers are not of concern for all scenarios. Based on the precautions and directions for use on product labels reviewed for this re-evaluation, risk estimates associated with mixing, loading and applying activities met the target dermal and inhalation MOEs and are not of concern.

Postapplication risks are not of concern for all uses.

Postapplication occupational risk assessments consider exposures to workers entering treated sites in agriculture. Based on the current use pattern for agricultural scenarios reviewed for this re-evaluation, postapplication risks to workers performing activities, such as scouting, met the target dermal MOEs and are not of concern. A minimum restricted entry interval of 12 hours is required for agricultural sites.

Polyethoxylated Tallow Amines (POEA)

POEA is a family of several compounds that are used as surfactants in many glyphosate products registered in Canada. No human health risks of concern were identified for these end-use products, provided that they contain no more than 20% POEA by weight. All of the currently registered glyphosate end-use products in Canada meet this limit.

Environmental Considerations

What Happens When Glyphosate Is Introduced Into the Environment?

When used according to revised label directions, glyphosate products are not expected to pose risks of concern to the environment. Labelled risk-reduction measures mitigate potential risks posed by glyphosate formulations to non-target plants and freshwater/marine/estuarine organisms.

When glyphosate is released into the environment, it can enter soil and surface water. Glyphosate breaks down in soil and water and is not expected to remain for long periods of time. Glyphosate produces one major break down product in soil and water, aminomethyl phosphonic acid (AMPA), which can last in the environment. Carryover of glyphosate and AMPA into the next growing season is not expected to be significant. Glyphosate and AMPA are not expected to move downward through the soil and are unlikely to enter groundwater.

Glyphosate dissolves readily in water but is expected to move into sediments in aquatic environments. Glyphosate is not expected to enter the atmosphere. Glyphosate and AMPA are unlikely to accumulate in animal tissues.

Certain glyphosate formulations include a surfactant composed of POEA compounds. At high enough concentrations, POEA is toxic to aquatic organisms but is not expected to remain in the environment. While, in general, glyphosate formulations that contain POEA are more toxic to freshwater and marine/estuarine organisms than formulations that do not contain POEA, they do not pose risks of concern to the environment when used as directed on the label.

In the terrestrial environment the only risk identified was for terrestrial plants, therefore, spray buffer zones are required to reduce exposure to sensitive terrestrial plants.

Glyphosate formulations pose a negligible risk to freshwater fish and amphibians, but may pose a risk to freshwater algae, freshwater plants, marine/estuarine invertebrates and marine fish if exposed to high enough concentrations. Hazard statements and mitigation measures (spray buffer zones) are required on product labels to protect aquatic organisms.

Glyphosate, AMPA and POEA do not meet all Toxic Substances Management Policy (TSMP) Track 1 criteria and are not considered Track 1 substances. Other than incident reports of damage to plants and one exceptional incident regarding fish in a river (PRVD2015-01, Section 4.2.3), there are currently no environmental incident reports involving glyphosate in Canada.

Value Considerations

What is the Value of Glyphosate?

Glyphosate plays an important role in Canadian weed management in both agricultural production and non-agricultural land management and is the most widely used herbicide in Canada.

Glyphosate is an important herbicide for Canadian agriculture:

- Due largely to its broad and flexible use pattern and its wide weed-control spectrum, it is the most widely used herbicide in several major crops grown in Canada, such as canola, soybean, field corn and wheat. It is also one of only a few herbicides regularly used in fruit orchards, such as apple.
- It is the essential herbicide for use on glyphosate tolerant crops (GTCs), including canola, soybean, corn, sweet corn and sugar beet. The combination of GTCs and glyphosate has been adopted as an important agricultural production practice in Canada.
- It has a wide application window ranging from pre-seeding to after seeding (prior to crop emergence), in-crop, pre-harvest or post-harvest, providing a flexible and effective weed management program.
- It is one of a few herbicides that can also be used as a harvest management and desiccation treatment.
- Post-harvest stubble treatment with glyphosate allows reduced or zero tillage, which has facilitated the adoption of conservation agriculture that results in improved soil quality.

Glyphosate is also an important weed management tool and is widely used for weed control in non-agricultural land management, such as forestry, industrial areas, and along rights-of-way. It is an effective tool for control of many invasive weed species and is also used in the control of toxic plants, such as poison ivy.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human health and the environment. These directions must be followed by law. As a result of the re-evaluation of glyphosate, the PMRA is requiring further risk-reduction measures in addition to those already listed on glyphosate product labels.

Additional risk-reduction measures are discussed below. Label amendments to be implemented are found in Appendix IV.

Human Health

- To protect commercial and residential applicators: glyphosate is not to be applied using hand-wicking or hand-daubing methods.
- To protect workers entering treated sites: a restricted-entry interval (REI) of 12 hours is required for agricultural uses.
- To protect bystanders: a statement is required indicating that the product is to be applied only when the potential for drift to areas of human habitation or areas of human activity, such as houses, cottages, schools and recreational areas, is minimal.

Environment

- Environmental hazard statements are added to inform users of toxicity to non-target species.
- Spray buffer zones to protect non-target terrestrial and aquatic habitats are required.
- To reduce the potential for runoff of glyphosate to adjacent aquatic habitats, precautionary statements for sites with characteristics that may be conducive to runoff and when heavy rain is forecasted are required. In addition, a vegetative strip between the treatment area and the edge of a water body is recommended to reduce runoff of glyphosate to aquatic areas.

What Additional Scientific Information is Being Requested?

There are no additional data requirements proposed as a condition of continued registration of glyphosate products.

International Regulatory Status and Updates on Glyphosate

The PMRA routinely works collaboratively with other member countries within the Organisation for Economic Co-operation and Development (OECD) on the regulation of pesticides. As part of the re-evaluation of an active ingredient, the PMRA takes into consideration recent developments and new information on the status of a pesticide in other jurisdictions. Glyphosate is currently acceptable for use in other OECD countries, including the United States, Australia and the European Union. As of 8 March 2017, no decision by an OECD member country to prohibit all uses of glyphosate for health or environmental reasons has been identified.

In March, 2015, the World Health Organization's (WHO) International Agency for Research on Cancer (IARC) published a summary of results of their hazard classification of five pesticides, including glyphosate. IARC classified glyphosate as probably carcinogenic to humans. It is important to note that the IARC classification is a hazard classification and not a health risk assessment. This means that the level of human exposure, which determines the actual risk, was not taken into account by IARC.

In November, 2015, the European Food Safety Authority (EFSA) finalized their re-assessment of glyphosate, concluding that glyphosate is unlikely to pose a carcinogenic hazard to humans. The EU also set an acute reference dose, which is the same as that set by the PMRA (PRVD2015-01). In May 2016, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures and that it is unlikely to pose a carcinogenic risk to humans from exposure through the diet. In March, 2017, the European Chemical Agency (ECHA) and the Australian Pesticides and Veterinary Medicines Authority (APVMA) released their determination that glyphosate is not a carcinogen. Currently, no pesticide regulatory authority, including Health Canada, considers glyphosate to be a carcinogenic risk of concern to humans.

Canada and the USEPA have been collaborating on the re-evaluation of glyphosate. In December 2016, the USEPA Scientific Advisory Panel (SAP) discussed the cancer potential of glyphosate, and Health Canada's PMRA participated as an observer. The final SAP meeting report was posted on March 17, 2017. The PMRA is continuing to monitor regulatory activities from other regulatory organizations, including the USEPA's review of the SAP recommendations and final determination regarding the potential carcinogenicity of glyphosate.

Health Canada's PMRA sets Maximum Residue Limits (MRLs) for pesticide residues on food, which is the maximum amount of residue that is expected to remain on food products when a pesticide is used according to label directions. These are set at levels well below the amount that could pose a health concern. In 2015, the Canadian Food Inspection Agency (CFIA) tested approximately 700 samples consisting of a variety of juice and juice blends, grains and grain products, beans, lentils, and a wide variety of fruit and vegetables. The CFIA also initiated a targeted survey of approximately 2,500 samples, looking at levels of glyphosate in bean, pea, lentil, chickpea and soy products, as well as less commonly consumed grains such as barley, buckwheat and quinoa. The results show a high degree of compliance with the MRLs established by the PMRA for glyphosate. The CFIA anticipates having the full analysis completed by Spring 2017.

Other Information

Any person may file a notice of objection regarding this decision on glyphosate within 60 days from the date of publication of Re-evaluation Decision RVD2017-01, *Glyphosate*. For more information regarding the basis for objecting (which must be based on scientific grounds), please refer to the Pesticides and Pest Management portion of Health Canada's website (Request a Reconsideration of Decision), or contact the PMRA's Pest Management Information Service.

List of Abbreviations

AD	administered dose
ADI	allowable daily intake
a.e.	acid equivalent
AFC	antibody forming cells
AHS	agricultural health study
AMPA	aminomethylphosphonic acid
APVMA	Australian Pesticide and Veterinary Medicines Authority
ARfD	acute reference dose
ASAE	American Society of Agricultural Engineers
ATAE	phosphate ester, tallowamine, ethoxylated
Atm	atmosphere
BAF	bioaccumulation factor
BCF	bioconcentration factor
Bt	*Bacillus thuringiensis*
BVL	The German Federal Office for Consumer Protection and Food Safety
CARC	Cancer Assessment Review Committee
CAS	Chemical Abstracts Service
CFIA	Canadian Food Inspection Agency
CHMS	Canadian Health Measures Survey
Cm	centimeter
DACO	Data Code
DAR	Draft Assessment Report
DIR	Directive
DMTT	PMRA drift mitigation technical team
DT_{50}	time required for 50% dissipation of the initial concentration
EC_{25}	effective concentration on 25% of the population
EC_{50}	effective concentration on 50% of the population
ECx	effective concentration on x (any number) % of the population
ECHA	European Chemicals Agency
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDTA	Endocrine Disrupters Testing and Assessment
EFSA	European Food Safety Authority
EP	end-use product
EU	European Union
EUP	end-use product
EUP + POEA	end-use products containing the surfactant POEA
EUP NO POEA	end-use products that do not contain POEA
FA	fraction of species affected
FAO	Food and Agriculture Organization of the United Nations
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLP	Good Laboratory Practices
GMO	genetically modified
Ha	hectare(s)

HC_5	hazardous concentration to five percent of species in a Species Sensitivity Distribution (SSD)
HD_5	hazardous dose to five percent of species in a Species Sensitivity Distribution (SSD)
Hr	hour(s)
HL	Hodgkin's lymphoma
IARC	International Agency for Research on Cancer
ICH	International Council on Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IgM	Immunoglobulin M
IPA salt	isopropylamine salt
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
JGTF	Joint Glyphosate Task Force
JMPR	Joint WHO/FAO Meeting on Pesticide Residues
K_{ow}	*n*-octanol-water partition coefficient
L	litre
Lab	laboratory
LC_{50}	lethal concentration on 50% of the population
LC_x	lethal concentration on x (any number) % of the population
Log	logarithm
LOAEL	lowest observed adverse effect level
m^3	meter cube
mg	milligram
mm	millimeter
Mn	Manganese
MOA	Mode of Action
MOE	Margin of Exposure
MRL	Maximum Residue Limit
MWCF	Molecular Weight Conversion Factor
N. bruchi	*Neochetina bruchi*
Ng	nanogram
NHL	Non-Hodgkin Lymphoma
NOAEL	no observed adverse effect level
NOEC	no-observed-effect-concentration
NOEL	no-observed-effect-level
NOI	notice of intent
NPAFC	North Pacific Anadromous Fish Commission
NTP	National Toxicology Program
NZEPA	New Zealand Environmental Protection Authority
OECD	Organization for Economic Co-operation and Development
OPP	Office of Pesticides
Pa	pascal
PCPA	Pest Control Products Act
PMRA	Pest Management Regulatory Agency
POEA	Polyethoxylated tallow amines
PPE	Personal Protective Equipment
ppm	parts per million

PRVD	Proposed Re-evaluation Decision
RAR	Renewal Assessment Report
ROS	reactive oxygen species
RD	Residue Definition
RED	Reregistration Eligibility Decision
REG	Regulatory Note
REI	Restricted-Entry Interval
REV	Re-evaluation Note
RVD	Re-evaluation Decision
SAP	Scientific Advisory Panel
SPN	Science Policy Note
spp.	species (plural)
SSD	species sensitivity distribution
Tech.	technical
TGAI	technical grade active ingredient
TSMP	toxic substances management policy
TTR	Turf Transferable Residue
UK	United Kingdom
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
VMG	Validation Management Groups
WHO	World Health Organization

Appendix I Comments and Responses

The PMRA received written comments from the technical registrants, the public and other stakeholders relating to the *Proposed Re-evaluation Decision PRVD2015-01, Glyphosate*. The comments and PMRA responses are summarized based on common scientific themes.

1.0 Comments Related to the Health Risk Assessments

1.1 Comments Related to Toxicology

In addition to specific comments related to the toxicological evaluation of glyphosate, comments related to broader considerations, were also received. These broader comments included questions on the established paradigms for the toxicological evaluation of chemicals in general, comments on the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals, concerns relating to the independence of the scientific findings, principles of Good Laboratory Practices (GLP), and other aspects of toxicological assessments. Although these broader types of comments were beyond the scope of the re-evaluation of glyphosate, every effort has been made to respond to the underlying concerns in the submitted comments as they relate to the toxicology review and health aspects of the glyphosate re-evaluation in Canada.

1.1.1 Salivary gland alterations and Acceptable Daily Intake (ADI)

Comment
The Joint Glyphosate Task Force (JGTF) proposed that the observation of cellular alterations in salivary glands results from oral irritation caused by dietary administration of glyphosate acid – a strong organic acid. New data was submitted to support this conclusion. In addition, it was noted that Canadian glyphosate formulations do not contain the technical acid, but instead contain neutral glyphosate salts (for example, potassium, ammonium, and isopropylamine). The JGTF requested that the PMRA consider the new data, re-assess the adversity of this finding, and base the ADI calculation on a more toxicologically relevant No Observed Adverse Effect Level (NOAEL).

PMRA Response
The newly submitted data consisted of a dose-range finding study and a non-guideline definitive study that examined the effects of citric acid administered to rats via gavage (to bypass direct oral exposure) or via diet, and trisodium citrate dihydrate given via diet for seven weeks. Rats treated with citric acid in their diet (a low pH diet) exhibited more pronounced changes in parotid glands (increased weight and histopathology severity) compared to rats receiving citric acid via gavage, or trisodium citrate dihydrate by diet (high pH diet).

However, an acidic diet did not appear to be the only factor responsible for changes in parotid glands, since these changes (albeit less pronounced) were also observed in both the high pH diet and gavage-treated citric acid (low pH) groups. Also, other organizations have conducted studies examining different modes of action (MOAs) that might explain changes observed in salivary glands of animals fed glyphosate-treated diets.

For example, as discussed in PRVD2015-01, (page 12), studies by the National Toxicology Program (NTP) indicated that glyphosate may be a β-adrenergic receptor agonist, as histological similarities were noted in salivary glands of animals treated with glyphosate acid, or a β-adrenergic receptor agonist (isoproterenol), and were reduced in severity by propranolol (a β-adrenergic receptor antagonist).

Additionally, the hazard assessment was based on the 'active substance' (glyphosate acid). Guideline toxicity data for "neutral" glyphosate salts, with particular attention to salivary gland examination in repeat-dose studies, were not available for selection of the toxicity endpoints.

The toxicological evaluation relied on a number of co-critical studies, rather than one 'key study', to establish each endpoint. The ADI (PRVD2015-01, page 20) is based on a 2-year study in rats with a NOAEL of 32/34 mg/kg bw/day, the highest (combined) NOAEL for all 2-year rat studies. The lowest (combined) Lowest Observed Adverse Effect Level (LOAEL) is 100 mg/kg bw/day, based on decreased body weight and increased incidences and severity of cellular alterations in the parotid and submandibular glands in one of the two-year rat studies. This choice of NOAEL and LOAEL is further supported by the NOAEL of 30 and LOAEL of 100 mg/kg bw/day, based on decreased body weight in three one-year dog studies. Thus, the selected ADI is based on two primary findings (decreased body weight as well as histological changes in the parotid salivary gland) observed in a number of different studies. No revision is required.

1.1.2 Acute Reference Dose (ARfD) for females 13-49 years of age

Comment
The endpoint selected for the ARfD for females 13-49 years of age was considered by the JGTF to be based on a spurious finding that is not reflected across developmental toxicity studies of glyphosate in rabbits. The JGTF presented an evaluation of seven rabbit developmental toxicity studies conducted by Kimmel et al. (2013), which concluded that the body of data failed to support an increased incidence of interventricular septal defects in the fetuses resulting from treatment with glyphosate during gestation in rabbits. Overall, the JGTF requested that the ARfD for this subpopulation be aligned with the ARfD for the general population.

PMRA Response
As noted in PRVD2015-01, the PMRA considered the evaluation conducted by Kimmel et al. (2013) in detail, as well as other available information, and based its conclusion on the overall weight-of-evidence in establishing an ARfD for the subpopulation of females 13-49 years of age.

Briefly, several limitations were noted in the analysis by Kimmel et al. (2013) including data tabulation errors and a lack of, or inadequately characterized, historical control data for key studies, including the study on which the PMRA based the ARfD. A re-analysis of this key study (Brooker et al. 1991, PMRA #1161779; PRVD2015-01) in conjunction with additional historical control data supplied by the JGTF resulted in the PMRA concluding that the incidence of cardiac malformations was increased relative to both concurrent and historical control data in high-dose animals, with an increase in variations at the mid-dose. The additional historical data provided by the JGTF did not alter the PMRA's original conclusions, thus, the ARfD for females 13-49 years of age was not revised.

1.1.3 Cancer Risk Assessment

Comments

1.1.3.1 International Agency for Research on Cancer (IARC) Glyphosate Monograph[5]

The majority of comments in relation to the 2015 IARC assessment, which classified glyphosate as 'probably carcinogenic to humans', requested that the PMRA review and re-assess the potential carcinogenicity of glyphosate, and restrict/ban its uses in Canada. Some comments noted that while the IARC assessment is a hazard classification, it also took into account the human exposure levels to glyphosate, largely by incorporating the epidemiological studies into the assessment. Some comments recommended that the PMRA apply the IARC classification in selecting a sensitive endpoint for occupational and bystander risk assessment in order to protect against the risk of developing non-Hodgkin's lymphoma and/or other cancers.

1.1.3.2 Ovarian Tubulostromal Tumours

The JGTF noted that PRVD2015-01 reported an increased incidence of ovarian tubulostromal tumours. The JGTF stated that these neoplasms arise out of the germinal epithelium of the ovarian stroma, are similar to those seen in epithelial hyperplasia, and therefore, do not provide sufficient evidence for oncogenicity. They also provided historical control data relevant to the strain of mice used, and noted that the reported incidence was within the range of Charles River historical control data for this finding. The JGTF requested that PMRA consider this finding as not related to glyphosate treatment and revise the text on page 89 of PRVD2015-01 from "equivocal evidence of oncogenicity" to "no evidence of oncogenicity"

1.1.3.3 Agricultural Health Study and Multiple Myeloma

The JGTF requested that the PMRA reconsider the suggested association between multiple myeloma and glyphosate use that was reported by the Agricultural Health Study (AHS) publication (De Roos et al. 2005, PMRA#:2391583). The comments indicated that it has been over 10 years since the study was conducted and a follow-up study, noted by De Roos as being necessary, has not been performed. The JGTF also noted that in an effort to understand how the conclusion of 'suggested association' was reached in the AHS study, the data were analyzed by a third-party expert (Sorahan, 2015) who determined that De Roos et. al., 2005 had pared down the AHS data set to come to the conclusion of 'suggested association'. When the full data set is analyzed, the risk ratio is 1.1, demonstrating no association between multiple myeloma and glyphosate use. Additionally, no association between multiple myeloma and glyphosate use was noted by the IARC review of glyphosate, which considered the Sorahan (2015) paper.

[5] IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 112 (2015). Some Organophosphate Insecticides and Herbicides: Diazinon, Glyphosate, Malathion, Parathion, and Tetrachlorvinphos. Available online from http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-09.pdf [last accessed June, 2016]

PMRA Response to Comments 1.1.3.1 – 1.1.3.3

Background

In March, 2015, the International Agency for Research on Cancer (IARC) published a summary of the basis for their hazard classifications of five pesticides, including glyphosate, which they classified as 'probably carcinogenic to humans'. The PMRA's position on the IARC's hazard-based classification was included in PRVD2015-01, published in April, 2015, however, the full IARC monograph only became available in July, 2015. The PMRA has since reviewed this document; a summary of the PMRA review is discussed below.

The IARC Assessment

The PMRA and IARC assessments of the carcinogenic potential of glyphosate were based on different datasets and considerations. As noted in Re-evaluation Note 2010 (REV2010-02), the PMRA collaborated with the United States Environmental Protection Agency (USEPA) on the re-evaluation of glyphosate, which included the examination of published scientific toxicity data according to the principles set out in USEPA guidance.[6] Additionally, considerations laid out in a second USEPA guidance[7] document were applied in the review of published epidemiology data.

The carcinogenic potential of glyphosate acid, the technical active ingredient, was assessed by the PMRA using a weight-of-evidence approach. Many registrant-supplied studies are available on the carcinogenic potential of glyphosate, which include lifetime cancer bioassays, as well as in vitro and in vivo mutagenicity studies. In addition, published data as well as epidemiological data were available for consideration. Results were then integrated and weighed according to their reliability, relevance and consistency. Note that studies conducted with glyphosate alone were considered more relevant in characterizing its inherent toxicity than were studies on the formulated products reported in the scientific literature, as the latter contained a variety of other constituents that, in most cases, were not identified. The compositions of formulated products are considered proprietary data, and often differ between countries. However, the composition of the formulated products must be disclosed to regulatory authorities in the country of registration; (see Genotoxicity section below). Although it is argued that formulated glyphosate products are more representative of 'real life' conditions, it is important to keep in mind that many different products (pesticide and non-pesticide) share many of these same constituents. In order to fully characterize a pesticide active ingredient, it is necessary to understand its inherent toxicity, which can only be characterized in the absence of these other constituents.

6 EPA (U.S. Environmental Protection Agency), 2012, Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment. Available online from http://www2.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf [last accessed February, 2016]

7 EPA (U.S. Environmental Protection Agency), 2010, February 2010 FIFRA SAP meeting minutes: Draft Framework and Case studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. Available online from https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2010-0125-0079 [last accessed February 2016]

In addition, studies that complied with internationally accepted test guidelines were considered by the PMRA to be more relevant and reliable than published studies conducted with methodologies not recognized by regulatory agencies or organizations, such as the OECD. In total, the PMRA, in cooperation with the USEPA, assessed a much larger and more relevant body of scientific information than was considered by the IARC.

Conversely, in its evaluation of the carcinogenic potential of glyphosate, the IARC considered only published sources of toxicology data, which included the scientific literature and certain documents published by regulatory agencies. The IARC did not directly consider, or did not consider at all, unpublished toxicology studies that were available to international regulatory agencies. It is the PMRA's understanding that unpublished registrant-sponsored studies are not requested by the IARC for their deliberations. Furthermore, the IARC classifications of carcinogenic hazard are based on scientific consensus related to the evidence examined, but do not provide risk information or recommendations for regulation or legislation. The IARC assessment relied on many studies that did not characterize the composition of the tested mixtures (formulated products) and/or grouped all glyphosate formulated products, regardless of their composition. The composition of glyphosate formulated products differs around the world, even in those marketed under the same trade name. This difference in the evaluation approach used by the IARC and the PMRA is an important distinction because some studies, mostly in vitro, with glyphosate formulated products suggest that certain formulations are genotoxic, while studies examining the active substance alone do not show this effect. This may indicate that genotoxicity observed in these studies is related to other constituents in the formulated product rather than glyphosate acid. The constituents of all pest control products registered in Canada are disclosed to the PMRA, and toxicity data (as well as other data) are also required for each formulated product, which are examined during the pre-market review process.

Genotoxicity

The PMRA did not identify any genotoxic potential for the active ingredient glyphosate acid. Negative results for in vitro and in vivo gene mutation and chromosomal effect assays in mammalian cells contributed to the overall conclusion that the active ingredient glyphosate was not genotoxic. In vitro studies are generally conducted to predict a potential effect in animal (in vivo) studies. In vivo studies are weighted more than in vitro studies based on relevancy and integrated metabolism of the whole animal.

A large battery of genotoxicity assays conducted according to the OECD test guidelines for glyphosate is available. Many studies have been replicated several times, and all indicated negative results for genotoxicity. The IARC assessment did not consider the majority of these studies. Instead, the IARC monograph reported mixed results for studies with glyphosate formulated products that examined DNA damage, gene mutation, and chromosomal aberrations, and included results from non-mammalian systems – for example fish, and plants, that are not considered relevant for human health hazard characterization.

The IARC monograph also noted that in several cases, positive results occurred at very high or toxic dose levels only. It is important to characterize the relationship of genotoxic results in the context of observed cytotoxicity. Positive results at very high or toxic dose levels indicate that the genotoxic effects are due to cytotoxicity rather than direct DNA-acting properties of glyphosate formulated products. High-dose cytotoxicity was one factor in the weight-of-evidence

approach used by the PMRA when considering the genotoxic potential of glyphosate, and is consistent with international approaches (EFSA 2011,[8] USEPA 1986,[9] USFDA, ICH S2(R1)[10]). The observed cytotoxicity is likely associated with surfactants that are present in many formulated products. For example, polyethoxylated tallow amines (POEAs), which are typical surfactant components of many glyphosate products, were shown to produce cytotoxic effects such as perturbation/disruption of the mitochondrial membrane in cultured mammalian cells (Levine et al. 2007,[11] Kier and Kirkland 2013[12]). A number of negative genotoxicity studies were reported by Kier and Kirkland (2013), but not considered by the IARC. It should be noted that genotoxic effects resulting from cytotoxicity exhibit a threshold, and carefully selected reference doses protect against this effect.

The IARC suggested other 'mechanisms of action' that might contribute to potential carcinogenicity, such as inflammation, immunosuppression, endocrine disrupting activity and oxidative stress, which were based mainly on in vitro studies. However, no evidence of glyphosate-induced immunosuppression was observed in a registrant-supplied guideline immunotoxicity study reviewed by the PMRA. In addition, no other studies in the extensive toxicity database suggested a concern for immunotoxicity, inflammation or oxidative stress. Glyphosate also showed no evidence of interaction with estrogen, androgen or thyroid endocrine pathways in studies conducted by the USEPA Endocrine Disruptor Screening Program (EDSP).

Carcinogenicity

1. Studies in Animals

As reported in PRVD2015-01, the PMRA also assessed the carcinogenic potential of glyphosate in several long-term animal studies, which included two mouse studies and four rat studies, as well as studies in the published literature. Although, not all available carcinogenicity studies on glyphosate were submitted to the PMRA, reviews, evaluation reports, and committee meeting documents from international regulatory authorities (EFSA and USEPA) for these particular studies were considered by the PMRA. No evidence of carcinogenicity was identified in any of the rat studies reviewed by the PMRA, or in the additional rat studies reviewed by other regulatory authorities.

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8 EFSA (European Food Safety Authority), 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Scientific Committee, EFSA journal, 9, 2379

9 EPA (U.S. Environmental Protection Agency), 1986. Guidelines for mutagenicity risk assessment. Fed. Register 51. 34006-34012.

10 FDA (U.S. Food and Drug Administration), 2012. Guidance for Industry. S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use. Available online from http://www.fda.gov/downloads/Drugs/Guidances/ucm074931.pdf [last accessed February, 2016]

11 Levine SL, Han Z, Liu J, et al. (2007). Disrupting mitochondrial function with surfactants inhibits MA-10 Leydig cell steroidogenesis. Cell Biology and Toxicology, 23, 385–400. Available online from http://link.springer.com/article/10.1007%2Fs10565-007-9001-6 [last accessed June, 2016]

12 Larry D. Kier & David J. Kirkland (2013) Review of genotoxicity studies of glyphosate and glyphosate-based formulations, Critical Reviews in Toxicology, 43:4, 283-315. Available online from http://www.tandfonline.com/doi/full/10.3109/10408444.2013.770820#.V2G7ZtJIiUk [last accessed June, 2016]

The IARC assessed seven long term studies in rats and two studies in mice. Pancreatic islet cell adenomas were noted in male rats in two of the rat studies. However, these findings were not dose-related and/or occurred at the low dose only. The IARC also reported a statistically significant positive trend for hepatocellular adenomas in male rats only (with no evidence of pre-neoplastic lesions or progression to carcinomas), and a statistically significant positive trend for thyroid C-cell adenomas in female rats only. None of these tumours were reproduced in other chronic studies in rats.

PRVD2015-01 reported a marginal increase in the incidence of ovarian tubulostromal hyperplasia and adenomas in mice. However, since adenomas were observed at the limit dose of testing, they were not considered relevant for human health risk assessment. Furthermore, additional historical control data submitted during the PRVD comment period indicated that the incidence of ovarian adenomas was actually within the historical control range for the conducting laboratory, which increased the likelihood that these tumours were not treatment-related.

For the two mouse studies, the IARC identified a positive trend for renal tubule adenomas and carcinomas in male mice in one study, and a positive trend for hemangiosarcoma in males in the other study. However, these tumours were not reproduced in other mouse studies, which used similar and higher doses (1000-4000 mg/kg bw/day).

Since the publication of PRVD2015-01, a review by Greim et al. (2015[13]) of 14 long-term glyphosate toxicity/carcinogenicity studies in rodents included four additional studies in rats and three additional studies in mice, which were negative for carcinogenicity. These seven studies were not considered acceptable by the IARC due to insufficient reporting of the study methods and results by Greim et al. The PMRA had access to detailed information for these studies, which were considered acceptable for hazard characterization; and the USEPA and EFSA also considered these studies as part of their assessment of the carcinogenic potential of glyphosate.

2. Epidemiological Studies

The PMRA, USEPA and the European Food Safety Authority (EFSA[14]) have concluded that the currently available epidemiological database does not support a causal relationship between exposure to glyphosate and cancer outcomes.

A general discussion of pivotal epidemiology studies, as identified in the IARC assessment, is presented below.

[13] Helmut Greim, David Saltmiras, Volker Mostert & Christian Strupp, (2015), Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies, Critical Reviews in Toxicology, 45:3, 185-208. Available online from http://dx.doi.org/10.3109/10408444.2014.1003423 [last accessed June, 2016]

[14] Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I. Literature review on epidemiological studies linking exposure to pesticides and health effects. EFSA (European Food Safety Authority), EFSA supporting publication 2013:EN-497, 159 pp. Available online from http://www.efsa.europa.eu/en/supporting/pub/497e [Last accessed February, 2016]

Multiple Myeloma

As a part of a larger study known as the Agricultural Health Study (AHS), a prospective cohort study examined cancer incidence in pesticide applicators in Iowa and North Carolina. As described in PRVD2015-01, the most relevant finding in this study was a non-statistically significant association between multiple myeloma and glyphosate exposure. The relative risk was 1.1 when adjusted for age (95% CI, 0.5-2.4; 32 cases; only 20 cases reported exposure to glyphosate), but was 2.6 (95% CI, 0.7-9.4) when adjusted for multiple confounders (age, smoking, other pesticides, alcohol consumption, family history of cancer, and education). Evidence for an exposure-response trend by duration or intensity of pesticide use was not observed during the relatively short period (enrollment in the study was 1993-1997 to end of 2001) of follow-up (PMRA#:2391583). In a follow-up analysis of male participants in the same cohort, no correlation was observed between exposure to glyphosate and risk of a pre-malignant plasma disorder (monoclonal gammopathy of undetermined significance) that typically precedes the development of multiple myeloma (Landgren et al., 2009). In multiple re-analyses of the AHS data, including that of Sorahan (2015), no definitive association between glyphosate exposure and multiple myeloma was observed.

Non-Hodgkin lymphoma (NHL)

In many case-control studies, as reported by IARC, the USEPA and EFSA, some investigators observed a positive, but generally non-statistically significantly association between glyphosate use and NHL cases, while others reported no association. Variation in the quality of exposure assessment, study design and methods, in addition to a lack of available information on confounding variables may explain inconsistencies in the data. NHL is also not a specific disease, as mentioned by most authors of these studies, but consists of multiple types of lymphoma that are classified for convenience as not being Hodgkin's lymphoma. For example, multiple myeloma can also be considered a type of NHL; however, the data on multiple myeloma was analysed separately by the IARC, instead of considering it with NHL studies. The World Health Organization has dismissed the dichotomous classification of lymphomas as NHL/HL (Hodgkin's lymphoma); and 43 different types of lymphomas have been characterized (Berry 2010[15]). Proper classification of the disease (for example, the type of cancer) is important in epidemiology studies in order to adequately link it with the exposure to a chemical.

The interpretation of available epidemiological studies involving glyphosate is problematic due to a lack of adequate characterization of glyphosate exposures, the small number of cancer cases, and other confounding variables. For example, glyphosate exposure was analyzed with several other pesticides, exposure was generally based on questionnaires, classification of the type of cancer was not consistent, and the contribution of toxicity from formulants could not be assessed.

[15] Berry, C.L. 2010. Relativism, regulation and the dangers of indifferent science. The Sir Roy Cameron lecture of the Royal College of Pathologists. Toxicology 267 (2010) 7-13. Available online from http://www.sciencedirect.com/science/article/pii/S0300483X09005812?np=y [Last accessed February 2016]

Only once an association is plausibly established can criteria, (such as Bradford Hill) be considered to determine whether a causal relationship exists[16]. Without a causal relationship, epidemiology data cannot be used to establish references doses or occupational endpoints.

Finally, it is important to note that the experts convened by the IARC to assess the carcinogenic hazard of glyphosate concluded that there is limited evidence of glyphosate-related carcinogenicity in humans based on the available epidemiological studies. This conclusion is consistent with the limited utility of epidemiology studies in selecting reference doses to conduct a human health risk assessment for glyphosate.

While epidemiology data have inherent limitations, reported findings have the advantage of being directly based on human exposures and population responses. Because of these advantages, epidemiological studies may provide valuable information in the Adverse Outcome Pathway framework[17]. The PMRA continues to support the conduct of well-designed epidemiological studies where exposure conditions are well characterized.

Conclusion

Overall, the IARC concluded that the evidence of carcinogenicity was limited in humans but sufficient in animals. This conclusion was reached based on statistically increased incidences of tumour findings in four chronic studies in rodents (two in rats and two in mice), as well results from genotoxicity (mostly in vitro) assays using formulated products. However, the IARC did not reflect the lack of dose-response relationships or other contextual information (for example, background/ historical control data, cytotoxicity) in their decision.

Based on a weight-of-evidence analysis that utilized all available carcinogenicity studies in animals, together with other contextual information, the PMRA did not consider any of the observed tumours to be treatment-related. The main aspects of this weight-of-evidence analysis are highlighted below:

- A clear dose-response was not observed for any of the noted tumours
- The statistically significant findings via pairwise comparisons were weighed against the lack of dose-response relationships.
- The statistically significant positive trend was weighed against the lack of consistency across several relevant studies from a total of fourteen long term toxicity/carcinogenicity studies in rodents.
- Slightly increased tumour incidences at dose levels at or above the limit dose of testing (1000 mg/kg bw/day) were not considered relevant for human health risk assessment.

[16] EPA (U.S. Environmental Protection Agency), 2010, February 2010 FIFRA SAP meeting minutes: Draft Framework and Case studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment. Available online from https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2010-0125-0079 [last accessed February, 2016]

[17] OECD, Organisation for Economic Co-operation and Development (OECD), 2012, Adverse Outcome Pathways, Molecular Screening and Toxicogenomincs. Available online from http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm [Last accessed February, 2016]

- Incidences fell within valid historical control data from the respective performing laboratories.
- There was a lack of pre-neoplastic lesions (for example, foci, hypertrophy, and hyperplasia) and/or other biologically plausible evidence (for example, mode of action data) to relate the noted tumours to glyphosate treatment.
- The weight-of-evidence from a wide range of assays, both in vitro and in vivo, that examined various endpoints such as gene mutation, chromosomal damage, DNA damage and repair, indicated no genotoxic concern for glyphosate.
- The currently available epidemiology evidence does not support a causal relationship between exposure to glyphosate and cancer outcomes.

The PMRA's determination on the carcinogenic potential of glyphosate is consistent with the most recent conclusions of other international regulatory authorities and intergovernmental organizations (USEPA CARC Report,[18] EFSA,[19] JMPR,[20] ECHA,[21] and NZEPA[22]), which concluded that glyphosate is unlikely to be genotoxic or carcinogenic. Therefore, the PMRA's conclusion with respect to the carcinogenicity of glyphosate acid, as outlined in PRVD2015-01, is unchanged.

1.1.4 Immunotoxicity

Comment

The JGTF noted that no statistically significant increase in T-cell dependent antibody response or total activity in the immunotoxicity study was observed. The JGTF requested that the statement regarding "evidence of immunotoxicity" be corrected to "no evidence of immunotoxicity." The JGTF also requested that additional wording be included to qualify PMRA's conclusion of "an altered function of the immune system could not be ruled out" to provide further context to PRVD2015-01.

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18 EPA (U.S Environmental Protection Agency), 2015, Cancer Assessment Document – Evaluation of the Carcinogenic Potential of Glyphosate. Final Report. Cancer Assessment Review Committee. Available online from http://src.bna.com/eAi [Last accessed June, 2016]

19 EFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. EFSA Journal 2015; 13(11):4302 [107 pp.] Available online from: https://www.efsa.europa.eu/en/efsajournal/pub/4302 [Last accessed June, 2016]

20 Pesticides Residues in Food, 2016. Special Session of the Joint FAO/WHO Meeting on Pesticide Residues – Report 2016. ISSN 2070-2515. FAO Plant Production and Protection Paper 227. Available online from http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/ [last accessed June, 2016]

21 ECHA (European Chemicals Agency). Public consultation on the harmonised classification and labelling proposal for Glyphosate. ECHA/NI/16/25. 2016. Available online from http://echa.europa.eu/view-article/-/journal_content/title/public-consultation-on-the-harmonised-classification-and-labelling-proposal-for-glyphosate [last accessed June, 2016]

22 NZEPA (New Zealand Environmental Protection Authority). Review of the Evidence Relating to Glyphosate and Carcinogenicity. 2016. Available online from http://www.epa.govt.nz/Publications/EPA_glyphosate_review.pdf [last accessed August, 2016]

PMRA Response

In the registrant-submitted immunotoxicity study, a dose-related increase in the T-cell dependent antibody response (IgM (Immunoglobulin M) AFC (Antibody Forming Cells)/10^6 spleen cells) was observed. The magnitude of increase was 10%, 18%, and 31% at 150, 449 and 1448 mg/kg bw/day, respectively, compared to the control group. The test guideline stated that a response of 800-1,000 IgM AFC/10^6 spleen cells should be noted in the negative control mice for the strain used in the AFC assay. Examination of individual animal data for T-cell dependent antibody response revealed that seven, six and eight animals in low, mid- and high dose groups, respectively, had a response higher than 1000 IgM AFC/10^6 spleen cells, compared to four animals in the control group, which indicated a treatment-related effect.

PRVD2015-01 also noted a dose-related increase in total spleen activity (IgM AFC/spleen x 10^3). The magnitude of increase for this effect was 13%, 50% and 54% @ 150, 449 and 1448 mg/kg bw/day, respectively, compared to the value of the vehicle control group. A non-dose-related increase in spleen cellularity (spleen cells × 10^7) of 20% and 10% in the mid- and high dose animals, respectively was noted. This increased immune response in the AFC assay was considered potentially treatment-related. However, immune effects were not observed in the rest of the toxicity database, and ultimately, this finding did not impact the risk assessment.

In summary, the PMRA examined trends (for example, dose-response relationships) as well as statistical significance in assessing the relevance of the above findings. Given that the variation (standard deviation) in the AFC assay data are generally large, key considerations other than statistical significance were important in developing an overall conclusion. The WHO (2012[23]) recommends considering unintended immune system stimulation as a noteworthy finding, but one that may be difficult to characterize or unambiguously define as adverse. Similarly, the USFDA (2002[24]) considers unintentional immunostimulation as a potentially adverse effect.

1.1.5 Aggregate Endpoint

Comment

A number of comments contested the endpoint selected by the PMRA for aggregate risk assessment, indicating that the NOAEL of 32/34 mg/kg bw/day from a 2-year rat study was inappropriate. The comments recommended that the endpoint be based on a NOAEL of 10 mg/kg bw/day due to an increased incidence of renal tubular dilation in F_{3b} offspring at the LOAEL in a three-generation reproduction toxicity study, as identified by the USEPA Integrated Risk Information System (IRIS).

[23] WHO (World Health Organization – International Programme on Chemical Safety), 2012. Guidance for Immunotoxicity Risk Assessment for Chemicals. Available online from http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf [Last accessed June, 2016]

[24] FDA (U.S Food and Drug Administration), 2012, Guidance for Industry – Immunotoxicology Evaluation of Investigational New Drugs. Available online from http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079239.pdf [last accessed June, 2016]

PMRA Response

Aggregate exposure is the total exposure to a single pesticide that may occur from food, drinking water, residential and other non-occupational sources, and from all known or plausible exposure routes (oral, dermal and inhalation). An initial step in performing an aggregate risk assessment is to review all available toxicity data and to identify the most appropriate toxicological endpoints of concern and their associated parameters (such as dose, duration, and route).[25]

Since histological changes in the salivary glands were observed in many repeat-dose oral studies over various durations in two species (rats and mice), it was considered a common endpoint of concern for aggregate risk assessment (as indicated in PRVD2015-01, page 27), particularly for potential aggregate exposure from food, drinking water and residential scenarios. In addition, this was considered appropriate for all durations since the same effects were observed from very short term dosing (28-day) or chronic dosing (two-year) studies. In reconciling the dosing routes, it was indicated that dermal toxicity studies did not examine salivary glands histologically and repeat dose inhalation studies were not available. As such, effects on salivary glands are assumed to occur via inhalation or dermal routes in the absence of route-specific and convincing mode of action data to support route-specificity of these findings.

Furthermore, the reproduction study in which renal tubular dilation was noted in the F_{3b} offspring, was not considered acceptable due to many reporting limitations. It is also important to note that this finding was observed macroscopically in a few animals only, and was considered a spurious finding in the USEPA Office of Pesticides (OPP), JMPR and EFSA assessments. Additionally, this finding does not meet the criteria for determining an appropriate toxicology endpoint for aggregate risk assessment (SPN2003-04[26]). Therefore, the endpoint chosen for aggregate risk assessment in PRVD2015-01 remains unchanged.

1.1.6 Cumulative Risk Assessment

Comment
A number of submitted comments recommended that PMRA conduct an assessment of the cumulative effects of the glyphosate pest control product and other pest control products that have a common mechanism of toxicity.

25 PMRA (Pest Management Regulatory Agency), 2003, General Principles for Performing Aggregate Exposure and Risk Assessments. Available online from http://www.hc-sc.gc.ca/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/pol-guide/spn/spn2003-04-eng.pdf [Last accessed February, 2016]

26 EPA (U.S. Environmental Protection Agency), 2001, General Principles for Performing Aggregate Exposure and Risk Assessments. Available online from http://www2.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf [Last accessed February, 2016]

PMRA Response

The *Pest Control Products Act* requires that PMRA assess the cumulative effects of pesticides. A cumulative assessment evaluates the potential adverse health effects from being exposed to more than one pesticide at a time from the same pesticide "group". These groups are created based on a common toxic effect that occurs by the same or similar mechanism. Glyphosate acid does not appear to share a common mode of toxicity with other pesticides. As such it does not belong to a 'pesticide group' that requires assessment of cumulative effects.

For more information and/or a description of the steps taken to determine a pesticide "group" for assessment of cumulative effects, refer to SPN2001-01.[27]

1.1.7 The *Pest Control Products Act* (PCPA) Hazard Characterization

Comment
A number of comments recommended that the PMRA apply a 10-fold *Pest Control Products Act* factor for human health risk assessment, as required under the *Pest Control Products Act*. The comments indicated that there was evidence of sensitivity of infants and children to glyphosate in the studies discussed in PRVD2015-01. In two of the three reproduction toxicity studies, decreased body weight in rat pups was noted at non-maternally toxic doses. The PMRA was also referred to studies in the published literature that reported endocrine effects and toxicity in the young.

PMRA Response

For assessing risks from potential residues in food or from products used in or around homes or schools, the *Pest Control Products Act* requires the application of an additional 10-fold factor to threshold effects to take into account completeness of the data with respect to the exposure of, and toxicity to, infants and children, and potential pre- and postnatal toxicity.

As indicated in PRVD2015-01 (page 17) with respect to the completeness of the toxicity database of glyphosate, many available guideline and non-guideline studies have investigated the potential developmental, reproductive, and endocrine effects of glyphosate. Recently, the USEPA completed an assessment of the results of their Endocrine Disrupting Screening Program (EDSP) Tier I testing and concluded that glyphosate showed no evidence of interaction with estrogen, androgen or thyroid endocrine pathways (USEPA, 2015). It is important to note that studies required in the EDSP program are of higher quality and reliability than certain studies available in the published scientific literature, including the in vitro assays cited in the comments received on PRVD2015-01.

With respect to potential pre- and postnatal toxicity, the two-generation reproduction toxicity studies in rats provided no indication of increased sensitivity of the young. In these studies, although offspring toxicity typically consisted of decreased body weight at doses that did not

[27] PMRA (Pest Management Regulatory Agency), 2001, Science Policy Notice (SPN2001-01) Guidance for Identifying Pesticides that have a Common Mechanism of Toxicity for Human Health Risk Assessment Available online from http://www.hc-sc.gc.ca/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/pol-guide/spn/spn2001-01-eng.pdf [Last accessed June 2016]

appear to produce maternal toxicity, it was noted that these same dose levels produced toxicity in adult animals in other studies available in the glyphosate database, (PRVD2015-01, pages 14, 17, 80, 81) lessening the level of concern for this finding. Additionally, the selected reference doses provide a sufficient margin (1000-fold) to the dose levels at which the pup bodyweights were affected.

In summary, based on the completeness of the database with respect to developmental and reproductive toxicity, the10-fold *Pest Control Products Act* factor was reduced to 1-fold for most populations. However, a 3-fold *Pest Control Products Act* factor was retained for the ARfD for females 13-49 years of age, for reasons discussed in PRVD2015-01 (page 17) and Section 1.1.2 of this document. For more information on the application of the *Pest Control Products Act* factor, please refer to SPN2008-01.[28]

1.1.8 General Comments on Health Effects and Toxicology Review

Comment
A number of comments from various stakeholder organizations (for example, Canadian Association of Agri-Retailers, the Canola Council of Canada, and Central Kootenay Invasive Species Society) acknowledged and supported the proposed re-evaluation decision on the health aspects of glyphosate. These comments emphasized the importance of a science-based approach in reviewing glyphosate and agreed with the proposed regulatory label changes.

PMRA Response

The PMRA re-evaluation drew upon a large, comprehensive body of scientific information that included data from registrants, published scientific studies, as well as information from other regulatory authorities, which formed the basis of its conclusions.

1.1.9 Glyphosate, GMOs (Genetically modified) and Health effects

Comment
A number of comments cited information from various non-governmental organizations or independent researchers, and requested that the PMRA use these sources of information as evidence for health risks of pest control products containing glyphosate in order to restrict or phase-out the uses of these products in Canada.

[28] PMRA (Pest Management Regulatory Agency), 2008, Science Policy Note (SPN2008-01): The Application of Uncertainty Factors and the *Pest Control Products Act* Factor in the Human Health Risk Assessment of Pesticide. Available online from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_pol-guide/spn2008-01/index-eng.php [Last accessed June, 2016]

PMRA Response

As noted in previous responses, the PMRA conducted a weight-of-evidence assessment that considered all relevant, hazard/toxicity data for glyphosate, including data from registrants, published scientific studies, and information from other regulatory authorities. In the PMRA assessment, published scientific toxicity data was evaluated according to the principles set out in a published USEPA guidance document.[29]

In contrast, while the documents/websites cited in these comments attempted to consolidate a wide range of sources of information, some of these studies were of low quality and reliability due to significant reporting limitations, and/or did not utilize accepted study methodologies, while others were anecdotal in nature. Also, as discussed in response to comments 1.1.3.1-1.1.3.3, studies based on formulated products are considered less relevant to characterizing the potential inherent toxicity of glyphosate itself, due to multiple and often unidentified constituents. Thus, the submitted citations did not result in a change to the toxicity assessment for glyphosate. The studies cited in these comments that were considered by the PMRA are listed in the reference list section of this document.

1.1.10 Glyphosate and Modern Diseases (such as Autism, and Celiac Disease)

Comment

A number of comments cited published articles that link glyphosate to various health problems such as autism, and celiac disease (for example, Samsel and Seneff 2013[30]; 2015[31]), and requested that PMRA restrict and/or phase-out the uses of pest control products containing glyphosate based on health effects reported in these articles.

PMRA Response

Correlations do not provide sufficient evidence of causation. These articles report disease frequencies in specific regions over several time periods. Although correlations were reported, these were difficult to interpret, as it could not be determined whether the health outcomes preceded or followed glyphosate application. These articles also lacked sufficient detail regarding the strength, consistency and specificity of the noted correlations. For example, in regions where glyphosate applications were low, it was not clear if the health outcomes occurred at lower incidences compared to those of the regions where glyphosate applications were at higher levels. Overall, due to the lack of adequate information regarding the amount, route or duration of exposure; or the timing between exposure and the onset of the symptoms, an association and/or causality relationship could not be assessed.

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29 EPA (U.S. Environmental Protection Agency), 2012, Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment. Available online from http://www2.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf [last accessed February, 2016]

30 Samsel A, and Seneff S. 2013. Glyphosate's suppression of Cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: pathways to modern diseases. Entropy. 15: 1416-1463.

31 Samsel A, and Seneff S. 2015. Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies. Surgical Neurology International. 6 (45).

1.1.11 Health Effects on the Gastrointestinal Tract and its Microbiome

Comment
A number of comments cited published articles that report an impact of glyphosate on the human intestinal microbiome, producing gastrointestinal effects which, some propose, may ultimately affect human health. Some comments noted that glyphosate is patented as an antibiotic, and requested information on the long term effects of ingesting glyphosate, on the human gut microbiome. Overall, the comments claimed that the PMRA did not address the implications of the chelation activity and antimicrobial properties of glyphosate.

PMRA Response

Glyphosate targets an amino acid synthesis pathway in plants that is shared by certain types of bacteria, but not humans. There is very little scientific evidence to support the claim that glyphosate has any direct impact on human gut microflora, or has any subsequent health effect. Several reports[32] [33] postulate that environmental chemicals may potentially lead to changes in normal gut microbiota. However, information to date is based on in vitro studies, with in vivo evidence being very limited and inconclusive.

The reference doses established by the PMRA, and documented in PRVD2015-01, include consideration of clinical signs of toxicity on the gastrointestinal tract and are considered protective of potential effects on the gastrointestinal tract.

1.1.12 Endocrine Effects

Comment
A few comments referred the PMRA to articles that indicated glyphosate was an endocrine disruptor and requested that the PMRA use this evidence to phase-out pest control products containing glyphosate.

PMRA Response

The cited articles were generally studies that examined the effects of glyphosate formulations on a specific biochemical pathway in in vitro tests. These studies frequently did not provide test material composition.

The PMRA considered multiple lines of evidence from various toxicity studies in assessing the potential for glyphosate to affect endocrine systems. Studies conducted by the NTP, guideline two-generation reproduction toxicity studies, as well as studies conducted under the US EDSP

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[32] Shehata AA, Shrödl W, Aldin AA, Hafez HM, Kürger M. 2013. The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Current Microbiology 66(4): 350-358. Available online from http://link.springer.com/article/10.1007%2Fs00284-012-0277-2 [Last accessed June, 2016]

[33] Dietert, RR. The Microbiome in early life: self-completion and microbiota protection as health priorities. Birth Defects Research (Part B) 101: 333-340 (2014). Available online from http://onlinelibrary.wiley.com/doi/10.1002/bdrb.21116/abstract [last accessed June, 2016]

program (United States Endocrine Disruptor Screening Program), were considered. Glyphosate has not been shown to interact with any specific endocrine pathway and has no physical / chemical properties or structural similarity to other chemicals that are known to interact with the endocrine system. Finally, as noted in response to comment 1.7, the USEPA completed a weight-of-evidence assessment on results obtained from the EDSP assays and concluded that glyphosate does not interact with estrogen, androgen, or thyroid pathways and that additional Tier 2 data was not triggered.

Thus, there is no compelling evidence to suggest that glyphosate has any significant adverse effect on endocrine-related pathways. See also response to comment 2.2.7.

1.1.13 Bioaccumulation

Comment
A few comments questioned whether glyphosate could accumulate in the body over time and how glyphosate is monitored to ensure levels do not go above acceptable limits that could cause health effects.

PMRA Response

No indication of glyphosate accumulation was reported in any of the toxicity studies, as summarized in PRVD2015-01. When animals received single or repeat doses (14 days), in each case, the administered dose (AD) was excreted within 7 days post-dosing and negligible levels (under 1% of AD) remained in the examined tissues. Overall, the metabolic studies indicated poor absorption from the gut, almost complete excretion, and very minor metabolism in animals. Published regulatory reports by EFSA and the USEPA confirm these results. In summary, glyphosate is not expected to accumulate in the body over time. Refer also to response 2.2.8.

1.1.14 Use of Independent Scientific Studies

Comment
A number of comments stated that the PMRA, in its review of glyphosate, appeared to consider only “seller sponsored science”. The comments referred the PMRA to a number of published studies that link glyphosate to health effects. Overall, these comments emphasized support for the use of “third party” data in assessing the health effects and making the final re-evaluation decision for glyphosate, in lieu of manufacturer-supplied data.

PMRA Response

Regulatory authorities world-wide regard studies that are performed under conditions of good laboratory practices (GLP) and according to internationally agreed upon study designs, such as the OECD test guidelines, as the most reliable, reproducible, and scientifically sound. Studies conducted according to these guidelines are of sufficient statistical power to detect effects of concern, they investigate many potential endpoints of toxicological concern, and have detailed individual animal results that enable regulatory authorities to thoroughly evaluate and interpret the data in an independent manner. Adherence to these guidelines produces studies in which regulators have a high degree of confidence.

Studies conducted by academic laboratories often have lower statistical power due to the use of fewer animals, investigate far fewer toxicological endpoints, and lack sufficient detail in their published form. These limitations prevent regulatory authorities from performing an in-depth analysis of study results.

As discussed in PRVD2015-01, the re-evaluation took into account all relevant sources of toxicity data in order to evaluate the potential health effects of glyphosate acid. This included an independent review of registrant-supplied data, which are required for the pesticide review and approval process in Canada, as well as consideration of scientific publications and information from other regulatory authorities.

For more information on the toxicology data requirements for registration of pest control products in Canada, please consult Guidance for Developing Datasets for Conventional Pest Control Product Applications: Data Codes for Parts 1 - 7 and 10[34] and/or 'OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring'.[35] Refer also to comment 2.2.9.

1.1.15 Health Effects of the Glyphosate Formulated Products

Comment

A number of comments questioned why glyphosate formulated products were not assessed for their health effects, stating that the health effects discussed in PRVD2015-01 were based on the active substance (glyphosate acid).

PMRA Response

Although the majority of mammalian toxicity studies for glyphosate were conducted using the active substance (glyphosate acid), toxicology studies that assess the acute hazard of formulated products are also examined. Individual formulated products are also used for other studies, such as in the generation of residue chemistry (field trial) data considered during the risk assessment phase. For more information on the data required for the active ingredient and formulated end use products for the registration of pest control products in Canada, please consult Guidance for Developing Datasets for Conventional Pest Control Product Applications: Data Codes for Parts 1-7 and 10.

In addition, as part of the glyphosate re-evaluation, an assessment was conducted on polyethoxylated tallow amines (POEA), which are a family of compounds often used as formulants in pest control products that function as surfactants. POEA substances (CAS no.

[34] Guidance for Developing Datasets for Conventional Pest Control Product Applications: Data Codes for Parts 1, 2, 3, 4, 5, 6, 7 and 10. Available online from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_pol-guide/data-guide-donnees/index-eng.php [Last accessed Dec, 2016]

[35] OECD (Organisation for Economic Co-operation and Development), 1997, OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Available online from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/mc/chem(98)17&doclanguage=en [Last accessed June, 2016]

61791-26-2) are included on List 4B of PMRA's list of Formulants (see REG2005-01[36] page 28). Currently, formulants are categorized into one of the five lists which rank them in descending order of concern. List 4B contains formulants are of minimal concern under specific conditions of use. For more details on the regulation of formulants in pest control products, refer to the PMRA Regulatory Directive DIR2006-02.[37]

As indicated in PRVD2015-01, the USEPA completed a human health risk assessment for phosphate ester, tallowamine, ethoxylated (ATAE), which is a subfamily of POEA. The PMRA considered the USEPA review, and reviewed the available toxicity studies that made up the USEPA assessment, including the pivotal study used in endpoint selection, which was a combined repeat-dose rat toxicity study with a reproduction/developmental toxicity screening component. As noted in the USEPA assessment, glyphosate products that contain no more that 20% POEA by weight are not of concern. Currently, all registered glyphosate products in Canada meet this limit.

1.2 Comments Related to Occupational / Residential Exposure

1.2.1 Bystanders

Comment
There were many general comments suggesting that the current level of non-dietary exposure to glyphosate is not safe for the general public (bystanders).

PMRA Response
Only those uses where human exposure to a pesticide is well below the level that cause effects in animal tests are considered acceptable for registration in Canada. This was confirmed with the re-evaluation of glyphosate

During the re-evaluation of glyphosate, it was recognized that there is potential for short-term exposure when entering treated non-cropland areas (in other words, hiking through forests or parks that have recently been treated with glyphosate). Calculated MOEs for all lifestages met the target MOE and are therefore not of concern to human health. In the interest of promoting best management practices and to minimize human exposure the following label statement is required:

"Apply only when the potential for drift to areas of human habitation or areas of human activity such as houses, cottages, schools and recreational areas is minimal. Take into consideration wind speed, wind direction, temperature inversions, application equipment and sprayer settings."

[36] PMRA (Pest Management Regulatory Agency), 2005. Regulatory Note: *PMRA List of Formulants*. Available online from http://publications.gc.ca/collections/Collection/H113-7-2005-1E.pdf [Last accessed February 2016]

[37] PMRA (Pest Management Regulatory Agency), 2006. Regulatory Directive: *Formulants Policy and Implementation Guidance Document*. Available online from http://www.hc-sc.gc.ca/cps-spc/alt_formats/pacrb-dgapcr/pdf/pubs/pest/pol-guide/dir/dir2006-02-eng.pdf [Last accessed February, 2016]

1.2.2 Restricted-Entry Interval

Comment
Comments questioned the basis for changing the "Restricted-Entry Interval" to 12 hours for commercial class products, when PRVD2015-01 states that postapplication risks are not of concern for all uses. Comments indicated that, in general, glyphosate dries on the plant very quickly and there are no residues that can be readily passed on to workers. It was recommended that the label not specify a time limit but should instead indicate that field entry is allowed once the herbicide application has dried.

PMRA Response
A restricted-entry interval (REI) is the period of time that agricultural workers, or anyone else, must not do hand labor in treated areas after a pesticide has been applied. This is to allow residues and vapours to dissipate to safe levels for work to be performed. Hand labour tasks involve substantial worker contact with treated surfaces such as plants, plant parts, or soil.

All pest control products with agricultural uses require a minimum REI of 12 hours to protect workers, and others, from potential risks that may occur from both immediate and longer-term exposures to pesticide residues, vapors, and particulates. A minimum 12-hour REI allows residues to dry and vapors to dissipate, limiting potential effects such as irritation or allergic reactions.

1.2.3 Personal Protective Equipment

Comment
It was noted that in the proposed label amendments for products containing glyphosate, as presented in Appendix XII of PRVD2015-01, there is no mention of proposed changes for protective clothing at the time of mixing and loading, application, clean-up and repair. For commercial formulations of glyphosate, the current label wording makes no requirement for use of personal protective equipment during application. The lack of proposed label changes for protective clothing is an important oversight, especially the lack of requirement for protective clothing during spraying.

PMRA Response
The exposure estimates for mixers, loaders, and applicators of glyphosate used in the agricultural exposure assessment presented in PRVD2015-01 were based on a baseline level of PPE (long pants, long sleeved shirts and chemical-resistant gloves). The calculated dermal, inhalation, and combined MOEs are greater than the target MOE for all mixing, loading, and applying activities and therefore are not of concern. As such, no additional requirements for protective clothing beyond the baseline level of PPE are needed, as the existing labels already include the appropriate PPE.

1.2.4 Application Rates in Aggregate Exposure Assessment

Comment
In PRVD2015-01, all three aggregate exposure scenarios initially assumed 2 applications with a 7 day interval at the highest rate. At that application rate, the calculated MOEs for adult and youth/children (6 to <11 years old) scenarios reached the target MOE of 100, but the MOE for

children (1 to <2 years old) for the post-application + incidental oral exposure + chronic dietary scenario did not. It was interpreted that the PMRA changed the aggregate assessment to one application of glyphosate with a seven-day time-weighted turf transferable residue average for the entire aggregate assessment for all populations. It was suggested to use the highest application rate and frequency of glyphosate use to assess the aggregate exposures, and, if safety margins (MOE) were not met, to propose meaningful and wide-ranging use restrictions to increase human health protection.

PMRA Response
When conducting the aggregate exposure assessment, 2 applications (with a 7 day interval) at the highest rate were assumed. All calculated MOEs reached the target MOE except for children (1 to <2 years old) for the post-application + incidental oral exposure + chronic dietary scenario. Therefore, dietary and non-dietary exposure refinements were required.

The dietary exposure assessment used US Tolerances or Codex MRLs for situations where these values were greater than Canadian MRLs. However, domestic production and import statistics indicated that barley, oats, and wheat consumed in Canada are almost totally produced in Canada (>99%), with <1% imported. Thus, it was considered reasonable to use Canadian MRLs for these crops as a refinement in the calculation of the chronic dietary exposure estimates for the purpose of aggregation with residential exposure only, rather than the US and Codex group tolerance of 30 ppm. The current Canadian MRLs in these cereal crops are as follows: barley (and barley flour) - 10 ppm, barley milling fractions (except flour) -15 ppm, oat (and oat flour) - 15 ppm, oat milling fractions (except flour) - 35 ppm, wheat (and wheat flour) - 5 ppm, and wheat milling fraction (except flour) - 15 ppm.

In addition, assuming 2 applications (with a 7 day interval) at the maximum application rate is a highly conservative exposure assumption, as it is unlikely that children would be exposed to turf residues of the highest rate, at the lowest interval of application immediately after application. Therefore, a refinement using 1 application of glyphosate along with a 7 day time-weighted TTR average was used (the average resides of glyphosate were calculated over a 7 day span) for the entire aggregate assessment for all populations.

These refinements are health protective and all calculated MOEs met the target MOE and are not of concern to human health.

1.3 Comments Related to Dietary Exposure

1.3.1 Genetically Modified Crops

Comment
A number of comments expressed concern regarding the potential for higher residue levels of glyphosate in genetically modified (GM) crops, as reported in the article "*Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM Soybeans. Bohn, T. et al., Food Chem. 2014, 153: 207-215.*"

PMRA Response

The residue chemistry of glyphosate, i.e. the nature and magnitude of residues of glyphosate in conventional (non-GM) crops, as well as in GM crops, is well understood and extensively documented. PMRA has received and reviewed all the metabolism studies required as per the PMRA Residue Chemistry Guidelines (Dir98-02[38]). The residue definition (RD) in plant commodities is based on scientifically sound metabolism studies conducted specifically in both types of crops. Whenever a new variant of GM crop is introduced on the market, the residue definition is reassessed based on mandatory supporting metabolism studies in that particular GM crop variant. The residue definition in animal commodities (resulting from feeding of the GM crop) is adjusted accordingly.

Currently there are three types of soybeans on the market: conventional (non-GM) soybean, EPSPS-GM soybean (containing the EPSPS gene) and GAT-GM soybean (containing the GAT gene). Based on metabolism studies in the respective crops, the RD in conventional and EPSPS soybeans are defined as the sum of glyphosate and its metabolite aminomethylphosphonic acid (AMPA). The RD in GAT soybean includes additional metabolites (acetylated glyphosate and acetylated AMPA) resulting from the specific biotransformation of glyphosate in GAT crops. As soybeans sold on the market cannot be distinguished with regards to whether they are conventional, EPSPS or GAT soybeans, the PMRA uses the most inclusive RD for soybeans, i.e., the RD in soybeans is the sum of glyphosate, AMPA and their acetylated counterparts.

All the metabolites included in the RD were deemed toxicologically equivalent to glyphosate. Consequently, in terms of residues, all the metabolites are expressed as the stoichiometric equivalent of glyphosate by using the appropriate molecular weight conversion factor (MWCF). The MWCFs are 1.5 for AMPA, 1.1 for N-acetyl AMPA and 0.8 for N-acetyl glyphosate. This means that the residue of glyphosate in soybeans (and in canola and corn comprising similar GM variants) is calculated as the sum: glyphosate + 1.5 AMPA + 1.1 N-acetyl AMPA + 0.8 N-acetyl glyphosate.

Residues of glyphosate (or any pesticide) in soybeans (or any crop) is a function of the agricultural practice by which they have been produced. GM soybeans are expected to have residue detects due to repeated spraying (in compliance with label directions) of plants throughout the production season. Conventional soybeans will contain lower residues levels because glyphosate is applied to weeds (before planting) and not on soybean plants. These facts are supported by field trial residue studies, which, as noted above, are required as per the PMRA Residue Chemistry Guidelines (Dir98-02). The field trial studies are conducted according to the petitioned-for use pattern and usage conditions (good agricultural practices) and constitute the basis for the registration and establishment of Maximum Residue Limits (MRLs). MRLs are established on the basis of worse case scenarios (maximum application rate, highest frequency of applications and shortest pre-harvest interval) within the agricultural practices. An MRL represents the maximum amount of residues that may remain on food when a pesticide is used according to label directions, and serves as a food safety standard. The results presented in the cited article did not exceed the established MRL of 20 mg/kg (20 ppm) for glyphosate in soybeans and confirm that current Canadian MRLs of glyphosate (including the metabolites) in

[38] PMRA (Pest Management Regulatory Agency), 1998. Regulatory Directive: *Residue Chemistry Guidelines*. Can be requested online from http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_pol-guide/dir98-02/index-eng.php [Last accessed August 2016]

soybeans are adequate. These MRLs were used in the estimation of short term (acute) as well as long term (chronic) dietary exposures. No dietary risk concerns were identified, as the levels of exposure estimates were well below the reference doses set for dietary risk assessment (the ARfD and ADI).

1.3.2 Mitigation Measures

Comment
A question was raised regarding a general (introductory) statement in Section 3.2 of PRVD2015-01 (Dietary Exposure and Risk Assessment) which reads: "*In situations where the need to mitigate dietary exposure has been identified, the following options are considered. Dietary exposure from Canadian agricultural uses can be mitigated through changes in the use pattern*." The comment indicated that this statement implies that there are concerns with the glyphosate use pattern and, therefore, requested clarity on what mitigation measures were proposed.

PMRA Response
This is a general statement which would apply to any pesticide presenting dietary risk concerns. As no dietary risk concerns were identified for glyphosate, no mitigation measures were required.

1.3.3 Food Labelling

Comment
A comment requested that "glyphosate content" be added to all food labels (in grocery stores) so that consumers could decide whether they want to buy food containing glyphosate residues or not.

PMRA Response
Although Health Canada and the Canadian Food Inspection Agency (CFIA) share the responsibility for food labelling policies under the *Food and Drugs Act*, food labelling does not fall within the mandate of the PMRA or the *Pest Control Products Act* (PCPA). Other areas of Health Canada are responsible for developing policy and setting standards related to the health and safety aspects of labelling under the *Food and Drugs Act and Regulations*, whereas the CFIA applies these policies and enforces the regulations. The CFIA also has the mandate to develop general food labelling policies and regulations not related to health and safety. In particular, the CFIA is responsible for protecting consumers from misrepresentation and fraud with respect to food labelling, packaging and advertising, and for prescribing basic food labelling and advertising requirements.

With respect to glyphosate residues in foods, the CFIA is responsible for monitoring the Canadian food supply for pesticide residues and the determination of compliance with MRLs specified by Health Canada. In addition, both Canadian and international producers are aware of these MRLs and must comply with them in order to sell their produce in Canada or export to other countries that also have MRLs established. Therefore, it is expected that foods with residues higher than the MRL would not be present in the Canadian food supply.

For more details, please visit the CFIA Website at http://www.inspection.gc.ca/food/labelling/food-labelling-for-industry/method-of-production-claims/genetically-engineered-foods/eng/1333373177199/1333373638071

1.3.4 Glyphosate Used as Desiccant and Residue

Comment
Comments expressed concern about the use of glyphosate for pre-harvest desiccation on conventional crops, the level of residues left on desiccated crops at harvest and the resulting long-term dietary exposure.

PMRA Response
Glyphosate is registered for pre-harvest use (desiccation) on a number of conventional crops including wheat, barley, oats, canola, flax, lentils, peas, dry beans, and soybeans. To support this use, field trial residue studies were required to determine the level of residues resulting from the pre-harvest desiccation conducted according to the requested use pattern. Maximum residue limits (MRLs) for these crops were established on the basis of the submitted studies. Those MRLs were included in the estimation of short term (acute) as well as long term (chronic) dietary exposures. During PMRA's assessment, no dietary risk concerns were identified, as the levels of exposure estimates were well below the reference doses set for dietary risk assessment (the ARfD and ADI).

1.3.5 Safety of GMO Crops

Comment
There were general questions as to whether GM crops are safe for human consumption.

PMRA Response
Health Canada conducts a rigorous and thorough science-based assessment of all GM food products before they are allowed to enter the Canadian marketplace. The assessments are conducted under the *Food and Drug Regulations*, which prohibit manufacturers of these products from selling them in Canada until Health Canada has completed a full safety assessment and has found them to be as safe and nutritious as conventional foods.

The approach taken by Health Canada in the safety assessment of GM foods is based upon scientific principles developed through expert international consultation over the last twenty years with agencies such as the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the Organization for Economic Co-operation and Development (OECD). This same approach is currently applied by regulatory authorities around the world in countries such as the European Union, Australia/New Zealand, Japan and the United States. For more details, please visit the Health Canada Website at http://www.hc-sc.gc.ca/fn-an/gmf-agm/index-eng.php.

1.3.6 Acceptable Level of Exposure

Comment
Comments included the question: "What is considered as acceptable level of exposure and how is that monitored to be sure that levels do not become unacceptable?"

PMRA Response
When assessing pesticide related health risks, two key factors are considered: the dose levels at which no health effects occur in animal testing (basis for the establishment of toxicological reference doses for humans) and the levels to which people may be exposed through diet, when handling and applying the pesticide, or by entering treated sites (in other words, level of exposure). The dose levels used to assess risks (in other words, toxicological reference doses) are established to protect the most sensitive human population (for example, children and nursing mothers). Only pesticide uses for which the level of exposure (through diet for example) is well below levels that cause no effects in animal testing are considered acceptable for registration.

Reference doses define levels to which an individual can be exposed to a pesticide residue over a single day (acute) or lifetime (chronic) and expect no adverse health effects. Generally, dietary exposure from food and water is acceptable if it is less than 100% of the acute reference dose or chronic reference dose (also known as acceptable daily intake).

The amount of pesticide to which an individual is exposed (in other words, exposure) is determined by determining the amount of pesticide that is in or on the food (in other words, residue levels) and combining that with the amount and type of foods that people eat (in other words, food consumption). Risk is then estimated by comparing the level of exposure to the reference doses described above. As previously noted, if the estimated intake is less than the reference dose, there are no dietary risks of concern.

In addition, inherent to pesticide registration is the establishment of maximum residue limits (MRLs) of the pesticide in/on foods on which the pesticide has been applied. An MRL represents the maximum amount of residues that may remain on food when a pesticide is used according to label directions, and serves as a food safety standard. The MRLs are calculated from residue data obtained from field trials that are conducted using the maximum application rate and the shortest pre-harvest interval. These MRLs, or field trial residue values, are used to estimate the level of dietary exposure at the time of pesticide registration. A pesticide is registered only if the calculated level of exposure is acceptable (in other words, exposure does not exceed the toxicological reference dose). The Canadian Food Inspection Agency (CFIA) is responsible for monitoring the Canadian food supply for pesticide residues and work very closely with Health Canada (PMRA) to ensure that the foods available on the Canadian market are compliant with the MRLs. In 2015, the Canadian Food Inspection Agency (CFIA) tested approximately 700 samples consisting of a variety of juice and juice blends, grains and grain products, beans, lentils, and a wide variety of fruit and vegetables. The CFIA also initiated a targeted survey of approximately 2,500 samples, looking at levels of glyphosate in bean, pea, lentil, chickpea and soy products, as well as less commonly consumed grains such as barley, buckwheat and quinoa. The results show a high degree of compliance with the MRLs established by the PMRA for glyphosate. The CFIA anticipates having their full analysis completed by Spring 2017.

1.3.7 Monitoring of Glyphosate Residue

Comment
Several comments noted: 1) the necessity to monitor amounts of glyphosate applied on fields, especially where resistant weeds have emerged; 2) the necessity to measure glyphosate residues resulting from ordinary field applications (field trial residue data); 3) the necessity to obtain glyphosate residue data that are reflective of foods as consumed through monitoring programs in

which food samples down the chain of commerce are sampled and analysed; 4) further information on maximum residue levels of glyphosate in food; and 5) the necessity to monitor glyphosate residues in body fluids and tissues (biomonitoring); as they are not included in the *Third Report on Biomonitoring of Environmental Chemicals in Canada*.

PMRA Response

As noted in response to comment 1.3.6, glyphosate residues on foods have been measured in field trial studies that are required to register a pesticide for specific uses, as per PMRA Residue Chemistry Guidelines (Dir98-02). These field trial data were used for the establishment of maximum residue limits (MRLs) for glyphosate, that is, the maximum legally allowed amount of glyphosate residue that may remain on foods when glyphosate is used according to label directions. The MRLs are enforced by law, and, the conditions of registration must be observed in all circumstances, regardless of whether resistant weeds have emerged or not. In cases of weed resistance, a higher rate than what is currently on the labels cannot be used, as this could lead to MRL exceedance and would be in violation of the *Food and Drugs Act*. The *Food and Drugs Act* prohibits the sale of adulterated food; that is, food containing a pesticide residue that exceeds the specified MRL.

The Canadian Food Inspection Agency (CFIA) is responsible for monitoring the Canadian food supply for pesticide residues and the determination of compliance with MRLs specified by Health Canada. As noted in response to comment 1.3.6, in 2015, the Canadian Food Inspection Agency (CFIA) tested approximately 700 samples consisting of a variety of juice and juice blends, grains and grain products, beans, lentils, and a wide variety of fruit and vegetables. The CFIA also initiated a targeted survey of approximately 2,500 samples, looking at levels of glyphosate in bean, pea, lentil, chickpea and soy products, as well as less commonly consumed grains such as barley, buckwheat and quinoa. The results show a high degree of compliance with the MRLs established by the PMRA for glyphosate. The CFIA anticipates having the full analysis completed by spring 2017. A complete list of MRLs specified in Canada can be found on the PMRA's MRL Database, an online query application that allows users to search for specified MRLs, regulated under the *Pest Control Products Act*, for pesticides, including glyphosate, or food commodities (http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php). For details on CFIA's monitoring program, please visit the CFIA website at http://www.inspection.gc.ca/food/fresh-fruits-and-vegetables/food-safety/chemical-residues/overview/eng/1374514433922/1374514696857.

Biomonitoring is a key tool used as an indicator and quantitative measure of exposure to chemicals in the environment. Human biomonitoring data contribute to our understanding of exposure and provide information to inform the management of the health risks posed by chemicals. The Canadian Health Measures Survey (CHMS) is an ongoing national biomonitoring survey led by Statistics Canada, in partnership with Health Canada and the Public Health Agency of Canada. Biomonitoring data have been reported for Cycle 1 (2007-2009), Cycle 2 (2009-2011) and Cycle 3 (2012-2013). Cycle 4 is currently underway, with data collection for this cycle having taken place from 2014 to 2015. These cycles are complementary, meaning that not all environmental chemicals (including pesticides) are included in a given cycle. For example, 55% of the chemicals measured in Cycle 2 were not included in Cycle 1 and about 31% of the chemicals measured in Cycle 3 were not included in previous cycles. Specific chemicals/pesticides are added to the list of measured chemicals in different cycles. Glyphosate, like many other pesticides, is being considered for inclusion in forthcoming cycles. For details on

the Canadian Health Measures Survey, please visit the Health Canada Website at http://www.hc-sc.gc.ca/ewh-semt/contaminants/human-humaine/chms-ecms-eng.php.

1.3.8 Glyphosate Use on Forest Vegetation and Effect on Health

Comment
One Aboriginal group provided the following comments:

I. Health Canada's glyphosate PRVD is based on dietary and occupational exposures that do not correspond with Anishinabek use of the territories for food, medicine and water;

II. Laboratory toxicological studies are based on reference values that do not conform to their own standards of risk, and do not take into account the cumulative effects of the environmental contaminants to which they are exposed;

III. They are concerned about the combined toxicity of glyphosate and the surfactants, solvents, and other additives.

PMRA Response
While the dietary risk assessment conducted by the PMRA does not directly assess the anticipated residues of glyphosate in edible forest vegetation, nor is the dietary burden to wild game specifically determined, based on assessments available, the PMRA does not expect that glyphosate residues from these foods would be of concern when ingested. This is because, in the dietary assessment that was conducted, residues in farm animal commodities were estimated and maximum residue limits (MRLs) were established by assuming the worst case scenario where the animal diet is considered to be comprised of 100% glyphosate-treated feedstuff, treated at the maximum application rate. This results in high-end residue estimates. For the same reason, residues in/on edible forest vegetation are expected to be low compared to MRLs established on conventional crops. These MRLs are established based on the worst case scenario, in other words, maximum application rate, shortest preharvest interval and maximum allowed number of applications per season. As noted in PRVD2015-01, using the above scenarios, there were no risk concerns from dietary exposure to glyphosate. The acute dietary exposure estimate (from food and drinking water) at the 95th percentile was 31% of the acute reference dose (ARfD) for females 13-49 years of age and ranged from 12% to 45% of the ARfD for all other population subgroups. The chronic dietary exposure estimate for the general population was 30% of the acceptable daily intake (ADI). Exposure estimates for population subgroups ranged from 20% of the ADI (for adults aged 50 years or older) to 70% of the ADI (for children 1-2 years old). Exposures less than 100% of the ARfD and ADI are not of concern. In the case of glyphosate, even when high-end (worst case) exposure estimates were used, no risk concerns to human health were identified.

The PMRA also conducted a health risk assessment for hikers walking through the forest immediately after application. The populations considered were adults, youths and children aged 6 to 10 years. From these estimates, no risk concerns were identified. As well, when exposures were aggregated (in other words, dietary exposure including from drinking water + non-dietary exposures as would occur from hiking in the forest), risks were also not of concern for the various population groups. Refer also to responses on environmental risk in Sections 2.2 and 2.4.

Regarding the cumulative effects of pesticides, please refer to the response to comments in Section comment 1.1.6 Cumulative Risk Assessment.

Regarding the combined toxicity of glyphosate and the surfactants, solvents and other additives, please refer to the response to comments in Section 1.1.15 Health Effects of the Glyphosate Formulated Products.

2.0 Comments Related to the Environmental Risk Assessments

2.1 Environmental Fate

2.1.1 Surficial and groundwater pollution and monitoring

Comment
Comments suggested or were concerned that glyphosate has the potential to leach to groundwater and natural areas, polluting water.

PMRA Response
In soil and water, glyphosate has been shown to break down quickly to aminomethylphosphonic acid (AMPA) through microbial processes and is considered to be non-persistent to moderately persistent. Glyphosate has low mobility in soil, giving it a low potential to contaminate groundwater systems, especially aquifers with low water hardness (Jayasumana et al. 2014). Glyphosate can enter surface waters when applied near water bodies or when carried in runoff, such as during a rain event on a steep slope. Glyphosate (without surfactant) and AMPA have comparable toxicological and ecotoxicological profiles, with both being considered to have low toxicity in general. According to the WHO (2004), the presence of glyphosate and AMPA at levels expected to be found in drinking water does not pose a risk to human health. Monitoring studies conducted throughout Canada indicate that glyphosate is rarely detected in groundwater. Although glyphosate is often detected in surface water, the concentrations detected are at relatively low levels that do not pose a risk of concern.

2.1.2 Glyphosate and AMPA persistence in soils and waters

Comment
Comments noted that glyphosate soil half-life values vary widely in terrestrial field dissipation studies in North America and that it may be more persistent than previously thought. Glyphosate may build up in soils and long-term negative effects are expected to occur. Glyphosate and AMPA are both frequently detected in soil and water in field dissipation studies from the United States (Battaglin et al. 2014).

PMRA Response
Glyphosate use per hectare in Canada is much lower compared to the US. Aquatic field studies conducted in Canada, including water monitoring studies, demonstrate glyphosate is detected less frequently and at lower concentrations than those reported in the US (Glozier et al. 2012, Hurley et al. 2012). The use of US field data for interpretation of the fate of glyphosate in Canada is challenging as the countries share only a few ecoregions, with climate and soil being different in much of the US where glyphosate is used as compared to Canada.

Terrestrial field dissipation studies

Laboratory studies conducted with glyphosate applied on different soils have DT_{50} (half-life) values ranging from 1 to 19.3 days, which classifies glyphosate as non-persistent to slightly persistent and indicates biotransformation by micro-organisms is effective.

Canadian terrestrial field dissipation studies show DT_{50} values ranging from 6 to 155 days for agricultural soils (average of less than 45 days) and from 24 to 82 days for forest soils (average of less than 55 days), similarly, in the US, DT_{50} values range from 1 to 174 days for agricultural soils (average of 41 days) and from <1 to 40.2 days for forest soils. The biotransformation of glyphosate is faster in forest ecosystems. In both environments, the compound is generally found in the upper soil horizons (0-15 cm depth) indicating overall that leaching to groundwater under field conditions is limited. The field data suggests glyphosate is non persistent to moderately persistent under field conditions and is not expected to carry over to the next year.

The wide range of dissipation rates, mainly in agricultural ecosystems, is likely a result of variation among soils, especially when considering foreign ecoregions (de Jonge et al. 2001; Vereecken, 2005, Borggaard and Gimsing, 2008, Farenhorst et al. 2009). Soil microbial activity may not always be efficient at transforming glyphosate or there may be other physical and chemical processes involved, reducing the rate of breakdown. Rapid adsorption to soil particles may play a role in preventing the transformation of glyphosate even in upper soil horizons where microbial activity is normally high and also when upper soil levels are not saturated with phosphate fertilizers (Helander et al. 2012). Preferential flow may play an important role, where root channels created by the death and decay of non-crop plants following glyphosate applications lead to the transport of glyphosate to lower soil horizons, however, leaching of glyphosate to deep soil horizons appears to be minimal.

Aquatic field dissipation studies

In general, aquatic field dissipation studies conducted in agricultural and forestry ecosystems in Canada and in the US indicate that glyphosate is non-persistent in natural waters (DT_{50} values ranging between ≤ 0.4 and 11.2 days).

Aquatic field dissipation studies conducted by Battaglin et al. (2014) and Battaglin and Koloc, (2014), show that glyphosate is readily transformed to AMPA by micro-organisms. Glyphosate was detected without AMPA in only 2.3% of samples, whereas AMPA was detected without glyphosate in 17.9% of samples. Both compounds were reported to be detected frequently in US soils and sediment, ditches and drains, precipitation, rivers, and streams, but less frequently in lakes, ponds, wetlands, soil water and groundwater. The study authors indicated that all concentrations of glyphosate measured were below the levels of concern for human and wildlife safety.

2.1.3 Runoff and aerial transport of glyphosate

Comment

Comments noted that the results of a runoff event studied in Argentina (Peruzzo et al. 2008) raise concerns about levels of glyphosate transported by runoff to aquatic environments. Glyphosate has been found in air and rain as demonstrated in a study conducted in Mississippi, USA (Chang et al. 2011, PMRA 2459642).

PMRA Response

The study of Peruzzo et al. 2008 suggests that rain events play an important role in transporting glyphosate present in the soil to stream water through runoff. In general, in the absence of mitigation measures to limit the run-off, especially when the ground is bare early in the season, this is not disputed. However, among all pesticides used in crop production in Argentina and elsewhere in the world, including Canada, glyphosate is among those that bind most strongly to soil. Despite glyphosate's high affinity for adsorption to soil particles, many studies have shown that the compound can find its way into water bodies, including studies from Italy (Screpanti et al., 2005; PMRA 2460734, Capri and Vicari, 2010; PMRA 2460735), the United States (Battaglin et al. 2005, PMRA 2423832, Scribner et al. 2007; PMRA 2460747, Newton et al. 1984; PMRA 1155371, Edwards et al. 1980; PMRA 2462226), Europe (Coupe et al. 2011; PMRA 2460748, Gregoire et al. 2010; PMRA 2462223, Siimes et al. 2006; PMRA 2462224), South America (Aparicio et al. 2013; PMRA 2462258) and Canada (Roy et al. 1989; PMRA 2460737, Struger et al. 2008; PMRA 1739313).

Many of the studies reported in the literature, including the one of Peruzzo et al. 2008, were conducted in ecoregions that are not equivalent to any Canadian ecoregions, meaning the soil and climatic conditions in study locations may not be relevant to conditions in Canada.

The amount of glyphosate applied in agricultural and forestry systems has increased since its first registration (about 40 years ago) and this is a factor in its frequent detection in surface waters and, more recently, in groundwaters of other countries outside North America (Sanchis et al. 2011, PMRA 2460750).

Examination of the factors controlling the transport of glyphosate to surface waters on a watershed scale is needed to determine which factors are important in this process and how these factors may change in importance, both spatially and temporally (Coupe et al. 2011, PMRA 2460748). The strong sorption of glyphosate to soil indicates that it expected to be poorly mobile. Recent studies on surface waters, both in Europe and in the Americas (North and South), suggest glyphosate could be transported to surface waters sorbed on soil particles. Detection in water may not only be a result of runoff, with drift, soil erosion, precipitation, and other processes having a role. In addition, the saturation of soils with phosphorus may play a role in reducing the sorption of glyphosate to soil particles, potentially increasing the amount carried in runoff.

Over the last two decades, Canadian growers have adopted best management practices on their farms (such as hedgerow, riparian strip, grass farm road, implementation of no till techniques leaving more plant biomass on the ground for runoff interception as well as the use of buffer zones) to avoid soil, fertilizer and pesticide losses from fields.

Runoff events can be difficult to predict and the presence of glyphosate in water as a result of runoff or spray drift is expected. Proper application timing and runoff/spray drift mitigation measures can reduce potential impacts.

Monitoring studies conducted throughout Canada indicate that glyphosate is rarely detected in groundwater. Although glyphosate is often detected in surface water, the concentrations detected are at relatively low levels that do not pose a risk of concern.

Glyphosate in the atmosphere

Available information indicates that limited amounts of glyphosate may enter the atmosphere at the time of spray application.

Glyphosate was not reported (among 49 compounds) in air or rain along the Mississippi river valley following an air survey campaign in 1995 (Foreman et al. 2000 and Majewski et al. 2000) but was recently reported to be frequently detected in air particles and rain from three agricultural areas of the Midwestern USA (Mississippi, Iowa and Indiana) with detection frequency ranging from 60 to 100% in air and rain in 2007 (Chang et al. 2011, PMRA 2459642 and Majewski et al. 2014). Glyphosate occurred at concentrations equal to or greater than the concentrations of other high-use herbicides previously studied in the Midwest (Waite et al. 2005). Unlike many other pesticides, the presence of glyphosate in air is reported to be due either to spray drift or wind erosion, because it is not volatile according to its low vapour pressure (1.3×10^{-7} Pa), Henry's law constant (2.1×10^{-9} Pa m^3/mole or 2.07 x 1014 atm. m^3/mole) and ionic character in moist soils (binding effect). Glyphosate was not measured or detected in the Canadian atmosphere during the Canadian Pesticide Air Sampling Campaign of 2003 (Yao et al. 2006).

In most studies, the maximum concentrations of glyphosate in air and rain correspond to the period of application and ranged from <0.01 to 9.1 ng/m^3 and from <0.1 to 2.5mg/L in air and rain samples, respectively. However, during a 2007 air survey by Majewski et al. (2000 and 2014) detectable concentrations of glyphosate were collected over the entire growing season, not just in spring as in previous years (before GMO's introduction around 1995), which is reported to be consistent with how glyphosate is now used on genetically modified crops for post-emergent weed control during the growing season. According to Chang et al. (2011), it is not known what percentage of the applied glyphosate was introduced into the air in 2007, but it is estimated that an average of 97% of the glyphosate in the air is removed by a weekly rainfall ≥30 mm. Based on the physical chemistry of glyphosate and the fact that the scale of use is lower in Canada as compared with the US, especially in the corn belt, the concentration of glyphosate in air is not expected to be of concern in Canada.

2.2 Ecotoxicological reviews

2.2.1 Beneficial insects impacted by the use of glyphosate

Comment
Comments noted that glyphosate negatively affects pollinator species (especially bees) and beneficial insect populations. GMO crops resistant to glyphosate, such as rapeseed crops or other GMO crops that include an insecticidal protein (for example, Bt) may have significant concentrations of these compounds in their flower pollen and nectar during the growing season following several applications of the herbicide. Bees foraging on these flowers may then transfer the glyphosate (with or without the insecticidal protein) through contaminated nectar and pollen when they feed young bees, which may have negative impact.

PMRA Response
The re-evaluation of glyphosate included a detailed analysis of studies to determine risks glyphosate may pose to pollinators and beneficial insects.

Acute oral and acute contact exposure of honey bees, and honey bee brood to technical glyphosate and glyphosate formulations obtained from the registrant did not result in mortality in laboratory studies. All acute oral and acute contact LD_{50} values were greater than the highest concentrations tested. The results of the studies indicate that glyphosate formulations and technical glyphosate are relatively non-toxic to bees. The use of glyphosate is expected to pose a negligible acute contact and oral risk to bees.

Direct exposure of bees to glyphosate through oral and contact tests represents a conservative exposure scenario as compared to the exposure bees receive from foraging on flowering rapeseed during a very specific time during the growing season.

A honey bee brood field study (Thompson, 2012) was reviewed by EFSA, 2015. Study results were also published in 2014 (Thompson et al. 2014), where the potential for glyphosate toxicity to developing honey bee larvae and pupae (tested with the Technical IPA salt and a glyphosate formulation (MON 52276)) when fed directly to honey bee colonies, showed a NOAEL (No Observed Adverse Effect Level) for brood development of honey bee colonies of 301 mg glyphosate a.e./L sucrose solution, the highest dose tested. EFSA concluded that glyphosate formulations (with POEA and without POEA) are relatively non-toxic to bees in terms of acute contact and acute oral routes to bees and honey bee brood.

Study results of Jadhav et al. 2008 showed no direct detrimental effects of glyphosate formulation with POEA on two water hyacinth biocontrol agents, *Neochetina eichhorniae* and *N. bruchi*. Jackson and Pitre (2004) demonstrated that the Roundup Ready soybean system, including applications of glyphosate, had no detrimental effects on pest and beneficial insects (*Cerotoma trifurcate* (Forster), *Spissistilus festinus* (Say), *Hypena scabra* (F.), and *Anticarsia gemmatalis* (Hübner) in wide-row soybean plantings. Study results of Hendrix and Parmelee (1985) showed that decomposition and microarthropod densities in glyphosate-treated grass litter (*Sorghum halepense*) were higher than untreated controls. Haughton et al. (2001a and 2001b) demonstrated that glyphosate spray applications were non-toxic to non-target spiders *Lepthyphantes tenuis* but that the loss of habitat was responsible for the reduction in abundance of the species. Similar observations and conclusions were found in tests carried out on the spider *Gonatium rubens* by Haughton et al. (1999).

Results of acute and chronic laboratory studies examining the toxicity of glyphosate formulations to the springtail *Folsomia candida* indicated that glyphosate formulations were not toxic to adult springtails up to the highest concentrations tested (Santos et al. 2012, PMRA 2469288). Results of acute and chronic laboratory studies examining the toxicity of glyphosate formulations to various other beneficial terrestrial arthropods on glass plates, leaf substrate and on artificial soil substrate generally indicate that glyphosate formulations were not toxic to the predatory mite (*Euseius victoriensis*) (Bernard et al. 2010; PMRA 2462245), the lacewing (*Chrysoperla carnea*) (SERA, 2010; PMRA 2469282), the hoverfly (*Episyrphus balteatus*) (Kedwards and Travis, 2001; PMRA 1213236), the carabid beetle (*Poecilus cupreus*) (Walker et al. 2000; PMRA 1213231) or the Staphylinid beetle (*Aleochara bilineata*) (Hermann, 2001; PMRA 1213232) up to the highest concentrations tested. Based on the weight of evidence, the risk to beneficial arthropods from the use of glyphosate is not expected to be of concern.

A study conducted by Murray et al. (2009) show that 50% of all wild bee species nest in a burrow in the ground. The intensification of agriculture may be contributing to the loss of foraging habitats and nesting sites for wild bees.

Studies by Duan et al. (2008) and Malone and Burgess (2009) show no adverse effects of glyphosate resistant Bt crops on exposed bees. These results are corroborated by Morandin and Winston (2003), Malone et al. (2007) and Babendreier et al. (2008), who looked at bumblebee colony exposure to Bt.

2.2.2 The Monarch Butterfly

Comment
Comments noted that the Monarch Butterfly is at risk due to the destruction of milkweed habitat resulting from the use of glyphosate.

PMRA Response
Monarch butterflies (*Danaus plexippus*) rely completely on plants in the milkweed family, especially the common milkweed (*Asclepias syriaca*) for both reproduction and larval food. Until recently, this plant was readily found in the Midwestern Corn Belt of the US and southern latitudes of Canada.

Monarch habitat has been documented to be in decline for the last 20 years in North America (Pleasants and Oberhauser, 2012, Brower et al. 2012, Bhowmik, 1994). Before the introduction of GMO crops, glyphosate was applied in spring at the pre-emergence stage of crops and had limited impact on the survival of the common milkweed (Waldecker and Wyse, 1985, Doll 1998). But recent introduction of GMO crops resistant to glyphosate enables herbicide treatments to be done very late in the growing season (Carpenter and Gianessi, 1999 and Duke and Powles, 2008), impacting the last emerged shoots of the common milkweed, and thus, compromising its survival.

For the monarch, the decline in milkweed represents a threat since the plant is now incapable of re-colonizing fields after GMO crop harvest, especially in the corn belt of the USA and now in the low latitude fields of Canada. The discussion is open as to what the grower should do regarding the competition of the milkweed and other weeds against his own crop within a specific field and/or the protection of the milkweed within the same field.

In fact, glyphosate is not meant to destroy monarch habitats outside of field limits. This is why buffer strips along agricultural fields close to hedgerows and other terrestrial and aquatic habitats exist, and why buffer zones are required to mitigate the impact of drift on non-target organisms located in aquatic and terrestrial habitats. In addition to agricultural pressures, Monarch habitat is also threatened by natural disasters (fire, drought, flood, etc.) and urbanization.

Canada is working with the US and Mexico to coordinate Monarch conservation efforts and is a member of the Trinational Monarch Science Partnership; the government of Canada's participation is led by Environment and Climate Change Canada. Domestically, the federal government has posted its proposed management plan for Monarch on the Species at Risk Public Registry, is funding research on Monarch habitat, and is using its Species at Risk funding programs to support Monarch and pollinator conservation.

2.2.3 Effect of glyphosate and its different formulations on soil microbes

Comment
Comments noted that PRVD 2015-01 did not address serious concerns related to glyphosate's chelation activity and antimicrobial (and antibiotic) properties. Recent published articles have reported that glyphosate and genetically modified (GM) crops can impact soil microbial populations (Fernandez et al. 2009). Glyphosate, like an antibiotic, may kill fungi in the soil, preventing soil microbes from delivering nutrients (minerals in particular) to plants and may increase plant diseases. Glyphosate may act on the shikimate pathway of gut bacteria. Research methods used in studies are not sensitive enough to properly determine the impact glyphosate has on soil microbial populations.

PMRA Response
Although the PMRA is aware that interactions between soil bacteria, fungi and plant root systems can improve plant health, the PMRA does not assess risks to soil microorganisms. Negative impacts have been observed on specific soil microbe strains, but overall, evidence suggests glyphosate end-use products have a low impact on deleterious and beneficial soil microbes following application. Glyphosate contributes to sustainable agricultural systems by reducing the need for cultivation (for example, no-till technique), increasing plant biomass on the ground, increasing the soil organic matter content, improving soil structure and reducing soil erosion and run-off. The fact that glyphosate use has been increasing since its first registration in Canada in 1976 demonstrates that growers have adopted the use of glyphosate and in turn the use of glyphosate-resistant crops very rapidly. If glyphosate had a meaningful negative impact on soil microbial activity over this 40 year use history, growers would not have been so quick to adopt and continue to use the product. The effects on soil microflora would have the strongest impact on crops grown on the fields. Areas away from the site of application are not likely to be negatively impacted.

2.2.4 Birds and mammals exposed to glyphosate and its formulations containing polyethoxylated tallow amine (POEA)

Comment
Comments noted that glyphosate has negative effects on non-target animals. Studies from the United Kingdom demonstrate that glyphosate contributes to a decline in bird species and is also believed to be responsible for increased livestock diseases, such as infertility, nutrient deficiencies (connected to Mn deficiencies), stillbirths, birth defects and abnormal bone formation. Glyphosate, in combination with surfactants used in glyphosate end use products (for example, POEA), is also more toxic to non-target organisms (animals and plants) than glyphosate alone.

PMRA Response
<u>Birds</u>
As presented in the PRVD2015-01, several oral, dietary and chronic toxicity studies were conducted with glyphosate technical and formulations on the bobwhite quail, *Colinus virginianus*, and the mallard duck, *Anas platyrhynchos*. Toxicity studies were also available for the canary, *Serinus canaria* (acute oral exposure with technical glyphosate) and the chicken (21-day dietary exposure with a glyphosate formulation). Glyphosate technical was not toxic to birds

on an acute oral, dietary or reproductive basis up to the highest concentrations or doses tested (PRVD2015-01). Similarly, glyphosate formulations are not particularly toxic to birds on an acute oral and dietary basis (reproduction tests were not available with glyphosate formulations). While acute oral exposure to glyphosate formulations resulted in bird mortality at high doses, glyphosate formulations were not toxic to birds up to the highest concentrations tested when exposure occurred through the diet. There is no indication that glyphosate formulations containing the surfactant POEA are more toxic to birds than formulations without it. Endpoints and risk quotients calculated using these studies are conservative as none of the toxicity studies conducted with technical glyphosate resulted in measured toxic effects in birds.

Although bird toxicity studies indicate that acute oral exposure to high doses of wet, unaltered, glyphosate formulations can result in effects, these effects are not observed when exposure occurs from dried residues of the formulation in the diet. Exposure to glyphosate formulations through the consumption of contaminated food items is a more relevant route of exposure for the environmental assessment than acute oral exposure to the wet formulation. The time period during which wet unaltered formulated product would be present on food items is very limited. Exposure is likely to be mostly from ingestion of dried residues on food items. It is noted that exposure via preening, which may be a relevant exposure route for wet formulation, is not considered in the current assessments. Thus, more weight is given to conclusions of the dietary assessment than to the acute oral assessment. The risk to birds from acute oral, dietary and reproduction exposure to glyphosate and its formulations is expected to be low.

One comment also reported the study of Newton (2004) as evidence of major farmland bird declines in the UK in connection with herbicide uses (not specifically glyphosate) and agricultural practices that would be responsible for the reduction of habitat and/or food available to many species.

Other studies indicate minimal impacts or even the absence of negative impacts on bird community structure and densities following glyphosate treatments in forests and vegetative changes after clearcuts (Morrison and Meslow, 1984; Mackinnon and Freedman, 1993). Other studies (Linz et al. 1992, Linz et al. 1994, Linz et al.1995, Linz et al. 1996a, Linz et al. 1996b, and Solberg and Higgins, 1993) show that glyphosate treatment in wetlands to control invasive species such as cattails (*Typha* spp.) was efficient and had positive impacts by restoring bird habitats (open water) and by increasing original population and diversity.

A review by Sullivan and Sullivan (2003; PMRA 2469318) reported that species richness and diversity of songbirds and small mammals were little affected by glyphosate-induced habitat alteration. Some species declined rapidly following treatment, whereas others increased in abundance. The effect of glyphosate on large mammalian herbivores was measured by the abundance of animals and food plants and by habitat use. Hares and deer were little affected, whereas reductions in plant biomass and related moose forage and habitat use generally occurred for the first few years after treatment, but not thereafter.

Studies in North America have identified habitat loss as the major cause of bird declines over the last 25 years (Santillo et al. 1989 and Hardy and Desgranges, 1990).

Mammals

Numerous acute oral toxicity studies on mammals were available for glyphosate technical and various glyphosate formulations. There is no indication that formulations containing the surfactant POEA are more toxic to mammals than formulations without POEA. Six multi-generation reproduction studies with exposure through the diet were available for technical glyphosate. No reproduction studies with glyphosate formulations were available.

Most mammalian toxicity studies show that exposure to high levels of glyphosate technical or its formulations does not result in toxic effects on mammals. Based on 60 acute oral studies, toxic effects were observed at high doses only in three studies conducted with glyphosate technical, and eight studies with glyphosate formulations. The majority of the available data indicate that risks to mammals following acute oral exposure to glyphosate and its formulations are low. Acute risks to mammals would be restricted to on-field exposure of only a few guilds (herbivores and insectivores). No reproductive risks to mammals are expected from the use of glyphosate. In addition, there are no incident reports for mammals related to the use of glyphosate.

2.2.5 Risk to Amphibians

Comment

Comments noted that glyphosate contributes to the decline of frog abundance. Glyphosate alone (Paganelli et al. 2010), and in combination with POEA, poses risks to amphibians according to studies of Relyea (2005a, 2005b and 2005c) and review of Annett et al. 2014.

PMRA Response

Toxicity data were available for 32 species of amphibians at various stages of development. As is shown with invertebrates and fish, the toxicity of technical glyphosate and its salts and glyphosate formulations containing non-POEA surfactants to amphibians is relatively low (acute LC_{50} = >17.9–7297 mg a.e./L) compared with glyphosate formulations containing POEA (acute LC_{50} = 0.8–51.8 mg a.e./L). Similarly, the results from subchronic and chronic laboratory studies and outdoor mesocosm studies with amphibians demonstrate that exposure to glyphosate formulations containing POEA elicit lethal and sublethal effects (for example, reduced body size, abnormal development, decreased time to metamorphosis) at relatively low concentrations (LC_{50} = 1.0–22.8 mg a.e./L, NOEC = 0.006 - >1.8 mg a.e./L).

Although acute studies showed no negative impacts on amphibians from glyphosate TGAI and formulations that do not contain POEA, a refined risk assessment conducted on amphibians (including frogs) exposed to glyphosate formulations containing POEA (lab tests) indicated that the level of concern was slightly exceeded (RQ = 1.1-1.2) for end-use products containing the surfactant POEA and tested in lab. Level of concern was not exceeded for refined mesocosm studies. Relyea (2005a and b) demonstrated a glyphosate formulation containing the surfactant POEA was responsible for the kill of 68-86% of juvenile amphibians exposed. This study, along with other amphibian studies, was considered in the re-evaluation of glyphosate and used to determine an HC_5 endpoint value from an SSD analysis. Results revealed an acute and chronic HC_5 of 0.93 and 0.86 mg a.e./L, respectively for glyphosate formulations containing the POEA surfactant that were used in the refined risk assessment. As a result, mitigation measures, in the form of no spray buffer zones, are identified on product labels and are required to protect amphibians. Risks to amphibians are not of concern if labelled spray buffer zone requirements are followed.

Annett et al. (2014), in their review, report the mode of action of different glyphosate formulations and their potential negative impact related to the inhibition of the enzyme acetylcholisesterase of some aquatic species as well as the oxidative stress due to Reactive Oxygen Species (ROS) causing damage to nucleic acid, lipids and proteins in aquatic species such as amphibian and fish that can lead to cell death. Studies reviewed, and reported by Annett et al. (2014) were also reviewed by the PMRA, with many of the reported endpoints being used by the PMRA in the risk assessment of glyphosate.

While there is evidence from laboratory studies suggesting that glyphosate products containing POEA are more toxic to amphibians than glyphosate alone, when considered in the context of all the studies available, particularly field studies conducted under actual use conditions, there is no compelling or credible evidence that gives rise to a serious possibility that glyphosate products containing POEA may cause an unacceptable environmental risk. In addition, while lower tier studies conducted in a laboratory showed potential for effects, a field study conducted under operational conditions (Thompson et al. 2004, PMRA 2032071) showed no significant adverse effects on amphibians. Moreover, glyphosate products containing POEA are used in forestry to prepare the site for reforestation which requires that the products be applied only once per silviculture cycle; typically equating to once every 50 to 80 years. As such, the potential for amphibian exposure to glyphosate products is limited in silviculture. Based on these findings, the PMRA concluded that there were no reasonable grounds to believe that the environmental risk to amphibians in small ephemeral forest wetlands from the spraying of glyphosate products was unacceptable.

2.2.6 Other Aquatic organisms

Comment
Comments noted that the following studies were not taken into account in the re-evaluation of glyphosate: Vera et al. 2010 (periphyton), Fairchild et al. 2002 (Atlantic salmon), and Sihtmae et al. 2013 (aquatic invertebrates).

PMRA Response
Periphyton
The study of Vera et al. 2010 entitled ‘’New evidence of Roundup impact on the aquatic periphyton community and the quality of freshwater ecosystems’’ (Ecotoxicology 19:710-721) was in fact considered qualitatively in the re-evaluation, but no endpoints were available in the study to be used as part of the SSD analysis. The study of Bonnineau et al. 2012 (PMRA# 2462244) on periphyton was preferred and the freshwater algae acute 6hr-EC_{50} endpoint of 8.7 mg a.e./L was used in the re-evaluation of glyphosate and presented in PRVD2015-01.

Atlantic salmon
The study of Fairchild et al. 2002, entitled “Effects of freshwater contaminants on marine survival in Atlantic salmon” (NPAFC Tech Report No. 4) was examined and it was determined that the study is related to the active atrazine and does not report on glyphosate.

Aquatic invertebrates
The study of Sihtmae et al. 2013 entitled "Ecotoxicological effects of different glyphosate formulations'' (Applied Soil Ecology 72:215-224) was indeed used in the re-evaluation of glyphopsate. The freshwater invertebrate endpoint values reported by Sihtmae et al. 2013 (PMRA 2574468) were used in the determination of HC_5 values from a SSD analysis. Refer to response 2.3.2 below.

2.2.7 Endocrine disruption

Comment
Comments noted that the PMRA should phase out the use of products containing glyphosate based on articles that have identified glyphosate as an endocrine disruptor.

PMRA Response
The USEPA's Endocrine Disruptor Screening Program (EDSP) is currently working to validate the assays proposed by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), many of which are being validated in coordination with the OECD through the Endocrine Disrupters Testing and Assessment (EDTA) and the Validation Management Groups (VMGs). The results of screening tests for glyphosate are available on the following website: (http://www2.epa.gov/sites/production/files/2015-06/documents/glyphosate-417300_2015-06-29_txr0057175.pdf).

Although the study by Antoniou et al. 2012 raised concerns regarding the potential impact of glyphosate as an endocrine disruptor, the conclusion is that glyphosate demonstrates no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways in mammals or wildlife. Based on weight of evidence considerations, mammalian or wildlife EDSP Tier 2 testing is not recommended for glyphosate. Also refer to response to comment 1.1.12.

2.2.8 Bioaccumulation

Comment
Comments questioned if glyphosate can accumulate in the body over time and how levels of glyphosate are monitored to ensure that it does not go above acceptable limits that could cause detrimental health effects to animals?

PMRA Response
Information available on the bioaccumulation potential of glyphosate is presented in the PRVD 2015-01. Glyphosate is not expected to bioaccumulate due to its high polarity (log K_{ow} = -2.8 to -0.67) and anionic character (Mensink and Janseen, 1994, PMRA 2462253 and Villeneuve, J., 2012 (PMRA 2203372)). A maximum bioconcentration factor (BCF) of 1.6 was reported for bluegill sunfish exposed to 0.6 mg/L for 28 days (Wang et al. 1994b; PMRA 2460743 and Takacs et al. 2002; PMRA 2462252). BCF values of 12 to 35.4 and 10 to 42.3 for tilapia and carp, respectively were also reported by Wang et al. 1994b (PMRA 2460743). Channel catfish, largemouth bass and rainbow trout exposed to 10 mg/L glyphosate for 14 d had BAFs of 0.18, 0.04, and 0.03, respectively (Kramer and Beasley, 1975, PMRA 1182548).

2.2.9 Science based approach and the use of independent scientific studies in the environmental risk assessment.

Comment
Various stakeholder organizations emphasized the importance of a science-based approach and agreed with the proposed regulatory label changes. Other commenters encouraged to use a number of different sources of information that claim glyphosate poses an environmental risk. Sources of information from various non-governmental organizations or independent researchers were provided. In addition to registrant submitted studies, work done by third parties (independent research) should be used in assessing the environmental effects of glyphosate and in making the final re-evaluation decision.

Some commenters believe that the environmental risk assessment for glyphosate was conducted using only studies provided by the registrants and that there has not been enough long-term testing of glyphosate done by independent scientists. Reviewing studies conducted and provided by the company that is seeking registration of the product is perceived as a conflict of interest and highly biased as these studies are not peer reviewed by the scientific community. Reference was provided to a number of published scientific studies that link glyphosate to environmental and agronomic effects.

PMRA Response
The environmental risk assessment of glyphosate was conducted using a science-based approach and included consideration of a large volume of literature. In addition to registrant supplied data, more than 1500 scientific articles related to glyphosate were examined, with approximately 250 of these studies being deemed relevant and useful for consideration in the environmental risk assessment. Values obtained from the public literature were used in combination with the registrant data set in order to strengthen the environmental risk assessment. Due to the tremendous amount of endpoint data available for different aquatic and terrestrial organisms, SSD analysis was employed to determine HC_5 and HD_5 values that were used in the risk assessment. Also refer to response to comment 1.1.14.

2.2.10 Assessment of formulations

Comment
Commenters questioned why the formulations of glyphosate products are not assessed for their environmental effects. Environmental effects discussed in the PRVD2015-01 were based primarily on the active substance (in other words, glyphosate).

PMRA Response
PRVD2015-01 includes risk assessments for not only the technical active ingredient, but also the various formulations, including those that contain POEA. Endpoints using values from EUPs were used to derive HD_5/HC_5 values from SSD calculations when possible. The risk assessment includes a comparison of the exposure of terrestrial and aquatic organisms to technical glyphosate and the formulations.

2.3 Risk assessment and methodology

2.3.1 Endpoint selection

Comment
Some endpoints used in the terrestrial and aquatic plant risk assessment as well as the risk assessment for aquatic organisms were inappropriate. The quality of some of the data used in the risk assessment was not clear and was questionable. Specific studies that were at issue were identified for the PMRA to reconsider. The process used to review and ensure the quality of open literature studies used in the risk assessment needs to be more transparent.

PMRA Response
Endpoints derived from unpublished registrant/applicant submitted data follow guidelines set by regulatory bodies and are subject to good laboratory practice standards. These studies have clear objectives, scientific and analytical protocols, and the data has been subject to appropriate statistical analysis. On the other hand, published scientific papers are written in a concise way in order to bring enough information and details for the reader to accept or reject the conclusion of the author(s). Although published scientific articles are subject to a scientific peer review that strengthens their validity, information in published studies must have sufficient detail so that the scientific methods (protocol) and the results obtained are reproducable. Unfortunately, many published scientific studies lack sufficient detail, reducing confidence in the conclusion reached by the author(s). As a result, some published scientific papers are rejected when reviewed by the PMRA during the re-evaluation process. (Refer also to response to comment 1.1.14).

That said, as a result of comments received during the comment period for the PRVD2015-01, endpoints questioned in the comments have been re-examined and changes to the risk assessment have been made based on a revised assessment of their validity. References associated with endpoint values are presented in the tables found in (Appendix III).

2.3.2 SSD model

Comment
The methodology for deriving Species Sensitivity Distributions (SSDs) is not fully described in the PRVD and the requirements for inclusion of endpoints is not discussed. The use of a combination of terrestrial plant EC_{25} and EC_{50} endpoints for vegetative vigour in SSD calculations should be reconsidered.

PMRA Response
The toxicity data analysis includes the determination of HC_5 or HD_5 values using an SSD or species sensitivity distribution. An SSD is a plot of all species' toxicity endpoints within a taxonomic group against a cumulative density function. An SSD is determined by fitting a theoretical distribution to the data set, such as a log-normal distribution, and allows the derivation of community level threshold concentrations such as the HC_5. The hazardous concentration (HC_5) or dose (HD_5) to five percent of species is calculated for acute and chronic data sets separately, using the acute LC_{50}/EC_{50} values and chronic NOEC/NOEL values, respectively. An SSD is constructed for acute and chronic effects for every taxonomic group where sufficient toxicity data are available. Acute toxicity data generally refers to short term studies, with the endpoints (LC_X or EC_x) being derived from effects on survival or other

endpoints considered to affect survival. Chronic and sub-chronic studies generally aim to determine sublethal effects and the associated NOEC or NOEL concentration. Different endpoints can also be used in SSDs such as the EC_{25} for terrestrial plants or other EC_X value such as an $EC_5/_{10}$ may be considered relevant and appropriate to the assessment. If SSDs cannot be calculated, the most sensitive endpoints with an appropriate uncertainty factor are used in risk assessment.

The software program ETX 2.1 is used with the log-normal model to generate SSDs where sufficient toxicity endpoints are available for different taxonomic groups. The median HC_5 values are reported for SSDs. The variability in the data sets is indicated not only by the upper and lower bound HC_5 estimates but also the confidence limit of the fraction of species affected (FA), which indicates the theoretical minimum and maximum percent of species that could be affected based on the available data when the population is exposed to the HC_5 concentration.

SSDs were determined for glyphosate herbicide for the following taxonomic groups (results are reported in Appendix III Tables 1 to 3):

- Freshwater organisms: invertebrates, fish, algae, amphibians, aquatic plants
- Marine organisms: fish, invertebrates and algae
- Terrestrial organisms: plants (crop and non-crop)

Where an HC_5 value cannot be determined due to insufficient species data or lack of model fit, etc., the most sensitive species endpoint is reported in summary tables without the use of uncertainty factors. Where multiple data points are available for one species, a geometric mean value is used to represent the species' sensitivity. The treatment of toxicity data is such that it allows quantitative comparisons and predictions including consistency of exposure concentration units, ecological relevance and comparability of measurement endpoints, and types of test chemicals, or duration of exposure.

All data sets were grouped by test material type including technical grade active ingredient (TGAI, includes all forms of glyphosate actives), end-use products containing the surfactant POEA (EUP + POEA), end-use products which do not contain POEA (EUP NO POEA), POEA alone and the glyphosate transformation product AMPA. All toxicity values were normalised to acid equivalent (a.e.).

Results of SSD analysis:
Glyphosate shows equal toxicity to many aquatic taxonomic groups, both acutely and chronically. The most acutely sensitive aquatic taxonomic groups are freshwater plant (overspray on aquatic macrophyte; Er_{50} of 38 g a.e/ha), freshwater and marine invertebrates, and freshwater algae (HC_5 = 0.1mg a.e./L). The lowest chronic toxicity threshold values were determined for freshwater and marine fish (NOEC = 0.28 and 0.1 mg a.e./L, respectively) and freshwater plants (chronic EC_{50} = 0.11 mg a.e./L). The most sensitive terrestrial plant endpoint for crops and non-crops is the HD_5 of EC_{50} value of 0.0658 kg a.e./ha for EUPs that contain, or do not contain POEA, based on plant vegetative vigor endpoints.

As observed for amphibian in previous section 2.2.5, it is noted that the formulated products of glyphosate are generally more toxic to some organisms than the active ingredient, as in the case of freshwater invertebrates which are two orders of magnitude (100x) more sensitive to formulations containing POEA vs. the active ingredient. Freshwater fish and plants are also more sensitive to EUPs. Marine fish on the other hand are most sensitive, on an acute basis, to the parent chemical.

Therefore the SSD analysis results indicate that the most sensitive population level aquatic toxicity threshold value (HC_5) is 0.1 mg a.e./L, based on acute and chronic endpoints for several taxanomic groups including freshwater and marine invertebrates, aquatic plants (except overspray), algae and fish. While the most sensitive population level terrestrial toxicity threshold value (HD_5 of EC_{50}) is 0.0658 mg Kg a.e./ha, based on acute toxicity to plants (crops + non-crops exposed to glyphosate formulations containing POEA + glyphosate formulations without POEA).

2.3.3 Buffer zone calculations

Comment
Comments noted that the buffer zone sizes should be recalculated based on reconsideration of acceptability of endpoints. Buffer zone sizes should be set based on scientific evidence and valid endpoints and no increase should be implemented if no such evidence exists. Please explain why buffer zones are different for treated areas of more than 500 ha and those that are less than 500 ha.

PMRA Response
The PMRA agrees with the fact that buffer zone sizes should be set based on scientific evidence and valid endpoints and no increase or decrease should be implemented if no such evidence exists. The methodology used by the PMRA to calculated buffer zones is based on scientific evidence and valid endpoints.

Endpoints were reconsidered following identification of questionable studies, which lead to changes in the endpoints included in the SSDs and the determination of HC_5 values, especially for aquatic organisms. Buffer zones have been recalculated as a result of the changes in the SSD calculations.

The reason why buffer zones are different for treated areas of more than 500 ha and those that are less than 500 ha. is the following:

The AGDISP software model (version 8.21) used by the PMRA to calculate aerial buffer zones takes into account the cumulative downwind drift associated with the number of flightlines made over a treated surface area with an aircraft. A forest surface area of more than 500 ha is considered as ‘woodland’ and is modelled using 50 flightlines as a realistic scenario. A forest surface area of less than 500 ha is considered as ‘woodlot’ and requires only 10 flightlines. As such, cumulative drift may be more significant in woodlands than in woodlots and consequently buffer zones may be larger in woodlands than in woodlots. Updated buffer zone tables are reported in Appendix IV, Tables 1 and 2.

2.4 Aerial spraying of forests

Comment
One Aboriginal group commented that aerial spraying of forests with glyphosate impacts the environment.

PMRA Response
As noted in response to comment 2.2.5, glyphosate is used for forest site preparation and plant release (conifers and deciduous trees) after trees are harvest. This use is expected to occur once every 50-80 years. As such, glyphosate exposure to forest is extremely low. In addition, glyphosate does not persist in the terrestrial environment, with DT50s ranging from 24 to 82 days in forest soils (average of less than 55 days).

For the protection of aquatic habitats, no spray buffer zones of 1 to 10 meters are required when glyphosate formulations that contain POEA are applied for forest site preparation and plant release by air. A buffer zone is defined as the distance between the point of direct pesticide application and the nearest downwind boundary of a sensitive habitat. Glyphosate does not persist in water (DT50s range from 0.4–11.2 days).

3.0 Comments Related to the Value Considerations

3.1 Glyphosate has value in contributing to Canadian agriculture and non-agricultural land management

Summary of Comments

- glyphosate is an important and cost effective weed management tool in crop production in that it can be applied at varying points of the cropping cycle from preplant to post-harvest.
- the application of glyphosate prior to harvest is important in terms of advancing the maturity and/or uniformly desiccating the crop and to control late season weeds that can interfere with harvesting operations and reduce crop quality.
- glyphosate with its unique mode of action remains an important tool for broad spectrum weed control, including of perennial, invasive and noxious weeds
- it allows the Canadian agricultural sector to remain competitive with those of its trading partners
- it remains an important tool for advancing conservation tillage, such as no-tillage and reduced tillage systems, that reduce soil erosion and increase soil organic matter
- it is used to control invasive plants to foster biodiversity by allowing native plant communities including those containing endangered or rare species, to be preserved or re-established.

PMRA Response
As stated in the PRVD2015-01, the PMRA acknowledges that glyphosate plays an important role in weed management in both Canadian agriculture and non-agricultural land management

3.2 Glyphosate has no value considering the risks to the environment and human health.

PMRA Response
The value of glyphosate to Canadian agriculture and non-agricultural land management is a result of this product's unique mode of action, diverse use pattern, and broad spectrum of weed control. As indicated in PRVD2015-01, based on a review of the science, the PMRA has concluded that this product is unlikely to affect human health or pose an unacceptable risk to the environment when used in accordance with label directions.

4.0 Other Comments Related to the Use of Glyphosate

4.1 Weed resistance

Comment
Comments noted that repeated use of glyphosate and heavy reliance on glyphosate to control weeds in today's agriculture practices increase weed resistance. PMRA has not addressed the issue of weed resistance in its re-evaluation of glyphosate. There is no mention of glyphosate-resistant weeds anywhere in the Environmental Considerations of the PMRA's Proposed Re-evaluation decision for glyphosate. A report recently published by the Canadian Biotechnology Action Network (CBAN) reveals that "there are five species of glyphosate-resistant weeds now found in Canada". An online survey of farmers from 2013 estimated that more than one million acres of Canadian farmland had glyphosate resistant weeds.

PMRA Response
The PMRA is aware of the fact that the current agricultural production system relies heavily on glyphosate, resulting in more and more occurrences of glyphosate-resistant weeds. Kochia, Canada fleabane, giant ragweed and common ragweed are examples of such resistant weeds reported in Canada. These glyphosate-resistant weeds are increasingly becoming challenge to the agricultural production system. In order to prevent or delay the development of glyphosate-resistant weeds, it is crucial to maintain diversity in weed management practices. From the regulatory perspective, the PMRA developed the resistance-management labelling program in 1999 with an aim to mitigate the risks for resistance development. Participation in this program is on a voluntary basis, but registrants are encouraged to add the resistance-management grouping symbols and resistance management statements to both new and existing product labels (Regulatory Directive DIR2013-04, *Pesticide Resistance Management Labelling Based on Target Site/Mode of Action)*. To date, the majority (about 95%) of labels for products containing glyphosate comply with the resistance-management labelling. Other organizations are more closely involved with improvements to agricultural practices.

4.2 Invasive species

Comment
Comments noted that herbicide treatments such as glyphosate are needed to control invasive species in standing water, such as *Phragmites australis* (2015 Resolution of the Canadian Federation of Agriculture Annual General Meeting).

PMRA Response
Before a pesticide is approved for use in Canada, it must undergo a thorough pre-market science-based risk assessment and meet strict health and environmental standards, and the product must have value. The use of glyphosate to control invasive species in standing water was not registered in Canada, and therefore was not considered during the re-evaluation.

The PMRA is aware of the rise of *Phragmites* in Canadian wetlands, and has been working with provincial partners to find solutions such as emergency registration where needed. An emergency use will be considered only if the product is efficacious and risks deemed acceptable.

4.3 Treaty rights and the duty to consult First Nations

Comment

One Aboriginal group commented that aerial spraying on traditional lands is a violation of treaty rights and it is a constitutional obligation for Health Canada to consult. The PMRA is obligated to hear oral testimony in their territory as a form of evidence.

PMRA Response

Concerns expressed by the aboriginal group in their written submission and in subsequent conversations, were identified as being related more to forest management practices and not specific to the use of this particular herbicide.

Following harvest, Canadian forests are either allowed to regenerate naturally or are re-planted with a crop tree species as part of a forest management plan. Glyphosate, or other herbicides, can be applied in a managed forest to control naturally occurring vegetation that could out compete newly planted crop tree seedling (for example, pine or spruce trees) for nutrients, light and space. Herbicides are also used in clearing logging roads and rights of way. As with other land management uses of pesticides such as agriculture, the use of herbicides in forestry operations can reduce biodiversity (for example, loss of grasses, raspberry and non-crop tree species, such as birch or aspen) in the application areas for a period of time.

Except on federal lands, the management of natural resources, such as forests, is the responsibility of provincial governments. Provincial ministries of natural resources are better informed about the local conditions and are generally responsible for approving sustainable forest-management plans. These plans indicate which land will be allowed to regenerate naturally and which will be re-planted and managed (with or without herbicides). If a herbicide is to be used, it must a product that is authorized by Health Canada's Pest management Regulatory Agency for forestry application. It the product is to be applied by air, permits are required, generally from provincial ministries of the environment, prior to application. Consultations with the aboriginal community on herbicide use in forestry can be most effectively done by considering forest management plans and the local land use requirements. It is recommended that the group continue to raise their concerns with the appropriate provincial authorities

Other concerns that were raised by this group regarding the impact of glyphosate use on human health and the environment were addressed under responses 1.3.8 and 2.4.

Appendix II Registered Products Containing Glyphosate in Canada as of 16 September 2016

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
ADAMA AGRICULTURAL SOLUTIONS CANADA LTD.	29219	GLYPHOGAN PLUS LIQUID HERBICIDE	GPI-356;	SN-SOLUTION	C+R
ALBAUGH LLC	28322	CLEAROUT 41 PLUS HERBICIDE SOLUTION	GPI-360;	SN-SOLUTION	C
	31913	GLYPHOSATE 480	GPI-480;	SN-SOLUTION	C
ALLIGARE, LLC	30093	ALLIGARE GLYPHOSATE 4+	GPI-360;	SN-SOLUTION	C
AGROMARKETING CO. INC.	30721	NASA 36	GPI-360;	SN-SOLUTION	C+R
AGRI STAR CANADA ULC.*	29995	CRUSH'R PLUS	GPI-360;	SN-SOLUTION	C
	32181	CRUSH'R 480	GPI-480;	SN-SOLUTION	C
	31655	AGRI STAR CRUSHR 540	GPP-540;	SN-SOLUTION	C
DOW AGROSCIENCES CANADA INC.	30958	ENLIST DUO HERBICIDE	GPX-204; DXJ-194;	SN-SOLUTION	C
	30960	GF-2726 TSOY HERBICIDE	GPX-204; DXJ-194;	SN-SOLUTION	C
	27394	PREPASS B HERBICIDE (A COMPONENT OF PREPASS HERBICIDE)	GPI-360;	SN-SOLUTION	C
	27615	VANTAGE PLUS MAX HERBICIDE SOLUTION	GPI-480;	SN-SOLUTION	C
	28245	MAVERICK II HERBICIDE SOLUTION	GPI-480;	SN-SOLUTION	C
	28540	ECLIPSE II B HERBICIDE	GPI-480;	SN-SOLUTION	C
	28977	MAVERICK III HERBICIDE	GPX-480;	SN-SOLUTION	C
	29033	ECLIPSE III B HERBICIDE	GPX-480;	SN-SOLUTION	C
	29652	PREPASS XC B HERBICIDE (A COMPONENT OF PREPASS XC HERBICIDE)	GPX-480;	SN-SOLUTION	C
	29994	VANTAGE XRT HERBICIDE	GPX-480;	SN-SOLUTION	C
	26171	VANTAGE PLUS HERBICIDE SOLUTION	GPI-360;	SN-SOLUTION	C+R
	26172	VANTAGE HERBICIDE SOLUTION	GPI-356;	SN-SOLUTION	C+R
	26884	VANTAGE FORESTRY HERBICIDE	GPI-356;	SN-SOLUTION	C+R
	29588	GF-772 HERBICIDE	GPI-360;	SN-SOLUTION	C+R
	29773	DEPOSE HERBICIDE SOLUTION	GPI-356;	SN-SOLUTION	C+R
	30516	VANTAGE MAX HERBICIDE	GPS-480;	SN-SOLUTION	C+R
	28840	VP480 HERBICIDE	GPX-480;	SN-SOLUTION	C+R
	29774	DURANGO HERBICIDE	GPX-480;	SN-SOLUTION	C+R
	30423	PREPASS 480	GPX-480;	SN-SOLUTION	C+R

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
		HERBICIDE			
	32314	GF-2018 HERBICIDE	GPX-480;	SN-SOLUTION	C+R
EZJECT, INC.	21262	DIAMONDBACK HERBICIDE SHELLS	GPI-0.15;	PA-PASTE	C
FMC CORPORATION	27287	GLYFOS AU SOLUBLE CONCENTRATE HERBICIDE	GPI-360;	SN-SOLUTION	C
	28925	CHEMINOVA GLYPHOSATE (TM) II	GPI-356;	SN-SOLUTION	C
	29363	GLYFOS BIO HERBICIDE	GPI-360;	SN-SOLUTION	C
	29364	GLYFOS BIO 450 HERBICIDE	GPI-450;	SN-SOLUTION	C
	30234	FORZA BIO SILVICULTURAL HERBICIDE	GPI-360;	SN-SOLUTION	C
	30235	FORZA BIO 450 SILVICULTURAL HERBICIDE	GPI-450;	SN-SOLUTION	C
	24359	GLYFOS SOLUBLE CONCENTRATE HERBICIDE	GPI-360;	SN-SOLUTION	C+R
	26401	FORZA SILVICULTURAL HERBICIDE	GPI-360;	SN-SOLUTION	C+R
	28924	GLYFOS SOLUBLE CONCENTRATE HERBICIDE II	GPI-360;	SN-SOLUTION	C+R
INTERPROVINCIAL COOPERATIVE LIMITED	26846	GLYPHOSATE HERBICIDE - AGRICULTURAL & INDUSTRIAL	GPI-360;	SN-SOLUTION	C
	29216	GLYPHOSATE WATER SOLUBLE HERBICIDE	GPI-309(+51);	SN-SOLUTION	C
	27988	IPCO FACTOR 540 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	31199	FORTRAN 540 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	31598	CO-OP VECTOR 540 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	29775	MATRIX HERBICIDE SOLUTION	GPX-480;	SN-SOLUTION	C+R
	30319	VECTOR HERBICIDE SOLUTION	GPX-480;	SN-SOLUTION	C+R
	31090	RIVET HERBICIDE	GPX-480;	SN-SOLUTION	C+R
JOINT GLYPHOSATE TASK FORCE, LLC	30678	JGTF GLYPHOSATE HERBICIDE	GPI-360;	SN-SOLUTION	C+R
LOVELAND PRODUCTS CANADA INC.	30076	MAD DOG PLUS	GPI-360;	SN-SOLUTION	C+R
MEY CANADA CORPORATION	29126	WISE UP HERBICIDE SOLUTION	GPI-360;	SN-SOLUTION	C
MONSANTO CANADA INC.	20423	MOCAN 943 WATER SOLUBLE HERBICIDE	GPI-120; DIC-86;	SN-SOLUTION	C
	21572	RUSTLER FALLOW LIQUID HERBICIDE	GPI-132; DIC-60;	SN-SOLUTION	C
	27200	RUSTLER LIQUID HERBICIDE	GPI-194; DIC-46;	SN-SOLUTION	C

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
	32274	ROUNDUP XTEND WITH VAPORGRIP TECHNOLOGY HERBICIDE	GPI-240; DIC-120;	SN-SOLUTION	C
	19536	RUSTLER SUMMERFALLOW HERBICIDE	GPI-108; DXB-182;	SN-SOLUTION	C
	25898	MON 77790 HERBICIDE	GPI-132; DXB-82;	SN-SOLUTION	C
	25604	ROUNDUP FAST FORWARD PREHARVEST HERBICIDE	GPI-300; GLG-16;	SN-SOLUTION	C
	25795	ROUNDUP FASTFORWARD PRESEED	GPI-300; GLG-10;	SN-SOLUTION	C
	25918	MON 77759 WATER SOLUBLE HERBICIDE	GPI-300; GLG-36;	SN-SOLUTION	C
	26625	MON 78027 WATER SOLUBLE HERBICIDE	GPI-180; GLG-131;	SN-SOLUTION	C
	26920	ROUNDUP TRANSORB MAX LIQUID HERBICIDE	GPI-480;	SN-SOLUTION	C
	29841	MON 76431 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C
	29868	MON 76429 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C
	19899	VISION SILVICULTURE HERBICIDE	GPI-356;	SN-SOLUTION	C+R
	25344	ROUNDUP TRANSORB LIQUID HERBICIDE	GPI-360;	SN-SOLUTION	C+R
	27487	ROUNDUP WEATHERMAX WITH TRANSORB 2 TECHNOLOGY LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	27736	VISIONMAX SILVICULTURE HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	27764	ROUNDUP ULTRA LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	27946	RENEGADE HC LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	28198	ROUNDUP TRANSORB HC LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	28486	ROUNDUP ULTRA 2 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	28487	RT/540 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	28608	MON 79828 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	28609	MON 79791 LIQUID HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	29498	START UP HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	30104	MON 76669	GPP-540;	SN-SOLUTION	C+R
	32209	POWERMAX HERBICIDE	GPP-540;	SN-SOLUTION	C+R
	32356	ROUNDUP CUSTOM FOR AQUATIC AND TERRESTRIAL USE	GPI-;	SN-SOLUTION	R

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
		LIQUID HERBICIDE			
NEWAGCO INC	29290	MPOWER GLYPHOSATE	GPI-356;	SN-SOLUTION	C
NUFARM AGRICULTURE INC.	30870	GLYKAMBA HERBICIDE	GPI-194; DIC-46;	SN-SOLUTION	C
	25866	NUFARM CREDIT LIQUID HERBICIDE	GPI-356;	SN-SOLUTION	C
	27950	CREDIT PLUS LIQUID HERBICIDE	GPI-360;	SN-SOLUTION	C
	29124	CREDIT 45 HERBICIDE	GPI-450;	SN-SOLUTION	C
	29125	NUFARM CREDIT 360 LIQUID HERBICIDE	GPI-360;	SN-SOLUTION	C
	29470	NUGLO HERBICIDE	GPI-450;	SN-SOLUTION	C
	29479	POLARIS	GPI-360;	SN-SOLUTION	C
	29480	NUFARM GLYPHOSATE 360 HERBICIDE	GPI-360;	SN-SOLUTION	C
	29888	CREDIT XTREME HERBICIDE	GPO-540;	SN-SOLUTION	C
	31316	CARNIVAL 540 HERBICIDE	GPO-540;	SN-SOLUTION	C
PRODUCTIERRA	31063	SMOKE 41% GLYPHOSATE	GPI-360;	SN-SOLUTION	C
RACK PETROLEUM LTD.	30442	THE RACK GLYPHOSATE	GPI-360;	SN-SOLUTION	C
	31314	RACKETEER	GPI-360;	SN-SOLUTION	C
SHARDA CROPCHEM LIMITED	31493	SHARDA GLYPHOSATE 360	GPI-360;	SN-SOLUTION	C
	32122	GLYFO SILVI HERBICIDE	GPI-360;	SN-SOLUTION	C+R
SYNGENTA CANADA INC.	29341	HALEX GT HERBICIDE	MER-25; GPP-250; AME-250;	SN-SOLUTION	C
	29552	TAKKLE HERBICIDE	GPI-140; DIC-70;	SN-SOLUTION	C
	30412	FLEXSTAR GT HERBICIDE	GPM-271; FOF-67;	SN-SOLUTION	C
	28802	CYCLE HERBICIDE	GPP-500;	SN-SOLUTION	C
	31711	CALLISTO GT HERBICIDE	MER-45.5; GPP-455;	SU-SUSPENSION	C
	27192	TOUCHDOWN IQ LIQUID HERBICIDE	GPM-360;	SN-SOLUTION	C+R
	28072	TOUCHDOWN TOTAL HERBICIDE	GPP-500;	SN-SOLUTION	C+R
	29201	TRAXION HERBICIDE	GPP-500;	SN-SOLUTION	C+R
TERAGRO INC	29022	WEED-MASTER GLYPHOSATE 41 HERBICIDE	GPS-356;	SN-SOLUTION	C
	29009	WEED-MASTER GLYPHOSATE FORESTRY HERBICIDE	GPI-356;	SN-SOLUTION	C+R
UNITED PHOSPHORUS INC.	30366	GLYPHO 41 HERBICIDE	GPI-356;	SN-SOLUTION	C+R
UNIVAR CANADA LTD.	32228	GUARDSMAN GLYPHOSATE	GPO-540;	SN-SOLUTION	C
DOW AGROSCIENCES CANADA INC.	27351	GLYPHOSATE 18% HERBICIDE SOLUTION CONCENTRATE	GPI-143;	SN-SOLUTION	D

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
	27352	GLYPHOSATE 0.96% HERBICIDE READY-TO-USE	GPI-7;	SN-SOLUTION	D
FMC CORPORATION	26609	GLYFOS HERBICIDE 143 CONCENTRATE	GPI-143;	SN-SOLUTION	D
	26610	GLYFOS HERBICIDE 7 READY-TO-USE	GPI-7;	SN-SOLUTION	D
	26827	GLYFOS CONCENTRATE 356 HERBICIDE	GPI-356;	SN-SOLUTION	D
MONSANTO CANADA INC.	22627	ROUNDUP CONCENTRATE NON-SELECTIVE HERBICIDE	GPI-143;	SN-SOLUTION	D
	22759	ROUNDUP SUPER CONCENTRATE GRASS & WEED CONTROL	GPI-356;	SN-SOLUTION	D
	22807	ROUNDUP READY TO USE NON-SELECTIVE HERBICIDE WITH FASTACT FOAM	GPI-7;	SN-SOLUTION	D
	24299	ROUNDUP READY-TO-USE GRASS & WEED CONTROL WITH FASTACT FOAM	GPI-7;	SN-SOLUTION	D
	26263	ROUNDUP READY-TO-USE WITH FASTACT FOAM PULL'N SPRAY NON-SELECTIVE HERBICIDE	GPI-7;	SN-SOLUTION	D
	27460	ROUNDUP READY-TO-USE NON-SELECTIVE HERBICIDE	GPI-7.2;	SN-SOLUTION	D
	27506	ROUNDUP READY-TO-USE PULL'N SPRAY NON-SELECTIVE HERBICIDE	GPI-14.0;	SN-SOLUTION	D
	27507	ROUNDUP READY-TO-USE PULL'N SPRAY TOUGH BRUSH & POISON IVY CONTROL NON-SELECTIVE HERBICIDE	GPI-14.0;	SN-SOLUTION	D
	28974	ROUNDUP PUMP 'N GO	GPI-7;	SN-SOLUTION	D
	29003	ROUNDUP READY-TO-USE POISON IVY & BRUSH CONTROL NON-SELECTIVE HERBICIDE	GPI-14;	SN-SOLUTION	D
	29034	ROUNDUP READY-TO-USE POISON IVY & BRUSH CONTROL WITH QUICK CONNECT SPRAYER	GPI-14;	SN-SOLUTION	D
	31153	REFILL FOR ROUNDUP READY-TO-USE WITH WAND APPLICATOR	GPI-7.0;	SN-SOLUTION	D
	31154	ROUNDUP READY-TO-USE WITH WAND APPLICATOR	GPI-7.0;	SN-SOLUTION	D
	31514	ROUNDUP READY-TO-USE REFILL	GPI-7;	SN-SOLUTION	D

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
	31997	ROUNDUP READY-TO-USE TOUGH BRUSH & POISON IVY CONTROL WITH WAND APPLICATOR	GPI-14.0;	SN-SOLUTION	D
	32041	REFILL FOR ROUNDUP READY-TO-USE TOUGH BRUSH & POISON IVY CONTROL WITH WAND APPLICATOR	GPI-14;	SN-SOLUTION	D
	23786	ROUNDUP QUIK STIK NON-SELECTIVE HERBICIDE TABLETS	GPS-60;	TA-TABLET	D
LES PRODUITS DE CONTROLE SUPERIEUR INC/SUPERIOR CONTROL PRODUCTS INC	28464	TOTALEX CONCENTRATE BRUSH, GRASS & WEED KILLER HOME GARDENER	GPI-143;	SN-SOLUTION	D
	28467	BYEBYE WEED CONCENTRATE BRUSH, GRASS & WEED KILLER	GPI-143;	SN-SOLUTION	D
	28469	BYEBYE WEED READY-TO-USE BRUSH, GRASS & WEED KILLER	GPI-7;	SN-SOLUTION	D
	28470	TOTALEX READY-TO-USE BRUSH, GRASS & WEED KILLER HOME GARDENER	GPI-7;	SN-SOLUTION	D
	28471	TOTALEX SUPER CONCENTRATE BRUSH, GRASS & WEED KILLER HOME GARDENER	GPI-356;	SN-SOLUTION	D
	28472	BYEBYE WEED SUPER CONCENTRATE BRUSH, GRASS & WEED KILLER	GPI-356;	SN-SOLUTION	D
	28574	TOTALEX RTU BRUSH, GRASS & WEED KILLER WITH 1 TOUCH POWER SPRAYER HOME	GPI-7.0;	SN-SOLUTION	D
	28575	BYEBYE WEED RTU BRUSH, GRASS & WEED KILLER WITH 1 TOUCH POWER SPRAYER	GPI-7.0;	SN-SOLUTION	D
	28576	TOTALEX EXTRA STRENGTH RTU BRUSH, GRASS & WEED KILLER WITH 1 TOUCH POWER SPRAYER HOME GARDENER	GPI-14;	SN-SOLUTION	D
	28577	TOTALEX EXTRA STRENGTH RTU BRUSH, GRASS & WEED KILLER WITH 1 TOUCH POWER	GPI-14;	SN-SOLUTION	D

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
		SPRAYER VIRTERRA			
SURE-GRO IP INC.	27013	WILSON TOTAL WIPEOUT MAX GRASS & WEED KILLER READY TO USE	GPI-7;	SN-SOLUTION	D
	27014	WILSON TOTAL WIPEOUT MAX GRASS & WEED KILLER CONCENTRATE	GPI-143;	SN-SOLUTION	D
	27015	LATER'S GRASS & WEED KILLER SUPER CONCENTRATE	GPI-356;	SN-SOLUTION	D
	29580	WILSON TOTAL WIPEOUT MAX GRASS & WEED KILLER READY TO USE BATTERY POWERED	GPI-7;	SN-SOLUTION	D
	31023	SMARTONES WIPEOUT MAX	GPI-7.0;	SN-SOLUTION	D
	32090	WILSON TOTAL WIPEOUT MAX GRASS & WEED KILLER REFILL	GPI-7;	SN-SOLUTION	D
DOW AGROSCIENCES CANADA INC.	26449	GLYPHOSATE 62% SOLUTION MANUFACTURING CONCENTRATE	GPI-46;	SN-SOLUTION	M
	27074	VANTAGE HERBICIDE SOLUTION MANUFACTURING CONCENTRATE	GPI-356;	SN-SOLUTION	M
	27075	VANTAGE PLUS HERBICIDE SOLUTION MANUFACTURING CONCENTRATE	GPI-360;	SN-SOLUTION	M
	28963	GLYPHOSATE 85% MANUFACTURING CONCENTRATE	GPS-85;	SN-SOLUTION	M
	28783	GF-1667 HERBICIDE MANUFACTURING CONCENTRATE	GPX-49;	SN-SOLUTION	M
FMC CORPORATION	25600	GLYPHOSATE CONCENTRATE HERBICIDE	GPI-46.3;	SN-SOLUTION	M
	27497	GLYFOS 356 MUC	GPI-356;	SN-SOLUTION	M
MONSANTO CANADA INC.	21061	MON 0139 SOLUTION HERBICIDE MANUFACTURING CONCENTRATE	GPI-46.0;	SN-SOLUTION	M
	26919	MON 77945 HERBICIDE MANUFACTURING CONCENTRATE SOLUTION	GPI-46;	SN-SOLUTION	M
	28625	MON 78087 HERBICIDE MANUFACTURING CONCENTRATE	GPI-356;	SN-SOLUTION	M
	32273	GLY 135EA HERBICIDE MANUFACTURING CONCENTRATE	GPI-45.6;	SN-SOLUTION	M

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
	27485	MON 78623 HERBICIDE MANUFACTURING CONCENTRATE	GPP-47.3;	SN-SOLUTION	M
	28603	MON 79380 HERBICIDE MANUFACTURING CONCENTRATE	GPP-540;	SN-SOLUTION	M
	28604	MON 79582 HERBICIDE MANUFACTURING CONCENTRATE	GPP-540;	SN-SOLUTION	M
	28605	MON 79544 HERBICIDE MANUFACTURING CONCENTRATE	GPP-540;	SN-SOLUTION	M
	27183	MON 77973 HERBICIDE MANUFACTURING CONCENTRATE	GPS-85;	SN-SOLUTION	M
NUA	29123	NUFARM GLYPHOSATE IPA MANUFACTURING CONCENTRATE	GPI-46;	SN-SOLUTION	M
SYNGENTA CANADA INC.	27871	GLYPHOSATE 600 SL MANUFACTURING CONCENTRATE	GPS-600;	SN-SOLUTION	M
WMW	29719	TERAGRO GLYPHOSATE MANUFACTURING CONCENTRATE	GPI-46;	SN-SOLUTION	M
ALBAUGH LLC	28321	CLEAROUT GLYPHOSATE TECHNICAL	GPS-94.8;	SO-SOLID	T
AGROMARKETING CO. INC.	29645	NASA GLYPHOSATE TECHNICAL	GPS-96.37;	SO-SOLID	T
CONSUS CHEMICALS, LLC.	31728	CONSUS GLYPHOSATE TECHNICAL	GPS-96.7;	SO-SOLID	T
DOW AGROSCIENCES CANADA INC.	26450	GLYPHOSATE TECHNICAL HERBICIDE	GPS-96.3;	SO-SOLID	T
	28967	TECHNICAL GLYPHOSATE HERBICIDE	GPS-96.2;	SO-SOLID	T
FMC CORPORATION	24337	GLYPHOSATE TECHNICAL	GPS-85.8;	SO-SOLID	T
	29143	GLYFOS SOLUBLE CONCENTRATE HERBICIDE 2	GPS-97.9;	SO-SOLID	T
	29326	CHEMINOVA GLYPHOSATE TECHNICAL II	GPS-95.7;	SO-SOLID	T
	29530	CHEMINOVA GLYPHOSATE TECHNICAL III	GPS-98.2;	SO-SOLID	T
JOINT GLYPHOSATE TASK FORCE, LLC	30638	JOINT GLYPHOSATE TECHNICAL	GPS-96.3;	SO-SOLID	T
LIBERTAS NOW INC.	29265	KNOCKOUT TECH	GPS-98.1;	SO-SOLID	T
MEY CORPORATION	29799	MEY CORP GLYPHOSATE TECHNICAL	GPS-98.5;	SO-SOLID	T
	30099	MGT GLYPHOSATE TECHNICAL	GPS-96.4;	SO-SOLID	T
	30617	MEY GLYPHOSATE SHANRG TECHNICAL	GPS-97.59;	SO-SOLID	T

Registrant Name	Registration Number	Product Name	Guarantee[1] (g a.e./L)	Formulation	Marketing Class[2]
MONSANTO CANADA INC.	19535	GLYPHOSATE TECHNICAL GRADE	GPS-96.3;	SO-SOLID	T
NEWAGCO INC	29381	NEWAGCO GLYPHOSATE TECHNICAL	GPS-96.0;	SO-SOLID	T
NUFARM AGRICULTURE INC.	28857	NUFARM GLYPHOSATE TECHNICAL ACID	GPS-96.5;	SO-SOLID	T
PRODUCTIERRA	31062	PRODUCTIERRA GLYPHOSATE TECHNICAL	GPS-98.0;	SO-SOLID	T
SHARDA CROPCHEM LIMITED	29980	SHARDA GLYPHOSATE TECHNICAL HERBICIDE	GPS-96.2;	SO-SOLID	T
SYNGENTA CANADA INC.	28983	TECHNICAL TOUCHDOWN HERBICIDE	GPS-97.1;	SO-SOLID	T
	29540	TOUCHDOWN TECHNICAL HERBICIDE	GPS-99;	SO-SOLID	T
UPI GLYPHOSATE TECHNICAL HERBICIDE	30634	UPI GLYPHOSATE TECHNICAL HERBICIDE	GPS-97.7;	SO-SOLID	T
TERAGRO INC	28882	GLYPHOSATE TECHNICAL HERBICIDE	GPS-97.5;	SO-SOLID	T

[1] GPS = glyphosate acid, GPI = glyphosate isopropylamine or ethnolamine salt, GPM = glyphosate mono-ammonium or diammonium salt, GPP = glyphosate potassium salt, GPX = glyphosate dimethylsulfonium salt, and GPO = GPI + GPP. Note that GPT (glyphosate trimethylsulfonium salt) has been voluntarily discontinued by the registrant Syngenta Canada Inc.
[2] C = Commercial Class, C+R = Commercial and Restricted Class, D = Domestic Class, M = Manufacturing Concentrate, T = Technical grade active ingredient.
[3] AME = s-metolachlor, DIC = dicamba, DIQ = diquat, DXB = 2,4-D (isomer specific), FOF = fomesafen, GLG = glufosinate ammonium and MER = mesotrione.

Appendix III Summary of Species sensitivity Distribution Toxicity Data

Table 1 Revised summary of Species Sensitivity Distribution (SSDs) toxicity data analysis for glyphosate herbicide: HC_5 [1] or the most sensitive endpoints are listed by taxonomic group for Fish, Aquatic Invertebrates and Amphibians [*]

Test material	Exposure	Freshwater invertebrates (mg a.e./L)[B]	Freshwater fish (mg a.e./L)[C]	Marine fish (mg a.e./L)[C]	Marine invertebrates (mg a.e./L)[B]	Amphibians (mg a.e./L)[C]	Amphibians Mesocosm/field (mg a.e./L)[C]
TGAI	Acute	HC_5: 15.9	HC_5: 70	HC_5: 19.9	HC_5: 4.7	HC_5: 14.9	-
	Chronic	NOEC: 13.0	NOEC: 22.4	NOEC: 0.1	-	-	-
EUP NON POEA	Acute	HC_5: 24.4	HC_5: 2.3	LC_{50}: 114.6	EC_{50}: 23.2	HC_5: 13.9	-
	Chronic	EC_{50}: 44.0	-	-	-	-	-
EUP WITH POEA	Acute	HC_5: 0.1	HC_5: 2.2	HC_5: 3.0	HC_5: 0.1	HC_5: 0.73	HC_5: 3.7 HC_5: 3.3 (kg a.e/ha)
	Chronic	NOEC: 0.2	NOEC: 0.28	-	-	HC_5: 0.43	HC_5: 1.9
AMPA	Acute	LC_{50}: 316.0	LC_{50}: 274.0	-	EC_{50}: 97.0	-	-
	Chronic	-	-	-	-		-
POEA	Acute	HC_5: 0.004	HC_5: 0.2	HC_5: 2.0	EC_{50}: 0.6	HC_5: 0.3	-
	Chronic	-	-	-	-	-	-

[*]Where SSDs could not be determined, the most sensitive species endpoint value is reported; [1]Hazardous concentration to 5% of species; POEA is a formulant, POEA concentrations cannot be directly compared to other data as the concentration in a formulation varies and not specified; [B] HC_5 is derived from EC_{50} values; [C] HC_5 is derived from LC_{50} values.
TGAI = Technical grade active ingredient, EUP NON POEA = End-use product that does not contain polyethoxylated tallow amine compound in their formulation, EUP WITH POEA = End-use product that does contain polyethoxylated tallow amine compound in their formulation, AMPA = aminmethylphosphonic acid compound, POEA = polyethoxylated tallow amine

Table 2 Revised summary of Species Sensitivity Distribution (SSDs) toxicity data analysis for glyphosate herbicide: HC_5 [1] or the most sensitive endpoints are listed by taxonomic group for Aquatic Plants, Algae, Terrestrial Plants [*]

Test material	Exposure	Freshwater Algae (mg a.e./L)[B]	Freshwater Plants (mg a.e./L)	Marine Algae (mg a.e./L)	Snails (mg a.e./L)
TGAI	Acute	HC_5: 6.6 EC_{50}: 10.1	EC_{50}: 17.3 Er_{50}: 0.38 kg a.e./ha	EC_{50}: 3.35	-
	Chronic	HC_5:21.6	-	EC_{50}:101.5	NOEC: 1000
EUP NON POEA	Acute	EC_{50}: 37	-	-	-
	Chronic	-	-	-	NOEC: 29.7 NOEC: 219 (mg a.e./kg soil)
EUP WITH POEA	Acute	HC_5: 0.1	EC_{50}: 2.1	EC_{50}: 0.43	LC_{50}: 2.3
	Chronic	HC_5:0.3		EC_{50}: 8.3	NOEC: 8.55
EUP NON POEA and WITH POEA	Acute	-	-	-	-

Test material	Exposure	Freshwater Algae (mg a.e./L)[B]	Freshwater Plants (mg a.e./L)	Marine Algae (mg a.e./L)	Snails (mg a.e./L)
AMPA	Acute	EC_{50}: 73	-	-	-
	Chronic	-	-	-	-
POEA	Acute	EC_{50}: 4	-	EC_{50}: 3.4	-

[*]Where SSDs could not be determined, the most sensitive species endpoint value is reported; [1]Hazardous concentration to 5% of species; POEA is a formulant, POEA concentrations cannot be directly compared to other data as the concentration in a formulation varies and not specified; [B] HC_5 is derived from EC_{50} values; [C] HC_5 is derived from LC_{50} values;
TGAI = Technical grade active ingredient, EUP NON POEA = End-use product that does not contain polyethoxylated tallow amine compound in their formulation, EUP WITH POEA = End-use product that does contain polyethoxylated tallow amine compound in their formulation, AMPA = aminmethylphosphonic acid compound, POEA = polyethoxylated tallow amine

Table 3 Revised summary of Species Sensitivity Distribution (SSDs) toxicity data analysis for glyphosate herbicide: HC_5 [1] or the most sensitive endpoints are listed by taxonomic group for Terrestrial Plants and Terrestrial Invertebrates.

Test material	Exposure	Terrestrial Plants (SE) EC_{50} (kg a.e/ha)	Terrestrial plants EC_{25} Mixed[D] (kg a.e/ha)	Terrestrial plants E_{C50} Mixed[D] (kg a.e/ha)	Earthworms (mg a.e./kg soil)
TGAI	Acute	EC_{50}: 0.07	-		690
	Chronic	-	-		-
EUP NON POEA	Acute	EC_{50}: 4.48	-		-
	Chronic	-	-		-
EUP WITH POEA	Acute	-	HD_5 = 0.035		0.253
	Chronic	-	-		-
EUP NON POEA and WITH POEA	Acute	-	HD_5 = 0.037	HD_5 = 0.0658	-

(SE) = seedling emergence, (VV) = vegetative vigor; [*]Where SSDs could not be determined, the most sensitive species endpoint value is reported; [1]Hazardous concentration to 5% of species; POEA is a formulant, POEA concentrations cannot be directly compared to other data as the concentration in a formulation varies and not specified; [B] HC_5 is derived from EC_{50} values; [C] HC_5 is derived from LC_{50} values; [D]Mixed = Crop and non-crop plants combined. Yellow highlight: most sensitive acute and chronic endpoint.
TGAI = Technical grade active ingredient, EUP NON POEA = End-use product that does not contain polyethoxylated tallow amine compound in their formulation, EUP WITH POEA = End-use product that does contain polyethoxylated tallow amine compound in their formulation, AMPA = aminmethylphosphonic acid compound, POEA = polyethoxylated tallow amine

Appendix IV Label Amendments for Products Containing Glyphosate

The label amendments presented below do not include all label requirements for individual products, such as first aid statements, disposal statements, precautionary statements and supplementary protective equipment. Information on labels of currently registered products should not be removed unless it contradicts the following label statements.

A) Label Amendments for Glyphosate Technical Products

The following label amendments are required on the Glyphosate Technical labels:

1) Add to the primary panel of the Technical product labels:

   The signal words "DANGER – EYE IRRITANT", and accompanying glyphs.

2) Before **STORAGE section, Add** the title "**ENVIRONMENTAL HAZARDS**" and the following statement:

   - **TOXIC** to non-target terrestrial plants
   - **TOXIC** to aquatic organisms

3) **Remove** the following statement under the "**DISPOSAL AND DECONTAMINATION**"

   "Canadian formulators of this technical should dispose of unwanted active and containers in accordance with municipal or provincial regulations. For information on disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in the case of a spill, and for clean-up of spills."

   and replace it with the following statement:

   "Canadian manufacturers should dispose of unwanted active ingredients and containers in accordance with municipal or provincial regulations. For additional details and clean up of spills, contact the manufacturer or the provincial regulatory agency."

B) For Domestic Products Containing Glyphosate

For all end-use products, the following statement is required:

"Glyphosate is not to be applied using hand-wicking or hand-daubing methods."

C) For Commercial and Agricultural Class Products Containing Glyphosate

1) Add to DIRECTIONS FOR USE:

   For all end-use products, the following statement is required:

   "Glyphosate is not to be applied using hand-wicking or hand-daubing methods."

   Restricted Entry Intervals

   "The restricted entry interval is 12 hours after application for all agricultural uses."

2) **Add to Use Precautions**

   "Apply only when the potential for drift to areas of human habitation or areas of human activity such as houses, cottages, schools and recreational areas is minimal. Take into consideration wind speed, wind direction, temperature inversions, application equipment and sprayer settings."

3) Add the following to ENVIRONMENTAL HAZARDS:

- **TOXIC** to aquatic organisms and non-target terrestrial plants. Observe buffer zones specified under DIRECTIONS FOR USE.
- To reduce runoff from treated areas into aquatic habitats, avoid application to areas with a moderate to steep slope, compacted soil or clay.
- Avoid application when heavy rain is forecast.
- Contamination of aquatic areas as a result of runoff may be reduced by including a vegetative strip between the treated area and the edge of the water body.

4) **Add to DIRECTIONS FOR USE**

The following statement is required for all agricultural and commercial pesticide products:

- **As this product is not registered for the control of pests in aquatic systems, DO NOT use to control aquatic pests**
- **DO NOT contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.**

5) Add to **DIRECTIONS FOR USE**

Field sprayer application: **DO NOT** apply during periods of dead calm. Avoid application of this product when winds are gusty. **DO NOT** apply with spray droplets smaller than the American Society of Agricultural Engineers (ASAE S572.1) coarse classification. Boom height must be 60 cm or less above the crop or ground.

Airblast or mist blower application: **DO NOT** apply during periods of dead calm. Avoid application of this product when winds are gusty. **DO NOT** direct spray above plants to be treated. **DO NOT** apply when wind speed is greater than 16 km/h at the application site as measured outside of the treatment area on the upwind side. For airblast applications, turn off outward pointing nozzles at row ends and outer rows.

Aerial application: **DO NOT** apply during periods of dead calm. Avoid application of this product when winds are gusty. **DO NOT** apply when wind speed is greater than 16 km/h at flying height at the site of application. **DO NOT** apply with spray droplets smaller than the American Society of Agricultural Engineers (ASAE S572.1) coarse classification. To reduce drift caused by turbulent wingtip vortices, the nozzle distribution along the spray boom length **MUST NOT** exceed 65% of the wing- or rotorspan.

Buffer zones:

Use of the following spray methods or equipment **DO NOT** require a buffer zone: hand-held or backpack sprayer and spot treatment, inter-row hooded sprayer, low-clearance hooded or shielded sprayers that ensure spray drift does not come in contact with orchard crop fruit or foliage, soil drench and soil incorporation.

For application to rights-of-way and for forestry uses, buffer zones for protection of sensitive terrestrial habitats are not required; however, the best available application strategies which minimize off-site drift, including meteorological conditions (for example, wind direction, low wind speed) and spray equipment (for example, coarse droplet sizes, minimizing height above canopy), should be used. Applicators must, however, observe the specified buffer zones for protection of sensitive aquatic habitats.

The buffer zones specified in the table below are required between the point of direct application and the closest downwind edge of sensitive terrestrial habitats (such as grasslands, forested areas, shelter belts, woodlots, hedgerows, riparian areas and shrublands) and sensitive aquatic habitats (such as lakes, rivers, sloughs, ponds, prairie potholes, creeks, marshes, streams, reservoirs, wetlands and estuarine/marine water bodies).

Table 1 Buffer Zones for the Protection of Aquatic and Terrestrial Habitats from Spray Drift of Glyphosate Products Formulated with POEA

Agricultural, forestry and non-cropland systems		Maximum number of applications	Buffer Zones (metres) Required for the Protection of:	
			Aquatic habitats	Terrestrial habitats
Agricultural crop system and ground boom application method				
Rye, cranberry, pasture, summer fallow, all other crops for pre-seeding treatments only, filberts or hazelnut at pre-seeding only, ginseng new garden		1	1	1
Ginseng - existing established garden, Canola – Roundup Ready hybrid for seed production		2	1	1
Filberts or hazelnut, sugar beets (glyphosate tolerant varieties)		4	1	1
Corn (glyphosate non-tolerant varieties including grain, silage and ornamental types), sugar beet (glyphosate non-tolerant varieties), strawberry, blueberry highbush and lowbush, walnut, chestnut, Japanese heartnut, Turf grass (prior to establishment or renovation)		2	1	2
Wheat, barley, oats, soybean (glyphosate non-tolerant varieties), corn-sweet (glyphosate tolerant varieties), canola (glyphosate non-tolerant varieties), peas, dry beans, flax (including low linoleic acid varieties), lentils, chickpea, lupin (dried), fava bean (dried), mustard (yellow/white, brown, oriental), pearl millet, sorghum (grain) (not for use as a forage crop), asparagus, corn (glyphosate tolerant varieties), forage grasses and legume including seed production		3	1	2
Canola (glyphosate tolerant varieties), soybean (glyphosate tolerant varieties)		4	1	2
Apple, apricot, cherry (sweet/sour), peaches, pears, plums, grapes		3	1	3
Agricultural crop system and airblast application method (including mist blower)				
Pasture		1	20	30
Turfgrass (Prior to establishment or renovation)		2	25	35
Forest plant system and ground boom application method				
Forest and woodlands > 500 ha Site preparation		2	1	NR
Forest plant system and airblast application method (including mist blower)				
Forest and woodlands > 500 ha Site preparation		2	1	NR
Non-cropland system and ground boom application method				
Non-crop land and industrial uses: Industrial and rights of way areas, Recreational and public areas		3	1	3*
Non-cropland system and airblast application method (including mist blower)				
Non-crop land and industrial uses: Industrial and rights of way areas, Recreational and public areas		3	1	30*
Agricultural crop system and aerial application method	**Wing type**			
Rye, corn (glyphosate non-tolerant varieties), corn-sweet (glyphosate tolerant varieties), chickpea, lupin (dried), fava bean (dried), mustard (yellow/white, brown, oriental), pearl millet , sorghum (grain) (not for use as a forage crop), sugar beet (glyphosate non-tolerant varieties), all other crops for pre-seeding treatments only	Fixed and rotary wing	1	15	20

Agricultural, forestry and non-cropland systems		Maximum number of applications	Buffer Zones (metres) Required for the Protection of:	
			Aquatic habitats	Terrestrial habitats
Canola (glyphosate tolerant varieties)	Fixed and rotary wing	3	20	40
Sugar beets (glyphosate tolerant varieties)	Fixed wing	2	20	30
	Rotary wing	2	15	30
Wheat, barley, oats, soybean (glyphosate non-tolerant varieties), canola (glyphosate non-tolerant varieties), peas, dry beans, flax (including low linoleic acid varieties), lentils	Fixed wing	2	20	35
	Rotary wing	2	20	30
Forage grasses and legume including seed production	Fixed and rotary wing	1	20	40
Soybean (glyphosate tolerant varieties)	Fixed wing	3	20	45
	Rotary wing	3	20	40
Summer fallow	Fixed wing	1	20	45
	Rotary wing	1	20	40
Corn (glyphosate tolerant varieties)	Fixed wing	2	20	50
	Rotary wing	2	20	45
Pasture	Fixed wing	1	30	70
	Rotary wing	1	30	55
Forestry system and aerial application method				
Forest and woodlands >500 ha Site preparation	Fixed wing	2	10	NR
	Rotary wing	2	1	NR
Forest and woodlands <500 ha Site preparation	Fixed wing	2	5	NR
	Rotary wing	2	1	NR
Non-cropland system and aerial application method				
Non-crop land and industrial uses: rights-of way areas only	Fixed wing	3	100	NR
	Rotary wing	3	60	NR

* Buffer zones for the protection of terrestrial habitats are not required for forestry uses or for use on rights-of-way including railroad ballast, rail and hydro rights-of-way, utility easements, roads, and training grounds and firing ranges on military bases.
NR = Buffer zones for the protection of terrestrial habitats are not required for forestry uses.

For tank mixes, consult the labels of the tank-mix partners and observe the largest (most restrictive) buffer zone of the products involved in the tank mixture and apply using the coarsest spray (ASAE) category indicated on the labels for those tank mix partners.

The buffer zones for this product can be modified based on weather conditions and spray equipment configuration by accessing the Buffer Zone Calculator on the Pest Management Regulatory Agency web site.

Table 2 Buffer Zones for the Protection of Aquatic and Terrestrial Habitats from Spray Drift of Glyphosate Products without POEA

Agricultural and non-cropland systems		Maximum number of applications	Buffer Zones (metres) Required for the Protection of:	
			Aquatic habitats	Terrestrial habitats
Agricultural crop system and ground boom application method				
Rye, cranberry, pasture, summer fallow, pasture, all other crops for pre-seeding treatments only, filberts or hazelnut pre-seeding only, ginseng new garden		1	1	1
Ginseng - existing established garden, Canola – Roundup Ready hybrid for seed production		2	1	1
Filberts or hazelnut, sugar beets (glyphosate tolerant varieties)		4	1	1
Corn (glyphosate non-tolerant varieties including grain, silage and ornamental types), sugar beet (glyphosate non-tolerant varieties), strawberry, blueberry highbush and lowbush, walnut, chestnut, Japanese heartnut, Turf grass (prior to establishment or renovation)		2	1	2
Wheat, barley, oats, soybean (glyphosate non-tolerant varieties), corn-sweet (glyphosate tolerant varieties), canola (glyphosate non-tolerant varieties), peas, dry beans, flax (including low linoleic acid varieties), lentils, chickpea, lupin (dried), fava bean (dried), mustard (yellow/white, brown, oriental), pearl millet, sorghum (grain) (not for use as a forage crop), asparagus, corn (glyphosate tolerant varieties), forage grasses and legume including seed production		3	1	2
Canola (glyphosate tolerant varieties), soybean (glyphosate tolerant varieties)		4	1	2
Apple, apricot, cherry (sweet/sour), peaches, pears, plums, grapes		3	1	3
Agricultural crop system and airblast application method (including mist blower)				
Pasture		1	20	30
Turfgrass (Prior to establishment or renovation)		2	25	35
Non-cropland system and ground boom application method				
Non-crop land and industrial uses: Industrial and rights of way areas, Recreational and public areas		3	1	3
Non-cropland system and airblast application method (including mist blower)				
Non-crop land and industrial uses: Industrial and rights of way areas, Recreational and public areas		3	20	30
Agricultural crop system and aerial application method				
Rye, corn (glyphosate non-tolerant varieties), corn-sweet (glyphosate tolerant varieties), chickpea, lupin (dried), fava bean (dried), mustard (yellow/white, brown, oriental), pearl millet , sorghum (grain) (not for use as a forage crop), sugar beet (glyphosate non-tolerant varieties), all other crops for pre-seeding treatments only	Fixed and rotary wing	1	15	20

Agricultural and non-cropland systems		Maximum number of applications	Buffer Zones (metres) Required for the Protection of:	
			Aquatic habitats	Terrestrial habitats
Sugar beets (glyphosate tolerant varieties)	Fixed wing	2	20	30
	Rotary wing	2	15	30
Wheat, barley, oats, soybean (glyphosate non-tolerant varieties), canola (glyphosate non-tolerant varieties), peas, dry beans, flax (including low linoleic acid varieties), lentils	Fixed wing	2	20	35
	Rotary wing	2	20	30
Forage grasses and legume including seed production	Fixed and rotary wing	1	20	40
Canola (glyphosate tolerant varieties)	Fixed and rotary wing	3	20	40
Soybean (glyphosate tolerant varieties)	Fixed wing	3	20	45
	Rotary wing	3	20	40
Summer fallow	Fixed wing	1	20	45
	Rotary wing	1	20	40
Corn (glyphosate tolerant varieties)	Fixed wing	2	20	50
	Rotary wing	2	20	45
Pasture	Fixed wing	1	30	70
	Rotary wing	1	30	55
Non-cropland system and aerial application method				
Non-crop land and industrial uses: rights-of way areas only	Fixed wing	3	100	NR
	Rotary wing	3	60	NR

* Buffer zones for the protection of terrestrial habitats are not required for use on rights-of-way including railroad ballast, rail and hydro rights-of-way, utility easements, roads, and training grounds and firing ranges on military bases.
NR = Buffer zones for the protection of terrestrial habitats are not required for forestry uses.

For tank mixes, consult the labels of the tank-mix partners and observe the largest (most restrictive) buffer zone of the products involved in the tank mixture and apply using the coarsest spray (ASAE) category indicated on the labels for those tank mix partners.

The buffer zones for this product can be modified based on weather conditions and spray equipment configuration by accessing the Buffer Zone Calculator on the Pest Management Regulatory Agency web site.

References

Studies and Information Considered in Relation to Human Health Risk Assessment

Toxicology

A. List of Additional Studies/Information submitted by Registrant – Unpublished

PMRA Document Number	Reference
1644044	2007, Surfactant 8184-92, acute dermal toxicity study in rabbits, DACO: 4.6.2
1644045	2007, Surfactant 8184-92, acute dermal toxicity study in rats, DACO: 4.6.2
1817835	2007, Surfactant, 8184-92, acute inhalation toxicity study in rats, DACO: 4.6.3
1817836	2007, Surfactant, 8184-92, skin sensitization study in guinea pigs, DACO: 4.6.6
1817838	2007, Surfactant, 8184-92, acute eye irritation study in rabbits, DACO: 4.6.4
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1817841	2007, Surfactant 8184-92, acute dermal irritation study in rabbits, DACO: 4.6.
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World Health Organization (WHO) and
Food and Agriculture Organization (FAO)

Joint FAO/WHO Meeting on Pesticide Residues

May 2016

JOINT FAO/WHO MEETING ON PESTICIDE RESIDUES

Geneva, 9–13 May 2016

SUMMARY REPORT

Issued 16 May 2016

Edited versions of these evaluations and general considerations will be published in the report of the May 2016 JMPR. They are reproduced here so that the information can be disseminated quickly. These drafts are subject to technical editing.

A Joint Meeting of the Food and Agriculture Organization of the United Nations (FAO) Panel of Experts on Pesticide Residues in Food and the Environment and the World Health Organization (WHO) Core Assessment Group on Pesticide Residues (JMPR) was held at WHO Headquarters, Geneva (Switzerland), from 9 to 13 May 2016. Diazinon, glyphosate and malathion were placed on the agenda by the JMPR Secretariat, based on the recommendation of the last session of JMPR to re-evaluate these compounds given the number of new studies that had become available since their last full assessments.

The following extracts of the results of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) are provided to make them accessible to interested parties at an early date.

More information on the work of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) is available at:

http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmpr/jmpr-rep/en/

http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/

1. *Evaluation of data for acceptable daily intake (ADI) and acute reference dose (ARfD) for humans*

1.1 Diazinon (22)

Diazinon is an insecticide with a wide range of insecticidal activity. Several epidemiological studies on cancer outcomes following occupational exposure to diazinon were available. The review of these studies provided no convincing evidence of a positive association between exposure to diazinon and non-Hodgkin lymphoma (NHL), but there was weak evidence of a positive association between leukaemia and exposure to diazinon and between lung cancer and exposure to diazinon from one large cohort study only. In studies submitted, diazinon was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Overall, these studies provided no convincing evidence of genotoxic effects, and the Meeting concluded that diazinon was unlikely to be genotoxic. The Meeting concluded that diazinon is unlikely to pose a carcinogenic risk to humans from exposure through the diet. After considering all previously evaluated data and the new studies, the Meeting established an ADI of 0–0.003 mg/kg body weight, based on inhibition of acetylcholinesterase activity as the most sensitive end-point. The Meeting reaffirmed the ARfD of 0.03 mg/kg body weight established by the 2006 JMPR based on acute (neuro)toxicity in rats.

1.2 Glyphosate (158)

Glyphosate is a broad-spectrum systemic herbicide. Several epidemiological studies on cancer outcomes following occupational exposure to glyphosate were available. The evaluation of these studies focused on the occurrence of NHL. Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the only large cohort study of high quality found no evidence of an association at any exposure level. Glyphosate has been extensively tested for genotoxic effects using a variety of tests in a wide range of organisms. The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg body weight by the oral route, the route most relevant to human dietary exposure, was not associated with genotoxic effects in an overwhelming majority of studies conducted in mammals, a model considered to be appropriate for assessing genotoxic risks to humans. The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures. Several carcinogenicity studies in mice and rats are available. The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses. In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet. The Meeting reaffirmed the group ADI for the sum of glyphosate and its metabolites of 0–1 mg/kg body weight on the basis of effects on the salivary gland. The Meeting concluded that it was not necessary to establish an ARfD for glyphosate or its metabolites in view of its low acute toxicity.

1.3 Malathion (49)

Malathion is an insecticide used to control insects on agricultural crops and stored commodities and for vector control. Several epidemiological studies on cancer outcomes in relation to occupational exposure to malathion were available. Overall, there is some very weak evidence of a positive association between malathion exposure and NHL; however, it is notable that the only large cohort study of high quality found no evidence of an association at any exposure level. The evidence is suggestive of a positive association between occupational exposure to malathion and risk of aggressive prostate cancer; however, the evidence base is limited to the one large cohort study. The Meeting concluded that there is some evidence that malathion is carcinogenic in rats and mice. However, the formation of nasal adenomas was due to a local irritancy caused by prolonged exposure to high concentrations of malathion absorbed via inhaled food particles. Scenarios of prolonged, direct and excessive exposure of human nasal tissue to malathion or malathion metabolites following ingestion of residues is unlikely, and therefore these tumours would not occur in humans following exposure to malathion in the diet. Malathion has been extensively tested for genotoxicity, including studies in exposed workers. The Meeting noted that there are numerous reports that malathion can induce oxidative damage in cells, and these results suggest that the observed genotoxic effects occur secondary to the formation of reactive oxygen species, which will exhibit a threshold. Based on consideration of the results of animal bioassays, genotoxicity assays and epidemiological data, the Meeting concluded that malathion and its metabolites are unlikely to pose a carcinogenic risk to humans from exposure via the diet. The current Meeting reaffirmed the ADI of 0–0.3 mg/kg body weight. The margins of exposure between this ADI and the doses causing cancer in mice and rats are 5000-fold and 1200-fold, respectively. The current Meeting also reaffirmed the ARfD of 2 mg/kg body weight. The Meeting concluded that the metabolite malaoxon is approximately 30-fold more toxic than malathion. On this basis, a 30-fold potency factor should be applied to the residue levels for use in both the acute and chronic dietary exposure estimates for malaoxon, and these should be added to the dietary exposures for malathion and compared with the ARfD and ADI for malathion, respectively.

2. General considerations

2.1 General considerations on the evaluation of genotoxicity studies

A large number of genotoxicity studies were evaluated during the present meeting. These were identified through direct submission to JMPR, searches of the publicly available literature and requests to the International Agency for Research on Cancer (IARC) Monographs Secretariat and industry groups. The studies evaluated included unpublished (primarily guideline) studies submitted to support pesticide registration as well as peer-reviewed studies published in the scientific literature. The number, quality and relevance of studies differed widely for each chemical and necessitated that a somewhat different approach be used to evaluate each pesticide. As a general strategy, the studies were separated into categories based largely on phylogenetic relevance and significance of the genetic

end-point measured. The categories used were human biomonitoring, in vivo mammals, in vitro mammalian cells, in vitro bacteria, phylogenetically distant organisms, metabolites in vivo and metabolites in vitro. The evaluation was conducted for the pesticide active ingredient, its formulation products and prominent metabolites, as data were available. For the three pesticides evaluated, the human biomonitoring studies were most often confounded by exposures to other pesticides or considered to have other limitations. Among the genotoxicity studies, in vivo studies in mammals were given the greatest weight, compared with cell culture studies or investigations in phylogenetically distant organisms. Studies of gene mutations and chromosomal alterations were also given more weight than studies measuring other less serious or transient types of genotoxic damage. With regard to route of exposure, studies in which chemicals were administered by the oral route were considered to be of most relevance for evaluating low-level dietary exposures.

Following an evaluation and weighting of the studies, taking the criteria described above and the quality of the studies into account, an overall weight of evidence approach was used to reach conclusions about the genotoxicity of the individual pesticides. An important aspect of the evaluation was whether the genotoxic effect would be likely to occur in humans exposed to low levels of the pesticide present as residues in food.

The Meeting recommended that a guidance document be developed for the evaluation of genotoxicity studies, taking the experience gained from this meeting into account.

2.2 Methods for the evaluation of epidemiological evidence for risk assessment

Identification of compound/cancer sites and screening of papers

There is a large body of literature regarding pesticide exposures and non-cancer outcomes (neurodevelopmental, neurodegenerative and reproductive outcomes, among other health outcomes), but the assessment of the epidemiological evidence on diazinon, glyphosate and malathion was restricted to studies of cancer outcomes. This restriction was partly driven by feasibility reasons: a clinically relevant adverse effect size (or an acceptable level of risk) for a non-cancer outcome must be defined, and the methodologies for hazard identification and characterization based on observational epidemiological findings of non-carcinogenic adverse effects are less well established than those for cancer.

The IARC Monographs on malathion, diazinon and glyphosate referred to a total of 45 epidemiological studies. Databases were searched for any relevant articles published after the studies cited in these Monographs using the following search terms: [(diazinon OR glyphosate OR malathion) AND cancer] and [(diazinon OR glyphosate OR malathion) AND (NHL OR lymphoma OR leukemia OR “lung cancer” OR “prostate cancer”)] in PubMed (limited to Humans; published in the last 5 years) and Scopus (limited to 2014–2016). Two studies published since the publication of the IARC Monographs that evaluated at least one of malathion, diazinon or glyphosate were identified in

relation to cancer outcomes. An additional study on prostate cancer, which was not included in the IARC Monographs, was also identified.

The pre-agreed evaluation process shown in Fig. 1 was used to (1) select compound/cancer site combinations to include in this evaluation; (2) screen papers for inclusion/exclusion in this evaluation (Tier 1 screening criteria); and (3) evaluate the information available for risk assessment. In this process, it was noted that there were stand-alone analyses for specific subtypes of non-Hodgkin lymphoma (NHL). The risk for subtypes of NHL was not evaluated separately, as there was insufficient evidence (too few studies or small numbers of cases); the risk for other haematopoietic and lymphoid tumours was also not evaluated separately, as the positive associations identified by IARC were for total NHL.

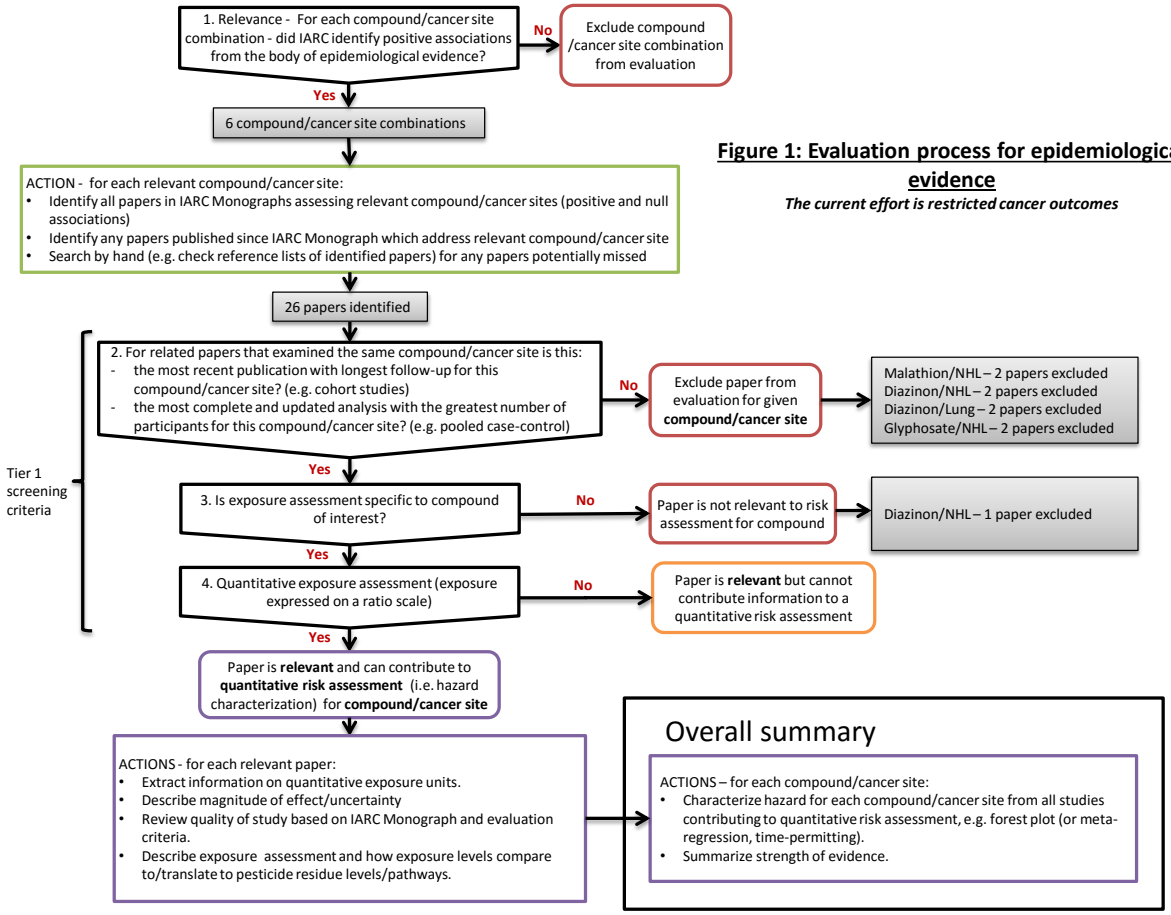

Figure 1: Evaluation process for epidemiological evidence

The current effort is restricted cancer outcomes

Evaluation of evidence for the compound/cancer site associations

Several aspects of each study and of all studies combined were considered in this evaluation, including factors that decrease the level of confidence in the body of evidence, such as risk of bias, unexplained inconsistency and imprecision; and factors that increase the level of confidence, such as large magnitude of effect, dose–response and consistency. The findings for each study were

summarized in tables, and risk estimates for non-quantitative exposure assessment (predominantly ever versus never use) were summarized in forest plots.

Evaluation of information available for risk assessment/hazard characterization

To evaluate overall evidence for dose–response relationships, risk estimates were plotted against quantitative exposure measures (for studies that had used these). The most commonly used quantitative exposure metric was days of use per year. Where studies had used other quantitative exposure metrics (e.g. lifetime days of exposure), data were requested from the authors on median "days of use per year" for the participants in each of the original exposure categories, although this information was not always forthcoming. These additional data allowed the translation and plotting of risk estimates from different studies on the same exposure scale (days of use per year).

New Zealand Environmental Protection Agency

Review of the Evidence Relating to Glyphosate and Carcinogenicity

August 2016

Lay Summary

The Environmental Protection Authority (EPA) commissioned Dr Wayne Temple, a toxicologist and former Director of the New Zealand National Poisons Centre, to undertake a review of the evidence relating to the possible carcinogenicity of glyphosate. This lay summary is to accompany the report he has produced *"Review of the Evidence Relating to Glyphosate and Carcinogenicity"*. The report also had input from Dr Michael Beasley, a toxicologist at the National Poisons Centre.

Dr Temple's report was peer reviewed by toxicologists from the EPA and the Ministry for Primary Industries.

The review took into account studies reviewed in the International Agency for Research on Cancer (IARC) report, as well as additional studies that were not reviewed by IARC but have been assessed by overseas regulators including the European Food Safety Authority (EFSA), US Environmental Protection Agency (US EPA) and the Joint FAO/WHO meeting on Pesticide Residues (JMPR)[1].

What are the conclusions of the review?

The review concluded that glyphosate is unlikely to be carcinogenic to humans or genotoxic (damaging to genetic material or DNA) and should not be classified as a mutagen or carcinogen under the HSNO Act.

This conclusion was based largely on consideration of the results of studies on humans (epidemiology studies) and studies in laboratory animals, as well as genotoxicity studies conducted by a range of methods. More details are provided below.

Studies on humans

The majority of human studies did not show an association between exposure to glyphosate and cancer. Although a small number of studies with a limited number of participants found a weak association between glyphosate exposure and increased risk of non-Hodgkin lymphoma (NHL), other studies did not. The studies that found no association between glyphosate exposure and NHL included the largest and most reliable study, which included over 50,000 participants.

There were also a number of limitations to many of the studies. These included only a small number of people being assessed, people also being exposed to other pesticides, and methodological limitations with how the amount of glyphosate people were exposed to was measured.

Based on the inconsistency in the results of the studies on glyphosate exposure and NHL, and the lack of any association in the largest, most robust study, it was concluded that there is no convincing evidence of an association between glyphosate exposure and the development of cancer in humans.

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1. The JMPR is an international expert scientific group administered jointly by the United Nations Food and Agriculture Organisation (FAO) and the World Health Organization (WHO). JMPR undertakes pesticide risk assessments for the purpose of establishing safe limits of pesticide residues in food.

Studies in laboratory animals

A small number of studies in laboratory animals found an increased incidence of cancers in rats or mice exposed to glyphosate. However, these findings were not considered to be reliable evidence of a carcinogenic effect by overseas regulators for a number of reasons including:

- There was a lack of dose response. Normally the incidence or severity of toxicological effects caused by chemicals increases as the amount of exposure to the chemical increases. This was not seen in the studies with glyphosate.
- In most cases tumours occurred only at very high doses which were at or above recommended maximum doses for animal studies so are not considered relevant for humans.
- The incidences of cancers in most studies were within the range of normal incidences of these cancers in the test animals.
- The carcinogenic effects seen in a small number of studies were not seen in other studies conducted in the same species at the same dose levels.

Therefore Dr Temple concluded that the overall weight of evidence indicates that glyphosate is not carcinogenic.

Genotoxicity studies

All studies done according to internationally agreed test guidelines did not find evidence of a genotoxic (damaging to DNA) effect of glyphosate. Some studies with pesticide formulations that contain glyphosate showed a genotoxic effect. However, in some cases these studies were conducted in test systems that have not been validated as relevant to assess genotoxicity. In addition, because genotoxic effects were not seen with glyphosate itself, it is possible that the effects were related to other components in the formulations that were tested.

It was concluded that the weight of evidence indicates that glyphosate is not genotoxic.

What does this mean?

Based on the information currently available, the EPA considers that glyphosate products approved in New Zealand are safe to use when following the instructions on the label.

Glyphosate is on the Chief Executive Initiated Reassessment (CEIR) programme list, which means that we are actively monitoring its status and international developments. If EPA staff consider a formal review is needed based on new information that becomes available, a reassessment may be initiated, but on the weight of evidence to date, glyphosate does not require classification under HSNO as a carcinogen or mutagen.

Where can I find out more about glyphosate?

If you need more information, visit www.epa.govt.nz/glyphosate or call 0800 HAZSUBS (0800 429 7827) or email: hazardous.substances@epa.govt.nz.

Environmental Protection Authority
Te Mana Rauhī Taiao

Review of the Evidence Relating to Glyphosate and Carcinogenicity

Prepared for the Environmental Protection Authority
by Dr Wayne Temple BSc (Hons), PhD, FNZIC, CChem, FRSC, MAACT

Published August 2016

Introduction

Glyphosate (N-phosphonomethyl glycine; CAS registry #1071-83-6) is the primary active ingredient in many generic herbicides. Glyphosate is formulated primarily as an isopropylamine, ammonium, or sodium salt in water soluble concentrates and water soluble granules. The relevant impurities in glyphosate technical concentrates are formaldehyde, N-nitrosoglyphosate and N-nitroso-N-phosphonomethylglycine. Surfactants and sulfuric and phosphoric acids may be added to formulations of glyphosate, with type and concentration differing by formulation. The United States (US) Environmental Protection Agency (EPA) and other regulatory agencies around the world have registered this chemical as a broad-spectrum herbicide for use on multiple food and non-food use crops. Glyphosate-based herbicides, which have been sold in the US since 1974, are now registered in over 130 countries.

Glyphosate is widely considered by regulatory authorities and scientific bodies to have no carcinogenic potential. The US EPA (1993) has classified glyphosate as a Group E carcinogen, which is defined as having ‘‘evidence of non-carcinogenicity for humans’’. This classification was based on ‘‘a lack of convincing evidence of carcinogenicity in adequate studies with two animal species, rat and mouse’’. Negative results were observed in genotoxicity studies that were conducted under good laboratory practice conditions and compliant with contemporary regulatory test guidelines.

However since that time, results of further studies have come to light, and the International Agency for Research on Cancer (IARC) Monograph 112 on glyphosate (released on 29 July 2015) came to the conclusion that glyphosate should now be classified as a carcinogenic substance in Group 2A (probably carcinogenic to humans). This classification was based on “limited evidence” from human data (regarding non-Hodgkin lymphoma (NHL)) but “sufficient evidence” in animal-experiments. The rationale identifies that the IARC working group (IWG) also notes mechanistic and other relevant data in support of the conclusion; in particular the IWG cites “strong evidence” that glyphosate can operate by two key characteristics of known human carcinogens, namely genotoxicity and oxidative stress.

This classification was initially published in a short report by Blair et al, (2015) in the “Lancet Oncology” on 20 March 2015.

This report discusses the relevant data on glyphosate, especially the more recent studies, and reviews the basis on which the IWG classified it as a probable human carcinogen (Group 2A). This involves review of the quality of evidence for carcinogenicity in humans and experimental animals and the mechanistic arguments.

Cancer in humans

The IWG found there was limited evidence in humans for the carcinogenicity of glyphosate. Some case-control studies of occupational exposure in the USA, Canada, and Sweden reported increased risks for NHL that persisted after adjustment for other pesticide exposures. However the Agricultural Health Study (AHS) cohort did not show a significantly increased risk of NHL. These studies are discussed below.

Case-control studies in the Midwest USA

Three case-control studies were conducted by the U.S National Cancer Institute in Iowa and Minnesota in the1980s using the same control series, but each investigating a different lymphohaematopoietic cancer. Brown et al, (1990) found a near null association between

glyphosate exposure and leukaemia among white males residing in the area (OR = 0.9; 95% CI 0.5–1.6). Among Iowa farmers reporting ever handling glyphosate, there was a slight non-statistically significant odds ratio for multiple myeloma (OR = 1.7; 95% CI 0.8–3.6) (Brown et al, 1993). Cantor et al, (1992) found an approximately null association between glyphosate exposure and NHL among males (OR 1.1; 95% CI 0.7–1.9).

The IWG reviewed a later study by De Roos et al, (2003) who used pooled data from three case-control studies of NHL conducted in the 1980s in Nebraska (Zahm et al, 1990), Iowa and Minnesota (Cantor et al, 1992), and Kansas (Hoar et al, 1986). Reported use of glyphosate as well as several other individual pesticides was associated with an increased risk of NHL. A total of 650 cases and 1,933 controls were included for the analysis of 47 pesticides. Reporting glyphosate exposure were 36 cases and 61 controls. After adjusting for other pesticide use, age, and study area, by two regression techniques, odds ratios of 2.1 (1.1–4.0) using logistic regression and 1.6 (0.9–2.8) using hierarchical regression were found.

In that regard, a later study by De Roos et al, (2005) where they reviewed the AHS cohort data is significant. They found no association between glyphosate and NHL. The authors noted that the aforementioned Midwest USA case control studies were retrospective in design and therefore potentially susceptible to recall bias as regards exposure reporting.

The cross-Canada case – control study

The IWG reviewed a report by McDuffie et al, (2001) who studied the association between NHL and exposure to specific pesticides in a multicentre population-based study with 517 cases and 1,506 controls among men of six Canadian provinces. The authors reported a slight, non-statistically significant increased risk for NHL from claimed glyphosate exposure, the OR being 1.26 (95% CI 0.87–1.80) for analysis adjusted for age and province, and 1.20 (95% CI 0.83–1.74) for analysis adjusted for age, province and high-risk exposures. The study also assessed the significance of different exposure durations. When stratified by greater than or less than two days of glyphosate exposure/year (< 2d/year), the values were 2.12 (95% CI 1.20–3.73) for >2d/year relative to those with < 2d/year (assigned OR of 1.0). The authors commented that although there was not a statistically significant finding for exposure to glyphosate per se, there was a dose-response relationship.

Case-control studies in Sweden

The IWG reviewed a study by Eriksson et al, (2008) who reported the results of a population-based case-control study of exposure to pesticides as a risk factor for NHL. Men and women aged 18–74 years living in Sweden were included from 1 December 1999 to 30 April 2002. In total, 910 (91%) cases and 1,016 (92%) controls participated. The authors found NHL associations with exposure to glyphosate. This exposure was reported by 29 cases and 18 controls, giving a reported odds ratio of 2.02 (95% CI 1.10–3.71) in a multivariate analysis. When restricted to a >10 year latency period the OR became 2.26 (95% CI 1.16–4.40). Odds ratios were also reported for lymphoma subtypes. For only two of the eight subtypes were odds ratios statistically significant; likely related to the small numbers. The IWG considered that this was a large study; that there was possible confounding from the use of other pesticides including MCPA, but this was controlled for in the analysis. Given the number of cases studied for glyphosate (29 cases and 18 controls) this study could hardly be considered as large. Twelve subjects were in a less than 10 days exposure group and 17 in a more than 10 days group. Therefore this study had limited power to detect an effect.

Other findings

In 2014 Schinasi and Leon reported their study of the association between NHL and occupational exposure to various agricultural pesticide chemical groups. Some findings on glyphosate were presented; for example the results from the studies by McDuffie et al, (2001), De Roos et al, (2005) and Eriksson et al, (2008) were given. This review included a series of meta-analyses, which they asserted showed consistent evidence of positive associations between NHL and carbamate insecticides, organophosphorus insecticides, lindane, and MCPA. As regards glyphosate (an “organophosphorus herbicide”), “in a handful of papers”, associations between pesticides and NHL subtypes were reported; B cell lymphoma was positively associated with phenoxy herbicides and glyphosate.

The Agricultural Health Study (AHS) cohort studies

These studies in Ohio and North Carolina involve a large cohort of private and commercial pesticide applicators (57,311 as at 2004–5). Several studies have been conducted using this cohort.

Alavanja et al, (2003) evaluated associations between specific pesticides and prostate cancer in the AHS. Glyphosate was listed as one of the pesticides with sufficient exposure data for analysis, but the findings for it were not listed, so that it has been assumed that no significant positive association was found with prostate cancer.

Flower et al, (2004) evaluated associations between pesticide application by parents and cancer among children born to Iowa participants in the AHS. There was no positive association between either maternal or paternal use of glyphosate and risk of childhood cancer.

De Roos et al, (2005) evaluated associations between glyphosate exposure and “all cancers” or any cancer site using the AHS cohort. This study did not show a significantly increased risk of NHL. In the group reportedly exposed to glyphosate, small, non-statistically significant relative risks of 1.2 (95% CI 0.7–1.9) adjusted for age (only) and 1.1 (95% CI 0.7–1.9) adjusted for age, demographic and lifestyle factors and other pesticide exposure were found for NHL, (De Roos 2005). There was no dose (exposure) response relationship.

De Roos et al, (2005) also found a non-statistically significant association between glyphosate exposure and multiple myeloma, with rate ratios (RR values) of 1.1 (95% CI 0.5–2.4) adjusted for age only, and 2.6 (95% CI 0.7–9.4) adjusted for age, demographic and lifestyle factors and other pesticides exposures. Such a finding had not previously been reported.

Comparisons were made between ever-exposed versus never-exposed groups, and between three equal sized groups (tertiles), formed by subdivision either on the basis of total days of exposure or intensity-weighted exposure days. In the intensity-weighted analysis of glyphosate and lung cancer, the relative risk for the highest tertile was only 0.6 (95% CI 0.3–1.0), for pancreatic cancer the RR for the highest tertile was 0.5, while for multiple myeloma the RR was 2.1, but the confidence interval was wide (0.6–7.0). None of these findings reached statistical significance at 95%. Regarding the whole group (ie ever used glyphosate), the RR for multiple myeloma was 1.1 (95% CI 0.5–2.4) adjusted for age only, and 2.6 (95% CI 0.7–9.4) adjusted for age, demographic and lifestyle factors and other pesticide exposures. Unremarkable, non-statistically significant results were found for the other cancer sites assessed.

Thus as regards this study, there was no evidence of a statistically significant positive association for any of the cancers for which data were reported (Mink et al, 2012). Furthermore De Roos et al, (2005) acknowledged in their paper that over 13,000 subjects were excluded from multivariate analyses because of missing data. In analyses of "ever" versus "never" exposed to glyphosate, the age-adjusted relative risk of multiple myeloma was 1.1. Lash (2007) assessed the study design and concluded that adjustment for confounders, which resulted in limiting the data set by 25% because of missing data on the adjustment variables, likely introduced selection bias, which was likely to have been in the direction away from the null (ie exaggerating any possible risk).

It is also known that multiple myeloma is often preceded by monoclonal gammopathy of undetermined significance (MGUS), a pre-malignant plasma cell disorder (Morgan et al, 2002). It is of interest to note that a decreased risk (albeit not statistically significant) of MGUS was observed in glyphosate applicators in the AHS.

Engel et al, (2005) evaluated breast cancer risk among wives of farmers in the AHS. No statistically significant association was found.

In an analysis of colorectal cancer and pesticide use, Lee et al, (2007) found no statistically significant association between glyphosate use and cancer of the colon or rectum.

Andreotti et al, (2009) reported no significant association of "ever" use (versus "never use") of glyphosate with pancreatic cancer among the combined group of AHS applicators and spouses (OR 1.1; 95% CI 0.6–1.07), nor was there evidence for a dose-response relationship.

Dennis et al, (2010) evaluated associations of 50 pesticides with cutaneous melanoma in the AHS cohort. Glyphosate was listed as one of the 22 pesticides on the enrolment questionnaire. The authors commented that none of these 22 pesticides was associated with melanoma.

None of the AHS cohort study analyses reported statistically significant positive findings for glyphosate exposure and total cancer or any site-specific cancer, in adults or children. In particular, the prospective AHS studies did not corroborate the positive association with NHL reported by the Swedish case-control studies. Analyses of increasing category of glyphosate exposure days and incidence of NHL produced rate ratios that were below the null value of 1.0 (De Roos et al, 2005 and Mink et al, 2012).

Discussion of review of epidemiological findings

In a review of glyphosate in 2006, the WHO observed that:
"widely used pesticides, like glyphosate, have recently become a focus of epidemiological research. In the past few years several epidemiological studies have been published that reported weak associations of glyphosate with lymphopoietic cancers, self-reported adverse reproductive outcomes and self-reported attention deficit hyperactivity disorder in children. However, the results of these studies do not meet generally accepted criteria from the epidemiology literature for determining causal relationships. Generally, the associations were rather weak and rarely statistically significant. Controlling for potential confounding factors, including other pesticides exposure, was not possible owing to limited available information and small numbers of subjects".

Whether or not there was any internal exposure or the extent of such exposure was not measured and, accordingly, a possible dose–response relationship could not be evaluated.

This seems a fair assessment of several of the studies regarding glyphosate and its formulations. De Roos et al, (2005) noted that the Midwest USA case control studies were retrospective in design and therefore potentially susceptible to recall bias as regards exposure reporting. Certainly a large prospective cohort study (such as that by De Roos et al, 2005) is much preferable to smaller case-control studies, the latter of which have much less statistical power to identify causal associations and are subject to more biases, including those regarding exposure assessment. Therefore much more weight should be given to the De Roos et al, (2005) cohort study than the much smaller De Roos et al, (2003) case-control study. In that regard, it is important to note that the cohort study found no association between glyphosate and NHL. There was, however, a small (non-statistically significant) increased risk of multiple myeloma in the 2005 study, but the point estimates of this risk may have been exaggerated. (Lash 2007.)

A re-analysis of some data from the De Roos et al, (2005) study has recently been undertaken, with a focus on multiple myeloma (Sorahan, 2015). Assessing the same data, Sorahan found no significant trends of multiple myeloma risk with reported cumulative days of glyphosate use, and unexceptional point estimates of risk for ever-use of glyphosate. This was irrespective of whether the analysis had made adjustment for a few basic variables (age and gender) or made adjustment for many other lifestyle factors or pesticide exposures; as long as data on all available pesticide applicators was used.

Sorahan (2015) argued that the elevated rate ratios (or relative risks) for multiple myeloma reported previously by Roos et al, (2005) arose from use of restricted data sets that, probably by chance, turned out to be unrepresentative. These restrictions were considered to be unnecessary and undesirable, as potentially informative data on the exposure or outcome under investigation were discarded. For example, it was asserted that there were a number of lost cases of multiple myeloma in the group of applicators who had never used glyphosate, because they were excluded by Roos et al, (2005) due to their not having data on for example use of alcohol, or smoking. These lost cases in the baseline category gave a false impression of elevated rates in ever-users. As a result Sorahan gave more weight to the point estimate of 1.1 as the RR (adjusted for age only) as opposed to the estimate of 2.6 as the RR for ever-use of glyphosate (adjusted for age, demographic and lifestyle factors, and other pesticides).

Mink et al, (2012) reviewed the epidemiological literature (and relevant methodological and biomonitoring studies) to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. Seven cohort studies and fourteen case-control studies examining a potential association between glyphosate and one or more cancer outcomes were subjected to a qualitative analysis.

The cohort studies were all based on analyses of participants or family members of the AHS cohort. Mink et al (2012), observed that none of the AHS cohort study analyses reported statistically significant positive findings for glyphosate exposure and total cancer or any site-specific cancer in adults or children. They found no consistent pattern of positive associations to suggest a causal relationship between human exposure to glyphosate and any cancer.

Overall, this 2012 review found no consistent pattern of positive associations between total cancer (in adults or children) or any site-specific cancer, and exposure to glyphosate. They suggested a cautious interpretation of the few positive associations reported, and concluded that the epidemiological data, when considered together, did not support a causal association between glyphosate exposure and cancer.

Similarly, the latest report of BfR (2015) to the European Food Safety Authority (EFSA)[1] based on the evaluation of over 30 epidemiological studies came to the overall assessment that there is no validated or significant relationship between exposure to glyphosate and an increased risk of NHL or other types of cancer.

A recent peer review by EFSA[2] (2015) essentially confirmed the conclusions in their re-evaluation of glyphosate. They noted that 10 cohort studies (which included the AHS, the largest series of prospective studies to date), found that glyphosate did not cause different types of cancer and did not increase risk of all cancers combined. (As noted earlier, the findings for NHL were negative in the AHS cohort.) Similarly nine case-control studies did not indicate an increased risk of carcinogenicity, or did not have sufficient power to assess this. With regard to NHL, the case-control studies exhibited poor consistency in their results and small numbers of cases limiting the statistical significance of findings in some studies. As noted above, case-control studies have less power, are more subject to various biases, and are less effective at assessing actual exposure levels than are cohort studies. EFSA concluded that there is very limited evidence for an association between glyphosate exposure and the occurrence of NHL.

Cancer in experimental animals

Mice studies

Glyphosate was tested in female and male mice by dietary administration in two studies. A skin application in one initiation-promotion study was conducted with male mice.

The IWG found that in male CD-1 mice, glyphosate induced a positive trend in the incidence of a rare tumour, renal tubule carcinoma. A second study reported a positive trend for hemangiosarcoma in male mice. A glyphosate formulation promoted skin tumours in an initiation-promotion study in mice.

The IWG noted there was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in male CD-1 mice in a glyphosate feeding study (0, 1,000, 5,000, or 30,000 ppm glyphosate *ad libitum* for 24 months). (This study was conducted prior to the institution of GLP.) The study was submitted to the US EPA which requested that a pathology working group (PWG) be convened to evaluate the renal tumours. In this second evaluation, the PWG found that the incidence of adenoma was not statistically significant but the incidence of carcinoma and the incidence of adenoma and carcinoma (combined) were significant. The IWG considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate.

However, this finding is at variance with the US EPA (1993) which reported in their glyphosate review that the occurrence of these adenomas was spontaneous rather than compound-induced because the incidence of renal tubular adenomas in males was not statistically significantly different when compared with the concurrent controls. An independent group of pathologists and biometricians also conducted extensive evaluations of these adenomas and reached the same conclusion. The US EPA concluded glyphosate was not considered to be carcinogenic in this study.

[1] The BfR (2015) report addressing the carcinogenicity of glyphosate is a report of Germany specifically, as Germany was the lead member state for the EFSA review of glyphosate.

[2] EFSA accepted the conclusion relating to glyphosate and cancer (including NHL), with one dissenting member state.

The IWG reviewed a second feeding study reported to the FAO/WHO Joint Meeting on Pesticide Residues (JMPR), and found there was a significant positive trend in the incidence of hemangiosarcoma in male CD-1 mice. Groups of 50 female and male mice were fed diets containing glyphosate at a concentration that was adjusted weekly for the first 13 weeks and every four weeks thereafter to give doses of 0, 100, 300, or 1,000 mg/kg body weight, *ad libitum* for 104 weeks.

In contrast JMPR (WHO 2006) found that owing to the lack of a dose-response relationship, the lack of statistical significance and the fact that the incidences recorded in this study fell within the historical ranges for controls, these changes were not considered to be caused by administration of glyphosate. They concluded administration of glyphosate to CD-1 mice for 104 weeks produced no signs of carcinogenic potential at any dose.

Initiation-promotion

The IWG found that in a study involving 20 male Swiss mice which had a glyphosate based formulation applied to their skin, it appeared to be a tumour promoter, but they concluded that this was an inadequate study because its design was poor, with short duration of treatment, no solvent controls, small numbers of animals, and a lack of histopathological examination.

However the BfR (2015) considered that generally testing of formulations should not be used for the toxicological evaluation of active substances because co-formulants may extensively alter the outcome. The BfR deemed that this IWG finding was not considered by the institutions in the EU to be evidence for the carcinogenic properties of glyphosate per se.

Review articles – mice studies

The IWG noted that Griem et al, (2015) had published a review article which included discussion of five long-term glyphosate feeding studies in mice. Two of the studies were discussed in the IARC monograph. The working group summarised the other three studies but claimed that it was unable to fully evaluate the other three studies because of the limited experimental data provided in the review article and supplemental information.

Griem et al, (2015) noted that the five mouse studies that they reviewed were submitted to support glyphosate renewal in the EU. They considered that all but the oldest study were reliable without restriction and were performed under conditions of GLP and OECD protocols.

During the EFSA peer-review process for the renewal of the approval of glyphosate, EFSA also received a complementary mandate from the EU to consider the findings by IARC regarding the potential carcinogenicity of glyphosate (EFSA 2015).

The EFSA peer review (2015) also evaluated the five mice studies. Only one of these suggested a potential carcinogenic effect, as evidenced by a statistically significant increased evidence of malignant lymphomas at the top dose level of 1,460 mg/kg/day. However the validity of the study was questioned, due to the occurrence of viral infection which could have influenced survival rates and the incidence of lymphomas. No carcinogenic effects were observed at the highest dose levels in any of the other studies. The IWG evaluated two of these studies and asserted positive trends in males for renal tubular carcinomas in one study and for hemangiosarcoma in the other. However EFSA took a weight-of-evidence approach; with considerations including the statistical significance being only found in trend analysis but not in pairwise comparison, lack of consistency in multiple

animal studies, the fact that the slightly increased incidences only occurred at doses higher than those recommended for the oral route in carcinogenicity studies, incidences in test animals generally being within the historical range for control groups, and the lack of pre-neoplastic lesions.

Rat studies

Five feeding studies in rats and two drinking water studies with glyphosate were reviewed by the IWG.

Drinking water

One study in Sprague-Dawley rats was considered by the IWG to be inadequate for evaluation because of its short exposure duration.

A glyphosate containing drinking water study with Wistar rats did not show any significant increase in tumour incidence.

Dietary administration

Two studies in Sprague-Dawley rats showed a significant increase in the incidence of pancreatic islet cell adenoma in male rats. One of these studies also showed a significant positive trend in the incidence of hepatocellular adenoma in males and of the thyroid C-cell adenoma in females. However two studies (one in Sprague-Dawley and one in Wistar rats) found no significant increase in tumour incidence at any site.

The IWG reviewed a chronic feeding study (provided by the US EPA) in which groups of 60 female and male Sprague Dawley rats were given diets containing glyphosate at a concentration of 0, 2,000, 8,000 or 20,000 ppm *ad libitum* for 24 months. In males at the lowest dose, there was a statistically significant increase in the incidence of pancreatic islet cell adenoma compared with controls. Additional analyses by the US EPA revealed a statistically significant higher incidence of pancreatic islet cell carcinoma in males at the lowest and highest doses compared with controls: lowest dose, 8/45 (18%); intermediate dose, 5/49 (10%); highest dose, 7/48 (15%) versus controls, 1/43 (2%). The range for historical controls for pancreatic cancer islet cell carcinoma reported in males at this laboratory was 1.8–8.5%. The IWG concluded that this study demonstrated a significant increase in the incidence of pancreatic islet cell adenoma in male rats.

However the US EPA (1993) had concluded that:
"these adenomas were not treatment-related and glyphosate was not considered to be carcinogenic in this study. With respect to pancreatic islet cells adenomas, there was no statistically significant positive dose-related trend in their occurrence; there was no progression to carcinomas; and the incidence of pancreatic hyperplasia (non-neoplastic lesion) was not dose-related. With respect to hepatocellular adenomas, the increased incidence of these neoplasms was not statistically significant in comparison with the controls; the incidence was within the historical control range; there was no progression to carcinomas; and the incidence of hyperplasia was not compound-related. With respect to thyroid C-cell adenomas, there was no statistically significant dose-related trend in their occurrence; the increased incidence was not statistically significant; there was no progression to carcinomas; and there was no significant dose-related increase in severity or incidence of hyperplasia in either sex".

Also, in the JMPR (WHO 2006) review of this study they reported:
"The historical-control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of studies reported recently in the literature revealed a prevalence of 0–17% in control males with several values being ≥8%. More importantly, the incidences of islet cell adenomas clearly did not follow a dose-related trend in the treated groups of males. There was no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a male in the control group, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support the conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to administration of glyphosate".

Review articles – rat studies

The IWG noted that Griem et al, (2015) had published a review article containing assessments of nine long-term glyphosate feeding studies in rats. Five of these studies were reviewed by the IWG. The remaining four studies were not evaluated by the IWG which stated that there was limited experimental data provided in the review article. These four studies had been submitted to various organisations for registration purposes. There was no evidence of a carcinogenic effect related to glyphosate treatment.

Its long-term toxicity and carcinogenicity was assessed in nine rat studies. The EFSA peer review concluded that no significant increase in tumour incidence was apparent. Three of these studies were not evaluated by the IARC panel. In two studies, increased incidences of pancreatic islet cell adenomas were found but were not dose-related. EFSA also noted that the significance of these findings depended on the statistical analysis: using a pairwise comparison (as planned for in the study protocol) no significant effect is observed, whereas a trend analysis performed by the IWG identified significant changes. EFSA noted that deviations from the statistical analysis used by the study authors should be limited and properly justified.

Other relevant data

The IWG group noted that soil microbes degrade glyphosate to aminomethylphosphonic acid (AMPA). Blood AMPA detection after glyphosate poisoning incidents suggests intestinal microbial metabolism in humans.

Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Neimann et al, (2015) published a critical review and comparison of data obtained in a total of seven studies from Europe and the US. They concluded that no health concern was revealed because the resulting exposure estimates were several magnitudes lower than the acceptable daily intake (ADI) or the acceptable operator exposure level (AOEL).

The measured internal exposure was clearly below the worst-case predictions made in the evaluation of glyphosate as performed for the renewal of its approval within the European Union.

This is consistent with the risk-based approach that regulatory agencies use when considering realistic dosages and real-life conditions. Those studies show that farmers and farm families are exposed to significantly lower doses of the herbicide than some model estimates would suggest.

It is also in keeping with an earlier review (Williams et al, 2000) of the animal data, in which dose levels from animal toxicity tests were compared to conservative, upper-limit estimates

of human exposure to glyphosate, to give a margin of exposure (MOE) value. MOE analyses compare the lowest NOAELs determined from animal studies to worst-case levels of human exposure; with MOEs of greater than 100 indicating confidence that no adverse health effects would occur. These authors found in their review that the MOEs for worst-case chronic exposure to glyphosate ranged from 3,370 to 5,420, and concluded that “under present and expected conditions of use, Roundup herbicide does not pose a health risk to humans”.

Genotoxicity

The IWG claimed that there is strong evidence that glyphosate is genotoxic. They tabulated numerous reports of tests relating to the genotoxicity of glyphosate and its formulations, with some showing a positive association, and some a negative association.

The evaluation of the large volume of genotoxicity data available requires consideration of assay system validation, test system species used, relevance of the endpoint to heritable mutation, reproducibility and consistency of effects and dose-response, and relationship of effects to toxicity. The guidelines for genetic toxicology tests developed for the OECD are a pre-eminent source of internationally agreed guidelines.

There were often inconsistent results reported (both positive and negative) from the same test systems in different laboratories. The relevance of many of the assays in test system species (fish, oysters, insects, snails, worms and caimans) which have never been validated for the assessment of genotoxicity in humans for regulatory purposes, is questionable. Additionally the *intraperitoneal* route of exposure for many of the mammalian *in vivo* studies is not appropriate since it does not reflect normal human exposure, with doses exceeding occupational exposure by orders of magnitude.

Kier and Kirkland (2013) published a review of the genotoxicity of glyphosate and glyphosate-based formulations. This review concluded that there was a strong weight of evidence that glyphosate and its formulations are predominantly negative in well-conducted, core bacterial reversion and *in vivo* mammalian micronucleus and chromosomal aberration assays. Although some positive results for glyphosate and glyphosate-based formulations were reported in DNA damage assays, and for the micronucleus endpoint for formulations in non-mammalian studies, the positive results were associated with high dose levels and/or overt toxic effects. The preponderance of negative results in core assays supports the conclusion that reports of DNA damage or non-mammalian micronucleus effects are likely to be secondary to cytotoxicity rather than indicative of DNA-reactive mechanisms.

The IWG found that glyphosate and glyphosate formulations induced DNA and chromosomal damage in mammals, and in human and animal cells *in vitro*. They referred to one study (Bolognesi, 2009) reporting increases in blood markers of chromosomal damage (micronuclei) in residents of several communities after spraying of glyphosate formulations, to support this contention of genotoxicity.

However, the authors of the Bolognesi (2009) study concluded that overall, data suggesting that genotoxic damage (as evidenced by the micronuclei test) associated with glyphosate spraying for control of illicit crops is slim, and any such effect appears to be transient. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for coca and poppy eradication is low. The attribution of a genotoxic effect due to glyphosate exposure rather than a multitude of other demographic and environmental causes seems rather tenuous given the uncertainty of actual exposure.

In a recent communication, EFSA summarised their appraisal of the genotoxicity studies. *In vitro* tests of mutagenicity gave consistently negative results. *In vitro* tests of mammalian chromosome aberration (all of those which had been performed under GLP conditions) were also negative. Positive results were found in some published *in vitro* studies of chromosomal aberrations, but these were not confirmed by *in vivo* studies addressing the appropriate endpoints, such as the micronucleus test.

As regards *in vivo* tests, all studies conducted according to internationally validated guidelines for good laboratory practice (GLP) and some non-GLP published studies gave negative results. Two non-GLP studies were positive in mice treated intraperitoneally, but at levels close to or above the LD_{50}[3] (possibly suggestive that this is a secondary effect), and one study had major flaws. No genotoxic effects on germ cells have been detected in rats or mice treated orally at dose levels up to 2,000 mg/kg/day (the maximum dose level recommended for such studies). EFSA concluded that, considering the weight of evidence, glyphosate is unlikely to be genotoxic *in vivo*.

As regards glyphosate-based commercial formulations, a number of formulations with unknown composition have given positive results when tested *in vitro* and *in vivo*. However some of the test systems are not validated and/or interpretation is difficult due to possible confounding, such as cytotoxicity, specific organ toxicity or unclear relevance to humans (such as tests in fish, amphibians, or invertebrates). Some of the co-formulants (such as polyethoxylated tallow amine (often abbreviated to POEA)) may be more systemically toxic than glyphosate. However EFSA concluded that the genotoxic potential of such complete formulations should be further assessed.

Kier (2015) reviewed genotoxicity biomonitoring studies of glyphosate-based formulations. He found that most of the human biomonitoring studies were not informative because there was either a very low frequency of exposure to glyphosate formulations or exposure to a large number of pesticides in addition to glyphosate without analysis of specific pesticide effects. One pesticide sprayer biomonitoring study indicated there was no statistically significant relationship between frequency of exposure to glyphosate formulations reported for the last spraying season and oxidative DNA damage. There were three studies of human populations in regions of glyphosate formulation aerial spraying. One study found increases for the cytokinesis-block micronucleus endpoint but these increases did not show statistically significant associations with self-reported spray exposure and were not consistent with application rates. A second study found increases for the blood cell comet endpoint at high exposures causing toxicity. However, a follow-up to this study two years after spraying did not indicate chromosomal effects.

Oxidative stress

The IWG found that glyphosate, glyphosate formulations, and AMPA induced oxidative stress in rodents and *in vitro*.

Oxidative stress was only found in one study in rats administered intraperitoneal glyphosate active ingredient (Astiz et al, 2009), and in numerous studies using *intraperitoneal* administration or *in vitro* methods with glyphosate-based formulations. However, these studies used doses that exceeded normal occupational exposures by orders of magnitude and the *intraperitoneal* route of exposure is not appropriate for evaluating human exposure. Glyphosate has low gastrointestinal absorption and poor dermal absorption. It therefore

[3] LD_{50} is the dose of the substance required (usually expressed in relation to body weight) that is estimated to kill 50% of the test population.

seems unlikely that human exposure would produce the sort of tissue levels used in the oxidative stress tests. There was also some inconsistency in results.

Most effects were seen when whole glyphosate formulations were tested. EFSA considered that generally testing of formulations should not be used for the toxicological evaluation of active substances because co-formulants may extensively alter the outcome. Thus any effects found cannot then be attributed to the glyphosate active ingredient present.

Discussion

The IARC WG (IWG) classified glyphosate as "probably carcinogenic to humans (Group 2A)" as the overall evaluation.
As set out in their evaluation section, this was based on:

- "*limited evidence*" in humans for the carcinogenicity of glyphosate, and
- "*sufficient evidence*" in experimental animals for carcinogenicity of glyphosate.

The rationale identifies that the IWG also notes mechanistic and other relevant data in support of the conclusion; in particular the IWG cites "strong evidence" that glyphosate can operate by two key characteristics of known human carcinogens, namely genotoxicity and oxidative stress.

This discussion section of the report will consider each of these sources of evidence in turn as contributing factors to the IWG's overall evaluation.

Human epidemiological evidence

The key cited studies in support of the "limited evidence" in humans for carcinogenicity of glyphosate consisted of three case-control investigations. The odds ratios (OR) for cases of NHL and glyphosate exposures are summarised in the following table.

Odds ratios (OR) for cases of NHL and glyphosate exposures

Study area	OR[1] and 95% CI[2]	Study reference
Midwest, USA	2.1 (1.1–4.0) [logistic regression] 1.6 (0.9–2.8) [hierarchical regression]	De Roos et al, 2003
Canada	1.26 (0.87–1.8) 1.20 (0.83–1.74) [adjusted for medical variables]	McDuffie et al, 2001
Sweden	2.02 (1.1–3.71) [univariate] 1.51 (0.77–2.94) [multivariate]	Erikson et al, 2008

1. OR is the odds ratio of outcome of interest between the relevant case group and the reference or control group.
2. The 95% CI are the confidence intervals round the OR representing the limits within which there is 95% confidence that the true value falls.

The first important observation is that depending on the statistical tests used only two studies (Midwest USA and Sweden) show OR values indicating statistical significance at the 95% level. In the Midwest USA, however, this is only true using logistic regression, while in the Swedish study only the univariate analysis showed statistical significance.

Some case control studies assessed data using dose (exposure)/response or intensity/response to determine whether or not there is a trend to a higher incidence of tumours in persons categorised as having higher exposures to glyphosate. While these approaches are desirable, the criteria of exposure seem low. For one case-control study, the criterion for high or lower glyphosate use was greater than or less than two days of glyphosate use/year (McDuffie et al, 2001), whereas in another the criterion was greater than or less than 10 days of glyphosate use/year (Eriksson et al, 2008). While the distribution of use category was not given in either study, 2–10 days of use per year seems a low benchmark for exposure comparisons. The direct glyphosate exposure findings with respect to NHL was not significant in the McDuffie et al, 2001 study, but they reported a dose response based on this dose comparison and quoted the OR for exposure >2 day/year as 2.12 (95% CI 1.20–3.73).

The direct glyphosate exposure findings with respect to NHL were significant in the Swedish study using univariate evaluation, and the effect of dose-response in the Swedish study appears to only be statistically significant using this approach (considering the data presented in the IARC Monograph in Table 2.2, p23) which reported a higher OR for “heavy” users (>10 days/year) of 2.36 (95% CI 1.04–5.37). It is noteworthy that the paper reports the highest OR, 2.81 (95% CI 1.27–6.22), for the association between exposure to MCPA and NHL. This may be the explanation for the difference between the results using univariate and multivariate evaluation. When considering the latency period, >10 years exposure to glyphosate had an OR of 2.26 (95% CI 1.16–4.4) in comparison to ≤ 10 years with an OR of 1.11 (95% CI 0.24–5.08), but these findings may be confounded by exposure to MCPA or other phenoxy herbicide exposures. There could be residual confounding from MCPA exposure if the participants under-reported earlier MCPA exposure. The apparent increased risk with latency for glyphosate exposure could be because participants who had sprayed pesticides for longer were more likely to have used the phenoxy herbicides (including MCPA) earlier in their working lives.

The AHS cohort study (De Roos, et al, 2005) had a more detailed assessment at different exposure intensities as they used cumulative lifetime days of use and an intensity measure (years of use x days/year x estimated exposure level). The data (presented in Table 2.1 of the IARC Monograph on p12) for this cohort study showed no statistically significant difference for the trend to increased exposure with exposure bands at 0–20, 21–56 and 57–2,678 cumulative days of exposure, despite the higher exposure levels in comparison to the case-control studies.

It is important in these circumstances to consider the overall data set. Rather than only highlighting the three case-control studies which identified a marginally statistically significant association between reported glyphosate use and NHL, the overall assessment needs to take into account other studies which did not demonstrate such an association. Also, it is particularly important to note the lack of significant finding in a large cohort study (the AHS) where the potential for recall bias is greatly reduced and should therefore be given greater weight than the case control studies. Cohort studies are generally considered more reliable than case-control studies, because the population is defined and the exposure parameters and the potential confounding exposures and lifestyle factors are established prior to the adverse outcome of interest so that the potential for recall bias is less likely.

Given the lack of confirmation of the small number of positive findings from case-control studies in the more powerful cohort study, the epidemiological support for the conclusion "limited evidence" in humans is not convincing.

Experimental animal studies

The key cited studies in support of the "sufficient evidence" in experimental animals for carcinogenicity of glyphosate consisted of three studies in mice. These comprised one oral study demonstrating a positive trend for increased incidence of renal tubule carcinoma, one oral study in mice demonstrating a positive trend for increased incidence of hemangiosarcoma; and a supporting skin study demonstrating tumour promotion using a glyphosate formulation. In addition, one rat study demonstrated an increased incidence of pancreatic islet cell adenomas.

In assessing these data, the IWG used different statistical tests to those in the original analysis (trend analysis rather than a pairwise comparison against controls). The original studies were designed with the intention to assess statistical significance by means of a pairwise comparison between the test and control groups, so use of the trend assessment by IARC to assess these data requires justification. IARC's use of the trend assessment gave a positive response, but in none of the studies are the positive effects statistically significant using the original statistical approaches. Also, the IWG did not take into account the generally accepted assessment of the same data by international panels of experts, which took into account additional historical incidence data for hepatocellular adenomas in the rats and the presence of a viral infection in the mouse study which could have influence survival rates and the incidence of lymphomas.

The promotion study using a glyphosate-based formulation should not be used as support for the carcinogenicity of glyphosate per se, since the test substance contains other components which might influence the outcome.

The IWG did not evaluate some other studies which have been used by other regulators. These did not support the view that exposure to glyphosate in long-term feeding studies was associated with an increase in tumours at any sites. While the IWG approach is consistent with the IARC pre-amble and policy on the selection of study data, in the current circumstances this attributes inappropriate weight to the three studies which IWG considered and for which their analysis found an increase in tumours. Firstly because other studies which other reputable bodies found to be negative were not considered, and secondly because the reasons why the above findings were not relied upon by other assessments were not taken into account by the IWG. In particular a lack of consistency (dose-response) in multiple studies, slight increases in incidence at the maximum tested dose only, or incidences within the historical control range.

Taking into account that the positive findings cited by the IWG were not assessed as evidence of a carcinogenic effect in the view of other reputable bodies, and that the total data set of long-term carcinogenicity bioassays were consistently negative, it is concluded that the overall weight of evidence does not indicate that glyphosate is carcinogenic.

Mechanism of action

The IWG cites what is described as "strong evidence" that glyphosate can operate by two key characteristics of known human carcinogens – genotoxicity and oxidative stress.
The studies used in support of this conclusion were primarily *in vitro* mammalian cell studies. In such studies the mammalian cells are directly exposed to the test substance (glyphosate or a glyphosate-based formulation) at high concentrations which would not be reasonably achieved in an *in vivo* exposure whether in animals or humans. All studies done according to internationally validated guidelines gave negative results, while studies using unvalidated

test method/species, or with glyphosate-containing formulations or using high *intraperitoneal* doses are inappropriate for assessment of genotoxicity to humans.

Other supporting evidence for this conclusion included DNA damage and micronuclei in various populations allegedly exposed to glyphosate from sprays. Attributing the effects found to the exposure to glyphosate is questionable when the exposure, if any, was to glyphosate-based formulations and unidentified demographic, geographical or lifestyle factors that could be responsible for the DNA damage.

In relation to oxidative stress this was only found in one study in rats administered *intraperitoneal* glyphosate active ingredient (Astiz et al, 2009), and in numerous studies using *intraperitoneal* administration or *in vitro* methods with glyphosate-based formulations. The *intraperitoneal* route of administration is not considered relevant to human exposures. Glyphosate has low gastrointestinal absorption and poor dermal absorption. There was also some inconsistency in results. So the evidence for glyphosate causing oxidative stress is considered weak.

Conclusion

The overall conclusion is that – based on a weight of evidence approach, taking into account the quality and reliability of the available data – glyphosate is unlikely to be genotoxic or carcinogenic to humans and does not require classification under HSNO as a carcinogen or mutagen.

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Australian Pesticides and Veterinary Medicines Authority (APVMA)

Regulatory position: consideration of the evidence for a formal reconsideration of glyphosate

September 2016

The APVMA's previous assessments of glyphosate

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has completed several scientific assessments of glyphosate since it was first registered:

Final regulatory position: Consideration of the evidence for a formal reconsideration of glyphosate – March 2017

Proposed regulatory position report – September 2016

Review of IARC Monograph 112 (Glyphosate): Tier 1 – September 2016

Review of IARC Monograph 112 (Glyphosate): Tier 2 – September 2016

1996 Reconsideration of Glyphosate

The APVMA's Regulatory Position Report – March 2017

The APVMA evaluated the International Agency for Research on Cancer (IARC) Monograph 112 (Glyphosate) in 2016. We considered the IARC report and other up-to-date scientific publications to decide whether a formal reconsideration of glyphosate was required. The APVMA concluded that glyphosate does not pose a carcinogenic risk to humans and that there was no reason to commence a formal reconsideration.

We invited comments from the public about the proposed regulatory position on glyphosate and assessed the comments received on this report. No scientific evidence relating to the potential carcinogenicity of glyphosate not already considered by the APVMA was submitted during the public consultation. You can read the full assessment in the Final regulatory position report.

Assessment of the 2016 IARC report by the APVMA

The APVMA conducted a weight-of-evidence[1] evaluation, which included a review of the IARC monograph completed by the Australian Commonwealth Department of Health. The review by the Department of Health was conducted in 2 phases. The first phase (Tier 1) identified studies relied on by IARC, which should be reviewed in more detail. The second phase (Tier 2) involved a detailed assessment of those studies. The result of these assessments is described in the Final regulatory position report.

We keep a close watch on new scientific studies that may indicate whether this position should be revised. A full list of peer-reviewed scientific articles about glyphosate considered by the APVMA is available for download from our website.

[1] In the weight-of-evidence assessment used by regulators, relevant observations are validated because different investigators reproduce them independently. A weight-of-evidence assessment considers both the number of studies reporting a particular conclusion and the quality of the study design and data analysis. A strength-of-evidence assessment can be based on a single study, even if the study protocol has limitations or does not comply with internationally accepted regulatory protocols, or if the results are not consistent with observations made in other well-designed studies. Regulators do not use strength-of-evidence assessments.

URL: https://www.apvma.gov.au/resources/chemicals-news/glyphosate/apvmas-previous-assessments-glyphosate
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SEPTEMBER 2016

Regulatory position: consideration of the evidence for a formal reconsideration of glyphosate

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FOREWORD

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. Its statutory powers are provided in the Agvet Codes scheduled to the *Agricultural and Veterinary Chemicals Code Act 1994*.

The APVMA has legislated powers to reconsider the approval of an active constituent, registration of a chemical product or approval of a label at any time after it has been registered. The reconsideration process is outlined in sections 29 to 34 of Part 2, Division 4 of the Agvet Codes.

A reconsideration may be initiated when new research or evidence raises concerns about the use or safety of a particular chemical, a product containing that chemical, or its label. The scope of each reconsideration can cover a range of areas including human health (toxicology, public health, occupational health and safety), the environment (environmental fate and ecotoxicology), residues and trade, chemistry, efficacy or target crop/animal safety. However, the scope of each reconsideration is determined on a case-by-case reflecting the specific issues raised by the new research or evidence.

The reconsideration process (illustrated in Figure 1) includes a call for information from a variety of sources, a review of that information and, following public consultation, a decision about the future use of the chemical or product. The information and technical data required by the APVMA to review the safety of both new and existing chemical products must be generated according to scientific principles. The APVMA conducts science and evidence-based risk analysis with respect to the matters of concern, analysing all the relevant information and data available.

When the APVMA receives or is made aware of a significant new piece of information that questions the safety (to target animals, humans or the environment) or efficacy of a registered chemical, the APVMA assesses the new information to determine whether a formal reconsideration of that chemical and/or products containing that chemical should be initiated.

In undertaking this process, the APVMA works in close cooperation with external experts including the Department of Health, Food Standards Australia New Zealand (FSANZ), the Department of the Environment and Energy and the state departments of agriculture, as well as other expert advisers as appropriate.

This document sets out the nomination assessment process for glyphosate that was initiated following the classification of glyphosate as ‘probably carcinogenic to humans’ by the International Agency for Research on Cancer (IARC) in March 2015.

This document and the technical reports relating to glyphosate are available from the APVMA website at www.apvma.gov.au. The technical reports are:

- Review of IARC Monograph 112 (Glyphosate): Tier 1
- Review of IARC Monograph 112 (Glyphosate): Tier 2.

1. Nomination	**Nomination.** Any person or group (including the APVMA and its partner agencies) may nominate an active constituent, product or label for reconsideration. The APVMA assesses the supporting scientific information and determines whether a reconsideration is warranted. Not all nominations will proceed to a formal reconsideration—there are other regulatory pathways available that may more efficiently address concerns. **The APVMA nominated glyphosate for reconsideration following the classification of glyphosate as 'probably carcinogenic to humans' by the International Agency for Research on Cancer in 2015**.	
2. Prioritisation	**Prioritisation.** The APVMA (with input from its advisory agencies) determines the priority of the reconsideration.	
3. Scoping and work plan	**Scope.** A scope document is prepared that outlines the areas of concern to be reconsidered. From 1 July 2015 the APVMA is legislatively required to publish a **work plan** for all reconsiderations to provide predictability about the timeframe for the reconsideration.	
4. Notice of reconsideration	**Notice of reconsideration.** To begin the reconsideration, the APVMA gives each holder a written Notice of Reconsideration that invites the holder to make a written submission to the APVMA. The holder is legally obliged to submit any available data relevant to the scope of the reconsideration. The APVMA supplements the submitted data with data available in the public domain (eg peer-reviewed scientific journal articles or international assessment reports).	
5. Assessment	**Toxicology assessment.** The toxicology assessment characterises all of the adverse health effects that a compound may cause and establishes health-based guidance values (also known as public health standards) for exposure to the chemical. The toxicology assessment recommends first aid directions, poisons scheduling and any necessary warnings for product labels.	**Environment risk assessment.** Where indicated in the scope of the reconsideration, an environmental risk assessment is conducted. The environmental risk assessment may include an evaluation of environmental fate and ecotoxicology.
	Human exposure assessment. The Toxicology assessment findings are used in the Occupational Health and Safety (human exposure) assessment. This assessment recommends safety directions, re-entry periods and restraints for all the uses supported by the assessment.	**Residues and dietary exposure risk assessment (includes trade).** The available residues data are used in the residues and dietary exposure risk assessment. This assessment recommends withholding periods, MRLs and restraints for all use patterns supported by this assessment. It also considers the potential trade risks arising from all the supported uses of products.
	Efficacy: If included in the scope of the review efficacy assessments are conducted by the APVMA.	

6. Draft regulatory measure	**Interim Regulatory Action.** At any time during a reconsideration, the APVMA may take regulatory action to mitigate any risks identified in relation to the use of a chemical. The aim of any such action is to protect human health or the environment (or both) while a final decision is being reached through the reconsideration process. **Proposed Regulatory Decision.** The APVMA considers all the assessments and develops draft recommendations for the reconsideration which summarise the results of the assessment, identified risks, risk mitigation measures, proposed review findings and draft regulatory decisions. The PRD and the component assessment reports are released for public consultation.
7. Consultation	**Consultation.** Further data or information may be submitted to the APVMA from a range of stakeholders including holders, users of the chemicals, peak industry bodies, interest groups, non-government organisations, state and territory governments or the public. Usually a 3-month public consultation period is conducted following publication of the PRD. Any further data or information submitted during consultation will be taken into consideration before making the final regulatory decision.
8. Regulatory decision	**Regulatory decision**. After the public consultation period has closed, the APVMA assesses all the comments received and amends the assessment, review findings and the proposed regulatory measures as necessary. We then make the final regulatory decision. There are three possible regulatory outcomes from a reconsideration: • affirm the approvals or registrations • vary the relevant particulars or conditions and affirm the approval or registration, or • suspend or cancel the approval or registration. The APVMA will affirm the approval or registration only if satisfied that it meets all statutory safety, efficacy, trade and labelling criteria and also complies with all requirements in the regulations If the active constituent, product or label does not meet the criteria as described above, the APVMA will examine whether the relevant particulars or conditions of the approval or registration can be varied so that the criteria can be met. This may include varying the instructions for use on the label. If product registrations or label approvals are cancelled the APVMA will examine whether a phase out period for dealing with or using cancelled products or products bearing cancelled labels is appropriate. Additional instructions may be applied during phase out. If a phase out period is not appropriate then recall action may be required.
END OF RECONSIDERATION (regulatory decision)	
9. Implementation	**Implementation.** Once the decision is made to affirm, cancel or vary conditions of registrations or approvals the APVMA will send written Notices to the holders of registrations and approvals and publish Notices of affirmation, variation of conditions, and cancellation of actives, products or label approvals. These Notices will include brief statements of the reasons for the actions, relevant particulars for any affirmed approvals or registrations and any appropriate instructions of use or phase-out periods for cancellations. The APVMA will publish details of any applicable phase out periods if any approvals of actives, registration of products or label approvals are cancelled. The maximum legislated phase out period is 12-months.

Figure 1: The chemical reconsideration process

SUBMISSIONS FROM THE PUBLIC ARE INVITED

This draft regulatory position report:

- outlines the APVMA chemical reconsideration process
- advises interested parties how to respond to the assessment
- summarises the nomination assessment methodology and outcomes
- outlines the proposed regulatory position to be taken in relation to the nomination for reconsideration of glyphosate and products containing glyphosate.

The APVMA invites persons and organisations to submit their comments and suggestions on this nomination assessment report directly to the APVMA. Comments on this report will be assessed by the APVMA before the report is finalised and the final regulatory position report is published.

Submissions can be sent to:

Director, Chemical Review
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182

KINGSTON ACT 2604

Telephone: +61 2 6210 4749
Facsimile: +61 2 6210 4776
Email: chemicalreview@apvma.gov.au
Website: www.apvma.gov.au.

Preparing your comments for submission

Please limit any comments you have to the scientific justification for the proposed regulatory position on glyphosate.

When making your comments:

- clearly identify the issue and clearly state your point of view
- give reasons for your comments, supporting them with relevant scientific information and indicating the source of the information you have used.

Please try to structure your comments in point form, referring each point to the relevant section in the regulatory position report. This will help the APVMA assemble and analyse all of the comments it receives.

When making a submission, please include:

- contact name

- company name or group name
- postal address
- email address (if available)
- the date you made the submission.

Finally, tell us whether the APVMA can quote your comments in part or full.

Please note that, subject to the *Freedom of Information Act 1982*, the *Privacy Act 1988* and the Agvet Code, all submissions received may be made publicly available. They may be listed or referred to in any papers or reports prepared on this subject matter.

The APVMA reserves the right to reveal the identity of a respondent unless a request for anonymity accompanies the submission. If no request for anonymity is made, the respondent will be taken to have consented to the disclosure of their identity for the purposes of Information Privacy Principle 11 of the *Privacy Act 1988*.

The contents of any submission will not be treated as confidential or confidential commercial information unless they are marked as such and the respondent has provided justification for the material to be classified as confidential or confidential commercial information in accordance with the *Freedom of Information Act 1982* or the Agvet Code, as the case may be.

THE CLOSING DATE FOR SUBMISSIONS IS FRIDAY 30 DECEMBER 2016.

EXECUTIVE SUMMARY

Introduction

Glyphosate is a broad-spectrum, non-selective, post-emergent, systemic herbicide that kills or suppresses all plant types (except those genetically modified to be resistant to glyphosate) and is commonly used to control annual and perennial broadleaf and grassy weeds in various agricultural and non-agricultural settings. Glyphosate acts by disrupting the shikimic acid pathway, which is unique to plants, to prevent protein biosynthesis and kill the plant.

The first product containing glyphosate was registered for use in Australia in the 1970s, under the trade name 'Roundup®'. Products containing glyphosate that are registered for use in Australia are formulated as solutions, granules, aerosols and gels and are generally applied using ground or aerial equipment.

Concerns have recently been raised about human exposure to glyphosate, following an assessment by the International Agency for Research on Cancer (IARC) that re-classified glyphosate as 'probably carcinogenic to humans'.

The APVMA chose to consider glyphosate for reconsideration following the publication of the IARC Monograph 112 in July 2015. Once a chemical has been nominated for reconsideration, the APVMA examines the new information to determine whether there are sufficient scientific grounds to warrant placing the chemical under formal reconsideration. This regulatory position report represents the outcome of that scientific nomination assessment process.

Evaluation methodology: a weight-of-evidence approach

The nomination assessment process involved a scientific weight-of-evidence evaluation of information in the IARC monograph, risk assessments undertaken independently by regulatory agencies in other countries and expert international bodies, in addition to Adverse Experience Reports (AERs) submitted to the APVMA. A weight-of-evidence assessment involves an examination of the quality, biological relevance and consistency of studies, assessment reports and scientific conclusions according to the scientific method.

The APVMA commissioned a review of the IARC monograph by the Office of Chemical Safety (OCS) within the Department of Health. This review was conducted in two phases: Tier 1 involved conducting a preliminary scoping review of the IARC monograph to ascertain the relevance of the carcinogenicity classification of glyphosate and any implications that this may have for glyphosate approvals and registrations in Australia; Tier 2 involved conducting a detailed assessment of those studies that were identified during the Tier 1 assessment as requiring further evaluation.

The APVMA also reviewed a number of very recent international assessments of glyphosate including those undertaken by the Joint Food and Agriculture Organisation of the United Nations/World Health Organisation (FAO/WHO) Meeting on Pesticide Residues, the European Food Safety Authority (EFSA), the European Chemicals Agency (ECHA), Health Canada and the New Zealand Environmental Protection Authority (NZ EPA).

Assessment of the IARC glyphosate monograph

The OCS undertook a screening level assessment of the IARC monograph (Tier 1) and identified 19 references relevant to the carcinogenicity classification of glyphosate requiring a more in-depth evaluation, with an additional 74 references requiring further review to determine their relevance—the APVMA utilised recent independent international assessments of these references. Following the assessment of the 19 studies relevant to the IARC carcinogenicity classification of glyphosate (Tier 2), the OCS concluded that there did not appear to be any new information to indicate that glyphosate poses a carcinogenic or genotoxic risk to humans.

Evaluation of international assessments of glyphosate

The JMPR, EFSA, ECHA and Health Canada assessments of glyphosate all evaluated the publicly available data that was considered in the IARC monograph, as well as other published and unpublished data not available to IARC. In addition, the NZ EPA assessed the publicly available data contained in the IARC monograph and assessments by JMPR and EFSA.

Carcinogenicity studies in laboratory animals: EFSA concluded that the weight-of-evidence is that there is no carcinogenic risk to humans related to the use of glyphosate. JMPR concluded that glyphosate is not carcinogenic in rats but was unable to exclude the possibility that glyphosate is carcinogenic in mice at very high doses. The assessment conducted by ECHA concluded that there was no evidence of carcinogenicity in mice or rats due to a lack of statistical significance in pair-wise comparisons, a lack of consistency across studies, that slightly increased tumour incidences were only evident at doses exceeding the maximum tolerated dose, the absence of early cellular changes or pre-neoplastic lesions and/or incidences that tumour incidences were in the range of normal biological variation. Health Canada concluded that there was no evidence that glyphosate was carcinogenic or genotoxic in rats but that there was some evidence for a marginal increase in the incidence of ovarian tumours in mice only at the highest tested dose—however, these results were considered to be of low concern for human health risk assessment. The assessment commissioned by the NZ EPA concluded that long-term carcinogenicity studies produced consistently negative results and that the IARC assessment attributed inappropriate weight to the studies included in its assessment, which did not demonstrate a dose-response relationship, reported only minor positive results at the maximum dose tested, did not to consider relevant historical control data and excluded some studies that did not report positive associations between glyphosate exposure and carcinogenicity.

Genotoxicity studies: JMPR concluded that the overall weight-of-evidence is that glyphosate is unlikely to be genotoxic to humans at anticipated dietary exposures. EFSA, ECHA, Health Canada and the NZ EPA similarly concluded that the weight-of-evidence does not support the hypothesis that glyphosate is genotoxic. Again, these assessments concluded that the evidence presented by IARC as representative of strong evidence for genotoxicity and oxidative stress was primarily based on exposure scenarios not relevant to humans.

Epidemiological studies: ECHA concluded that the value of the human data for hazard classification purposes is questionable and limited because it is difficult to distinguish between the effects of the active constituent and co-formulants, as humans are never exposed to the active constituent alone, and humans are exposed to a many environmental chemicals, making it difficult to attribute health effects to one specific chemical. The JMPR, EFSA, ECHA and NZ EPA assessments concluded that while there was some evidence of a positive statistical association between glyphosate exposure and the risk of non-Hodgkin's lymphoma (NHL) in some retrospective

case-control studies, the one large, high-quality prospective cohort study found no statistical association at any exposure level. The EFSA assessment further noted that it was not possible to differentiate between the effects of glyphosate and the co-formulants in the epidemiological data available. The ECHA assessment describes a number of papers that did not identify a risk between glyphosate exposure and various specific cancer types, including NHL, lymphomas in general or multiple myeloma. The ECHA concluded that a comprehensive review of epidemiological studies assessing the possible association between glyphosate exposure and cancer found no consistent pattern of positive associations that would suggest a causal relationship between glyphosate exposure and the development of cancer in adults or children. The ECHA further concluded that, while epidemiological data is of limited value for detecting the carcinogenic potential of a pesticide, the data do not provide convincing evidence for an association between glyphosate exposure in humans and any cancer type. The Health Canada assessment concluded that the majority of epidemiological data considered by IARC lacked adequate characterisation of glyphosate exposure and that as a result these studies were of limited use for supplementing the hazard assessment of glyphosate.

Assessment of adverse experience reports (AER)

Between 1996 and 2013, a total of four AERs relating to human safety were submitted to the APVMA's Adverse Experience Reporting Program (AERP). All were classified as 'possible' or 'probable' by the APVMA. Of the four reports, one was of skin irritation while the remaining three were reports of eye irritation. The APVMA is confident that the current safety and use directions included on approved labels for products containing glyphosate are sufficient to mitigate these known adverse effects.

Proposed regulatory position

Based on this nomination assessment, the APVMA concludes that the scientific weight-of-evidence indicates that:

- exposure to glyphosate does not pose a carcinogenic or genotoxic risk to humans
- there is no scientific basis for revising the APVMA's satisfaction that glyphosate or products containing glyphosate:
  - would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues
  - would not be likely to have an effect that is harmful to human beings
  - would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment
  - would be effective according to criteria determined by the APVMA by legislative instrument, and
  - would not unduly prejudice trade or commerce between Australia and places outside Australia.
- **there are no scientific grounds for placing glyphosate and products containing glyphosate under formal reconsideration**
- the APVMA will continue to maintain a close focus on any new assessment reports or studies that indicate that this position should be revised.

1 INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine) is an aminophosphonic analogue of glycine, which is a naturally occurring amino acid. Glyphosate is classified as an organophosphate as it contains carbon and phosphorous; however, it does not affect the nervous system the way other organophosphates do. Glyphosate is a broad-spectrum, non-selective, post-emergent, systemic herbicide that kills or suppresses all plant types, except those that have been genetically modified to be resistant to glyphosate, and can be used as a plant-growth regulator/desiccator at lower dose rates. Herbicide products that contain glyphosate are commonly used to control annual and perennial broadleaf and grassy weeds in various agricultural and non-agricultural settings. Glyphosate binds strongly to soil particles and is readily metabolised by soil microorganisms, therefore when applied post-emergence, glyphosate demonstrates no pre-emergence or residual activity.

The water solubility of technical-grade glyphosate acid can be increased by formulating it primarily as its isopropylamine salt, or less commonly as monoammonium, potassium, trimesium, monoethanolamine or dimethylammonium salts, or various combinations of those salts. Furthermore, commercial formulated products contain various non-ionic surfactants to facilitate uptake by plants. Some commercial formulations also contain other active constituents in an attempt to mitigate herbicide resistance.

Glyphosate is taken up by the leaves and other green parts of the plant and translocated to the entire plant systemically. As a result, glyphosate is capable of total destruction of the plant. Glyphosate binds to and blocks the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), thereby disrupting the shikimic acid pathway and preventing the plant from synthesising the essential aromatic amino acids required for protein biosynthesis (phenylalanine, tyrosine and tryptophan), killing the plant. As this pathway is unique to plants and therefore is not present in mammals, glyphosate demonstrates low vertebrate toxicity.

The first product containing glyphosate was registered for use in Australia in the 1970s, under the trade name ‘Roundup’. Products containing glyphosate that are registered for use in Australia are formulated as solutions, granules, aerosols and gels (Table 1) and can be applied using ground or aerial equipment, as well as some specialised application methods (eg aerosol).

1.1 Current regulatory status of glyphosate in Australia

As of February 2016 there were 80 active constituent approvals for glyphosate and 471 registered products containing glyphosate. Of the 471 registered products, 130 are for home garden use and 370 are for commercial/agricultural use (Table 1). In these registered products, glyphosate is present at varying concentrations and is formulated in various salt forms, including ammonium, dimethylammonium, isopropylamine, mono-ammonium, monoethanolamine and potassium salts. Some registered products contain additional active constituents, including amitrole, ammonium thiocynate, butafenacil, carfentrazone-ethyl, diflufenican, imazapyr and oxyfluorfen.

Glyphosate is approved for use in Australia to control various annual and perennial broadleaf, grassy and woody weeds, trees and brush and is used in a variety of different situations, such as:

- croplands for the control of emerged weeds prior to crop and fallow establishment, minimum tillage farming, direct drilling into seedbed, for pre-harvest desiccation

- non-cultivated land (eg industrial, commercial, domestic and public service areas) and rights of way
- forests, orchards, vines and plantations
- home garden use on rockeries, garden beds, driveways, fence lines, firebreaks, around buildings and prior to planting new lawns and gardens
- aquatic areas (restricted to dry drains and channels, dry margins or dams, lakes and streams)
- aquatic weed control and control of weeds on margins of dams, lakes and streams or in channels, drains or irrigation (selected products only).

Glyphosate is applied by ground boom, knapsack/handgun, gas/splatter gun, wiper equipment, controlled droplet application equipment, aerial spraying, aerosol spray, ready to use spray bottle and ready to use gel dispenser.

Table 1: Formulation types for glyphosate products

Formulation type	Level of active constituent	Product type
Aqueous concentrate	3.6 g/L	Home garden
	7.2 g/L	Home garden
	60 g/L	Commercial
	100 g/L	Home garden
	150 g/L	Commercial
	300 g/L	Commercial
	360 g/L	Home garden and commercial
	450 g/L	Home garden and commercial
	470 g/L	Commercial
	480 g/L	Commercial
	490 g/L	Home garden and commercial
	500 g/L	Home garden and commercial
	510 g/L	Commercial
	540 g/L	Home garden and commercial
Soluble concentrate	7.2 g/L	Home garden
	15.2 g/L	Home garden
	143 g/L	Home garden
	150 g/L	Commercial

Formulation type	Level of active constituent	Product type
	360 g/L	Home garden and commercial
	450 g/L	Commercial
	470 g/L	Commercial
	480 g/L	Commercial
	490 g/L	Home garden
	495 g/L	Commercial
	500 g/L	Commercial
	510 g/L	Commercial
	517 g/L	Commercial
	535 g/L	Commercial
	540 g/L	Home garden and commercial
	570 g/L	Commercial
	600 g/L	Commercial
Emulsifiable concentrate	360 g/L	Commercial
Suspension concentrate	225 g/L	Home garden and commercial
	360 g/L	Home garden and commercial
	450 g/L	Commercial
	510 g/L	Commercial
	600 g/L	Commercial
	700 g/L	Commercial
Water dispersible granule	680 g/kg	Home garden and commercial
	690 g/kg	Commercial
	700 g/kg	Commercial
	835 g/kg	Commercial
Water soluble granule	680 g/kg	Commercial
	700 g/kg	Commercial
	720 g/kg	Commercial

Formulation type	Level of active constituent	Product type
	800 g/kg	Commercial
	840 g/kg	Commercial
	900 g/kg	Commercial
	875 g/kg	Commercial
Aerosol	10 g/kg	Home garden
Liquid	7.2 g/L	Home garden
	360 g/L	Home garden and commercial
	450 g/L	Commercial
Liquid concentrate	570 g/L	Commercial
Emulsion, oil in water	4.8 g/L	Home garden
	25.6 g/L	Home garden
	432 g/L	Commercial
Gel	7.2 g/L	Home garden
	40 g/L	Home garden
Dry flowable	225 g/L	Home garden
Other liquids to be applied undiluted	7.2 g/L	Home garden
	7.4 g/L	Home garden
	16 g/L	Home garden

Previous reconsideration of glyphosate by the APVMA in 1996

A formal reconsideration of glyphosate was initiated following concern by the then Commonwealth Environment Protection Agency that certain surfactants in glyphosate formulations were acutely toxic to tadpoles at concentrations that are likely to occur in shallow water when products were used according to approved label instructions. Seventy five products were placed under review and all 27 holders were invited to provide information to the APVMA (then the National Registration Authority; NRA) relating to the review.

The scope of the review was limited to:

- reviewing application methods of glyphosate formulations adjacent to aquatic environments of all registered agricultural products

- a proposal to include a warning statement on all agricultural glyphosate product labels precluding use on or adjacent to waterways unless otherwise authorised
- a proposal to only allow use of glyphosate formulations in sensitive aquatic situations where it can be demonstrated that there is no significant risk to the aquatic environment.

The conclusions of the reconsideration were that the aquatic toxicity of registered glyphosate formulations was undesirably high and was mainly due to the surfactants in the formulations. Therefore, a number of conditions of registration were modified to describe more clearly the situations in which products registered for use in aquatic situations could be used to avoid the risk of significant aquatic contamination. Use of the formulated products was restricted to dry drains and channels and dry margins of dams, lakes and streams. Warning statements on labels were amended to minimise any possible aquatic contamination. Only formulations with an acceptable margin of aquatic safety would be registered for controlling weeds growing in or over water. Holders were provided 12 months (until 30 June 1997) to make the necessary changes to their products. No changes were made to products registered solely for home garden use, as the risk of significant aquatic contamination was considered very low. The final reconsideration report is available on the APVMA website.

Response to claims that glyphosate is responsible for causing birth defects

In June 2011, Earth Open Source (EOS) published a document titled 'Roundup and birth defects: is the public being kept in the dark?' In this document, EOS questioned the safety of glyphosate and products that contain it. The claims made by EOS were:

- exposure to concentrations of glyphosate lower than those commonly used in agriculture and the home garden have been linked to developmental malformations affecting the skull, face, brain and spinal cord in frog and chicken embryos
- a range of developmental malformations, as well as endocrine disruption and reproductive toxicity have been observed in humans and experimental animals following exposure to glyphosate
- a variety of *in vitro* test systems have demonstrated that glyphosate can induce damage to DNA and genetic material in laboratory animals and humans
- glyphosate exposure has been linked to cancer of the testis in rats, skin cancer in mice and blood system cancers in humans
- glyphosate exposure has been linked to neurotoxicity and the development of Parkinson's disease in humans.

The APVMA commissioned an expert review of that document, which was published in July 2013, to address the concerns raised in the EOS article. In doing so, the APVMA evaluated both the published studies cited in the EOS document and other more recent publications and archived toxicology studies of glyphosate, compared the EU reviews of glyphosate with reviews prepared by other regulators, assessed the scientific merit of the claims made by EOS and the research upon which those claims were based and considered whether there were implications for the registration of products containing glyphosate in Australia. The full review of the EOS document can be found on the APVMA archive website.

A number of conclusions were made in the review of the EOS document. These included:

- The available data do not indicate that glyphosate products registered for use in Australia and used according to label instructions present any unacceptable risks to human health, the environment or trade.
- The weight- and strength-of-evidence demonstrate that glyphosate is not genotoxic, carcinogenic or neurotoxic.
- Developmental malformations caused by glyphosate in toad and chicken embryos are not predictive of a developmental hazard to humans because of the routes of administration used. Some studies have reported fetal skeletal abnormalities, toxicity to the male reproductive tract during puberty and interference with the maturation of the male reproductive organs during puberty; however, these studies were affected by flawed design, methodology and/or reporting and the claimed effects on puberty are inconsistent.
- Glyphosate is extremely unlikely to cause reproductive or developmental toxicity in humans under normal conditions of exposure.
- At present, there is no scientific justification for classifying glyphosate as an endocrine disrupter.
- Effects on hormonal regulation and cellular toxicity observed *in vitro* may have been confounded by surfactants present in formulated products.
- Most studies utilising formulated products containing glyphosate have not identified which chemical constituent was responsible for causing the reported effects, or characterised their mode of action.
- The toxicological studies cited by EOS do not demonstrate a need to revise the current Australian Acceptable Daily Intake (ADI) of 0.3 mg/kg bw/day for glyphosate.
- New information that emerges from the United States (US) and Canadian reviews of glyphosate will be considered by the APVMA.

The Poisons Standard (SUSMP)

The Poisons Standard, or the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) controls how medicines and poisons are made available to the public and classifies them into Schedules according to the level of regulatory control that is required in order to maintain public health and safety. Scheduling of medicines and poisons in Australia is a legislative requirement administered by the Therapeutic Goods Administration (TGA). However, the scheduling controls are implemented through State and Territory legislation, therefore the implementation of any restrictions imposed by the TGA may differ between States and Territories. Model provisions about packaging and labels, a list of products recommended to be exempt from the provisions and recommendations about other relevant controls are also included.

When making a scheduling decision, various criteria are considered, including toxicity, purpose of use, potential for abuse, safety in use and the need for the substance. Medicines and poisons are classified in one of ten Schedules. Agricultural, domestic and industrial poisons are generally listed in Schedules 5 (caution), 6 (poison) or 7 (dangerous poison), which represent increasingly stricter container and labelling requirements. Products for domestic use must not be listed in Schedule 7.

Glyphosate is classified as a Schedule 5 (caution) substance, which is defined as a substance with a 'low potential for causing harm, the extent of which can be reduced through the use of appropriate packaging with strong warnings and safety directions on the label'. To classify as a Schedule 5 poison, the substance must adhere to the following criteria:

- the substance is non-corrosive and has a low toxicity
  - acute oral toxicity (rat): 2000 mg/kg to 5000 mg/kg
  - acute dermal LD_{50}: > 2000 mg/kg
  - acute inhalation LC_{50} (rat): > 3000 mg/m^3 (4 hours)
- the substance has a low health hazard from repeated use and is unlikely to result in irreversible toxicity
  - no other significant toxicity (eg carcinogenicity, mutagenicity, etc)
- the substance is capable of causing only minor adverse effects to humans in normal use
  - specialised personal protective equipment should not be necessary for safe use
- the likelihood of injury during handling, storage and use can be mitigated through appropriate packaging and label warnings
- the substance has a low potential for causing harm
  - potential harm is reduced through the use of appropriate packaging with simple warnings and safety directions on the label.

1.2 Health-based guidance values for glyphosate

Health-based guidance values are established by regulatory authorities (and international bodies such as the JMPR) for the purpose of determining whether human exposure (via the diet or occupationally) to a particular chemical is safe. Health-based guidance values provide quantitative information to risk managers to enable them to make informed, scientific decisions related to protecting human health.

Acceptable Daily Intake (ADI)

The ADI is the amount of a chemical that can be ingested daily over a lifetime without any appreciable risk to health. The ADI is based on the lowest NOAEL (No Observed Adverse Effect Level) for the most sensitive adverse effect relevant to humans.

The ADI for glyphosate in Australia is 0.3 mg/kg bw/day based on the No-Observed-Adverse-Effect Level (NOAEL) of 30 mg/kg bw/day (the highest tested dose) in a 3-generation reproduction dietary study in rats and using a 100-fold safety factor to account for extrapolation from animals to humans as well as variation in sensitivity within the human population.

Acute Reference Dose (ARfD)

The ARfD is an estimate of the amount of a substance in food and drinking water, expressed on a milligram per kilogram bodyweight basis, which can be ingested in a period of 24 hours or less without appreciable health risk to the consumer. In 1998, JMPR concluded that an ARfD must be determined for all pesticides, unless the toxicological profile indicated that the pesticide was unlikely to present an acute hazard. As the toxicology assessments of glyphosate indicate that there is no likelihood of glyphosate presenting an acute hazard to human health, an ARfD has not been established for glyphosate in Australia or overseas.

Maximum Residue Limits (MRL) and National Residue Survey (NRS)

The maximum amount of a chemical that is legally permitted in a food is known as the MRL. The MRL is based on good agricultural and chemical use practices to ensure that an agricultural or veterinary chemical has been used according to the directions on the approved label. The MRL is set well below the level that would result in the health-based guidance values being exceeded if the chemical is used according to the approved label instructions. Therefore, while exceedance of the MRL may indicate a misuse of the chemical, it does not normally indicate that there is a public health or safety concern. The APVMA sets MRLs for agricultural and veterinary chemicals in agricultural produce. The states and territories are responsible for enforcing MRLs.

The *Agricultural and Veterinary Chemicals Code Instrument No. 4 2012* (MRL Standard) lists MRLs for chemicals that may arise from the approved use of products containing that chemical, and outlines the definitions of those residues. The glyphosate residue definition is the sum of glyphosate, *N*-acetyl-glyphosate and aminomethyphosphonic acid (AMPA) metabolite, expressed as glyphosate.

As a part of the Department of Agriculture and Water Resources strategy to minimise chemical residues in agricultural product, the NRS facilitates testing of animal and plant products for pesticide and veterinary medicine residues, and environmental contaminants. In the 2013–14 NRS report, glyphosate residues greater than half of the MRL were not detected in any samples of barley, canola, chickpea, faba bean, field pea, lentil, lupin, maize, sorghum, triticale, wheat, wheat durum or macadamias. In 1/28 samples of oats, glyphosate residues above the MRL were detected (NRS 2014b), while in 1/37 almond samples, glyphosate residues lower than the MRL were detected (NRS 2014a). In the 2014–15 report (not yet published), glyphosate residues above the MRL were reported in 1/42 oat samples and residues below the MRL (above half of the MRL) were reported in 4/42 oat samples (NRS 2015). No residues greater than half of the MRL were detected in any samples of barley, chickpea, faba bean, canola, cowpea, field pea, lentil, maize, lupin, maize, mung bean, sorghum or wheat.

Australian Total Diet Study (ATDS)

The ATDS is coordinated by FSANZ to monitor Australia's food supply and ensure that food regulatory measures are protecting consumer health and safety. The ATDS assesses dietary exposure to pesticide residues, contaminants and other substances and is conducted approximately every two years.

The 23rd ATDS examined dietary exposure to 214 agricultural and veterinary chemicals, nine contaminants, 12 mycotoxins and 11 nutrients in 92 commonly consumed foods and beverages in 2008 (FSANZ 2011a). Glyphosate residues were detected in 2/12 samples of multigrain bread (mean concentration 0.016 mg/kg) (FSANZ 2011b). Based on these results, FSANZ estimated the mean consumer dietary exposure to glyphosate as 0.12, 0.81, 0.87, 0.97 and 1.4 µg/day in children aged 9 months, 2–5 years, 6–12 years and 13–16 years and adults aged 17 years and above, respectively (FSANZ 2011b). These estimated exposures are well below (214–25 000 times) the ADI of 0.3 mg/kg indicating that there are no safety concerns for Australian and New Zealand consumers.

Drinking water standards

The Australian Drinking Water Guidelines (the Guidelines) are a joint publication of the National Health and Medical Research Council (NHMRC) and the Agricultural and Resource Management Council of Australia and New Zealand. The Guidelines are not legally enforceable but provide a standard for water authorities and state health authorities to ensure the quality and safety of Australia's drinking water.

The health-related guideline value (expressed as mg/L) is the concentration or measure of a water quality characteristic that, based on present knowledge, does not result in any significant risk to the health of the consumer over a lifetime of consumption (NHMRC 2011). Health values are derived so as to limit intake from water alone to approximately 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult having a daily water consumption of 2 litres over a lifetime. The current health-related guideline value for glyphosate in drinking water is 1 mg/L—excursions above this value would need to occur over a significant period of time to be of a health concern (NHMRC 2011). Glyphosate is generally not reported in the analysis of Australian waters and is unlikely to be found at levels that may cause health concerns.

1.3 Legislative basis for a reconsideration of glyphosate

The basis for a reconsideration of the registration and approvals for a chemical is whether the APVMA is satisfied that the safety, efficacy and trade criteria listed in sections 5A, 5B and 5C of the Agvet Code for continued registration and approval are being met. These requirements are that the use of the product, in accordance with instructions approved, or to be approved, by the APVMA for the product or contained in an established standard:

- would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues
- would not be likely to have an effect that is harmful to human beings
- would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment
- would be effective according to criteria determined by the APVMA by legislative instrument, and
- would not unduly prejudice trade or commerce between Australia and places outside Australia.

The APVMA may also consider whether labels for containers for chemical products containing glyphosate meet the labelling criteria as defined in section 5D of the Agvet Code which requires that labels have adequate instructions relating to:

- the circumstances in which the product should be used
- how the product should be used
- the times when the product should be used
- the frequency of the use of the product
- the re-entry period after use of the product
- the withholding period after the use of the product
- disposal of the product and its container
- safe handling of the product and first aid in the event of an accident
- any matters prescribed by the regulations.

2 INTERNATIONAL REGULATORY STATUS

Glyphosate is approved for use throughout the world, including in Europe and the United Kingdom (UK), the US, Canada, Australia, New Zealand, China, Brazil etc.

2.1 United States

The United States Environmental Protection Agency (US EPA) registers pesticides under the Federal Insecticide, Fungicide and Rodenticide Act and periodically (at least every 15 years) re-evaluates pesticides to ensure that they continue to meet registration standards, noting that new scientific information may be generated that should be taken into consideration. The registration of glyphosate is currently being reviewed as a part of this process. The re-assessment began in 2009 and was originally scheduled for completion in 2015; however, finalisation of the assessment was delayed following the re-classification of glyphosate by IARC. The final report is currently expected to be completed and published in 2016. The US EPA utilises a risk assessment process for evaluating the potential for health and ecological effects of a pesticide. The human health risk assessment process utilises the National Research Council's process for human health risk assessments, which is the procedure outlined by the International Programme on Chemical Safety (IPCS) and adopted by JMPR, as described in Section 4.3. In addition, the US EPA has developed a framework to incorporate epidemiological information into its risk assessment, which is based on peer-reviewed, robust principles and tools. The framework methodology was reviewed in 2010 by the Federal Insecticide, Fungicide and Rodenticide Act Scientific Advisory Panel. Chemicals are assessed for carcinogenicity using the US EPA's Guidelines for Carcinogen Risk Assessment (2005).

In February 2016, the US Food and Drug Administration (US FDA) announced that they would begin testing for residues of glyphosate on various foods, including soybeans, corn, milk and eggs. Concurrently, the US Fish and Wildlife Service announced that they would commence an analysis in conjunction with the US EPA of the impacts of four commonly used pesticides (including glyphosate) on 1500 endangered species, which is due for completion by December 2022.

Glyphosate-based formulations are currently registered in the US to control weeds in various fruit, vegetable and other food crops, glyphosate-resistant transgenic crops, ornamental plantings, lawns and turf, greenhouses, aquatic areas, forest plantings and roadside rights of way. Products registered in the US that contain glyphosate are formulated as liquids, solids and ready-to-use formulations, and can be applied using ground and aerial equipment as well as small hand-held sprayers.

2.2 Canada

The registration of pesticides in Canada is regulated by Health Canada's Pest Management Regulatory Agency (PMRA). In 2010 Health Canada's PMRA commenced a re-evaluation of glyphosate in collaboration with the US EPA's re-evaluation of glyphosate. In April 2015, the PMRA published its Proposed Re-evaluation Decision (PRVD2015-01) for glyphosate. In that document, the PMRA proposed continued registration of products containing glyphosate for sale and use in Canada. However, as a condition of the proposed continued registration, new risk reduction measures were proposed for end-use products, aimed at protecting both human health and the environment (Table 2).

Table 2: New measures to minimise risk of glyphosate exposure proposed by Health Canada's Pest Management Regulatory Agency

Human health	Environment
A restricted-entry interval of 12 hours for agricultural uses to protect workers	Environmental hazard statements to inform users of toxicity to non-target species
Apply only when potential for drift to areas of human habitation or activity (eg houses, cottages, schools and recreational areas) is minimal, to protect bystanders	Spray buffer zones to protect non-target terrestrial and aquatic habitats
	Precautionary statements for sites with characteristics that may be conducive to runoff and when heavy rain is forecast are proposed to reduce potential for runoff to adjacent aquatic habitats
	A vegetative strip between treatment area and edge of a water body to reduce runoff to aquatic areas

Following the publication of the proposed re-evaluation decision, the PMRA accepted written comments on the report for 60 days from the date of publication. The PMRA will consider all submissions prior to making a final, scientific decision on the registration of glyphosate in Canada.

2.3 Europe and the United Kingdom

All active constituents used in pesticide products in the EU are subject to approval by the European Commission (EC). However, individual Member States are responsible for authorising the final formulated pesticide products containing those active constituents in its territory. Therefore, whilst a chemical may be registered for use in the EU, Member States have the power to restrict use of that product in its territory. The EC approval is limited to a maximum of ten years—therefore, if manufacturers wish to continue using that active constituent in pesticide products, they must apply for renewed approval prior to the end of these ten years. The EC appoints a member state to act as the Rapporteur Member State (RMS) to conduct the assessment of a chemical.

The European Food Safety Authority (EFSA) is an agency that is funded by the EU but operates independently of the European legislation and member states. Legally established in 2002 by the EU, EFSA provides scientific advice and communication on risks associated with the food chain in Europe and is responsible for risk assessment of available science, but is not involved in legislative risk management or policy determination. Instead, the risk assessment conducted by EFSA is used to inform European policy and legislation by the EU risk managers, including the EC and the European Parliament (EP).

Glyphosate is registered for use throughout Europe and the UK and in August 2014 was subjected to a re-assessment by the RMS, Germany, as mandated by the EC and coordinated by EFSA. The Federal Republic of Germany was appointed as the RMS to conduct the assessment. The Federal Office of Consumer Protection and Food Safety was appointed by the German government as the lead authority for drafting the Renewal Assessment Rapport (RAR). The Federal Institute for Risk Assessment (BfR) was subsequently commissioned to assess the potential health risks of glyphosate. Once completed, the draft report was presented to EFSA and a consultation

period commenced. All comments and additional data resulting from the consultation period was incorporated into the draft, which was then submitted to EFSA in December 2014.

In February 2015, the BfR prepared a revised health risk assessment report on glyphosate, which was subsequently revised in April 2015 to include additional evaluation tables and clarify some factual information following consultation with EFSA. The assessment by EFSA was published in November 2015. The report concluded that glyphosate was 'unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential' (EFSA 2015).

In April 2015, the EC provided EFSA with a second mandate, to consider the findings of the IARC regarding the potential carcinogenicity of glyphosate or products containing glyphosate in the original assessment. In July 2015, the German government and EFSA commissioned BfR to review the IARC monograph on the re-classification of glyphosate. The review was completed in August 2015 as an addendum to the original RAR and was peer reviewed by EFSA. A detailed discussion of the BfR's review of the IARC monograph is provided below in Section 4.4).

Briefly, the BfR agreed with IARC's conclusion that there is 'limited evidence in humans for the carcinogenicity of glyphosate' but noted that no consistent positive association between glyphosate exposure and the development of cancer was demonstrated, and the most powerful study reported no effect. The BfR disagreed with IARC's conclusion that there is 'sufficient evidence in animals for the carcinogenicity of glyphosate', concluding that the weight-of-evidence suggests that there is no carcinogenic risk related to the use of glyphosate and that no hazard classification for carcinogenicity is warranted according to the Classification, Labelling and Packaging of Substances and Mixtures (CLP criteria) (Germany 2015). The BfR also disagreed with IARC's conclusion that there 'is mechanistic evidence for genotoxicity, oxidative stress, inflammation, immunosuppression, receptor-mediated effects, and cell proliferation or death of glyphosate' and concluded that the mechanistic and other studies do not provide evidence for a carcinogenic mechanism. The BfR concluded that the weight-of-evidence suggests that neither glyphosate nor AMPA (a metabolite of glyphosate) induce mutations *in vivo* and that no hazard classification for mutagenicity was warranted according to CLP criteria (Germany 2015).

The initial registration of glyphosate was scheduled to expire on 31 December 2015 (EC 2015). Following an expert meeting of EFSA, the EU member states, WHO, IARC and the US EPA, and in consideration of the revised RAR and addendum, EFSA completed its report for the assessment of glyphosate for the purpose of renewed approval and recommended that a renewal of the registration of glyphosate be granted. The EFSA RAR and addendum were subject to a thorough peer review by the competent authorities of the EU Member States and to accommodate that peer review process, the registration of glyphosate was provisionally extended until 30 June 2016. All but one of the Member States experts agreed that glyphosate is unlikely to be genotoxic or pose a carcinogenic risk to humans. The EC postponed a vote by EU member states to renew approval of glyphosate, which was originally scheduled for the meeting on 7 and 8 March 2016 of the EU Standing Committee on Plants, Animals, Food and Feed (hereafter referred to as the Standing Committee) until after the European Parliament vote in April 2016.

In March 2016, the EU Environment Committee Members of the European Parliament (MEPs) voted in favour of a resolution for the EC to abandon its proposal to renew approval of glyphosate in the EU for a further 15 years with no restrictions. The Environment MEPs instead requested that the EC conduct an independent review and disclose all of the scientific evidence used by EFSA in its assessment of glyphosate. They added that the EU Food and Veterinary Office should also be mandated to test and monitor glyphosate residues in food and drink.

The resolution was put to a vote at the plenary session of the EP scheduled for 11–14 April in Strasbourg, which again resulted in a postponement of the vote to re-register glyphosate, as a qualified majority consensus could not be reached. The Standing Committee again met on 18–19 May 2016 to discuss a 10 year re-registration for glyphosate in the EU. Again, the vote was postponed because a qualified majority was not reached. On 2 June 2016, the EC announced a proposal for the Standing Committee to meet on 6 June 2016 to consider a 2-year extension to the current registration of glyphosate so that the ECHA could complete an assessment of the carcinogenicity and potential for endocrine disruption of glyphosate. The EC also proposed banning polyethoxylated tallow amines (POEA; in glyphosate-based formulations only), minimising the use of glyphosate in public parks, playgrounds and gardens, and minimising pre-harvest use of glyphosate. In order for the proposal to pass, 55% of Member States (representing 65% of the EU's population) would be required to vote in favour. Of the 28 Member States, 20 voted in favour of the proposal, 7 abstained (did not vote for or against) and 1 (Malta) voted against the proposal. As a result of the relatively large populations of some of the countries that abstained from voting, the favourable votes accounted for only 52.91% of the EU's population and the proposal did not pass.

On 24 June 2016, the EC convened an Appeals Committee to consider the re-approval of glyphosate for 18 months to allow the ECHA to gather additional data and undertake a comprehensive analysis of the health risks association with its use. Again, a qualified majority position was not reached, with 19 countries in favour of the extended approval, two against (France and Malta) and seven abstaining, representing 51.49% of the EU's population in favour of the extension.

When a qualified majority is not obtained, the EC may bring forward its own decision to authorise the re-approval of a chemical. On 29 June 2016, the EC extended the approval of glyphosate in the EU to allow the ECHA to complete its assessment of glyphosate. This approval will expire either 6 months following the date of receipt of the ECHA report or 31 December 2017, whichever occurs first (EC 2016). On 11 July 2016, Member State experts voted as a qualified majority in favour of two recommendations proposed by the EC as conditions to the registration extension, at a meeting of the Standing Committee in Plants, Animals, Food and Feed. These restrictions included:

- an EU-wide ban on POEAs contained in some glyphosate-based formulations
- restricted use of glyphosate-based formulations in public parks, playgrounds and home gardens and for pre-harvest application.

In July 2016, the pesticide regulator in Malta (the Malta Competition and Consumer Affairs Authority) began implementing a policy decision by the Environment Ministry to withdraw authorisation for all glyphosate and glyphosate-based formulations.

Glyphosate is currently authorised throughout the EU and UK, predominantly for uses in agriculture (cereals, vineyards, olives, citrus, nuts etc), but also to manage weed growth on non-cultivated areas (eg railway tracks, verges), public amenities, forestry and aquatic environments, and in home gardens. Glyphosate is authorised for weed control use after harvest or sowing, before a new crop is planted. Glyphosate is also authorised for pre-harvest weed control use and dessication (to promote the maturation of crops) in crops such as oilseed rape and cereals. It is not currently clear which uses will be affected as a result of the recently announced use restrictions described above.

2.4 New Zealand

In New Zealand, the registration of herbicides is the responsibility of the Environmental Protection Authority and the Ministry for Primary Industries. Glyphosate is listed on the Chief Executive Initiated Reassessment (CEIR) Programme and as such is being actively monitored by the Environmental Protection Authority.

Glyphosate has been registered in New Zealand since 1976 and is used in various settings, including orchards, vineyards, pastures, vegetable patches, along roadways and in parks, sporting fields and home gardens.

3 EVALUATION METHODOLOGY: THE WEIGHT OF SCIENTIFIC EVIDENCE

Consistent with the scientific method, a weight-of-evidence approach should be used to determine whether a chemical is carcinogenic. To conduct an initial quality assessment of each individual study, the study design should be assessed, taking into account OECD (Organisation for Economic Co-operation and Development) or national test guidelines where appropriate. In a weight-of-evidence assessment, any observation should be reproducible: the strength of any finding will be increased if it can be replicated under the same conditions in more than one laboratory. Plausible patterns in the hierarchy of the results will also strengthen the finding—ie where a finding *in vitro* is reproduced *in vivo*.

In toxicological science, there are a number of criteria that are used to determine whether an effect, such as cancer, is treatment-related and adverse:

- *Dose-response relationship*—the number of animals or subjects showing the effect and/or the severity of the effect should increase with dose. There should be a progression to a more severe state of toxicity as the dose and duration of dosing increases.
- *Consistency of the effect*— the effect should be observed consistently across studies of similar exposure duration and sexes (in unusual cases an effect may be sex-specific). Additionally, an effect should be corroborated by related toxicological endpoints – for example, increases in malignant neoplasms should be preceded by cellular changes that should be observed at lower doses or following shorter exposure durations.
- *Statistical significance*—differences between treated groups and the concurrent control group should be statistically significant. However, statistical significance on its own does not imply biological significance and the absence of statistical significance also does not necessarily mean the absence of an effect (for example a rare type of tumour may be highly biologically relevant).
- *Biological plausibility*—an observed effect needs to be mechanistically plausible based on the characteristics of the chemical and principles of biology/physiology.
- *Natural variation and incidental findings*—the normal range of natural variation of a parameter in the test species needs to be understood through the use of age- and sex-matched historical control data. All laboratory animal strains used in rodent bioassays have a background incidence of age- and sex-related neoplasms at different tissue sites. It is critical that this normal range of biological variation is documented and understood.

When assessing toxicological data associated with chemical residues in food, the APVMA has regard to the principles and methods outlined by the IPCS, described below in Section 4.3 (IPCS 2009) including guidance on the interpretation of toxicological data by JMPR[1] and OECD[2]. For the evaluation of carcinogenicity via dietary or other exposure routes, the IPCS has published a mode-of-action (MOA) framework for chemical carcinogenesis (Meek et al 2013). In this framework, treatment-related cancer must first be demonstrated in laboratory animals

[1] http://www.who.int/foodsafety/publications/jmpr_guidance_document_1.pdf?ua=1
[2] http://www.oecd-ilibrary.org/docserver/download/9750321e.pdf?expires=1472172141&id=id&accname=guest&checksum=28F68D5204F38A1B96055A611D12C4DF

before proceeding to examine genotoxicity data, human epidemiological and mechanistic data in order to determine the mechanism for how cancer arises and the human relevance of adverse effects observed in laboratory animals.

The APVMA considered aspects of study design and reporting that may either increase or decrease confidence in the data. The presence of a dose-response relationship, consistency and reproducibility were considered to increase confidence in the data, while any unexplained inconsistencies and significant deviations from international test guidelines were considered to reduce confidence in the data. Therefore, those studies that demonstrated a dose-response relationship, adhered to international test guidelines (where appropriate) and were consistent and reproducible within and/or between laboratories were given more weight in the assessment.

For epidemiological data, the APVMA considered prospective cohort studies to be more powerful than retrospective case-control studies, which are more prone to recall bias and confounding by exposure to other chemicals and environmental situations. It is well known that study participants' memory may not be reliable: participants are often asked to provide information about use patterns that occurred many years previously, participants may be providing information relating to a family members' usage (not their own) and it is possible that a participant with cancer may have spent more time thinking about possible causes and exposure scenarios than participants without cancer. It is also very difficult to separate usage of one pesticide from another: those who routinely use glyphosate-based formulations are likely to have been using many other types of agricultural and/or industrial chemicals, or be exposed to other occupational scenarios that may confound the data.

3.1 Use of international test guidelines

All scientific studies considered by the APVMA are assessed on their scientific merits. However, studies that have been conducted according to principles of Good Laboratory Practice (GLP) and comply with international test guidelines are preferred because of the assurance of their scientific quality.

To ensure the scientific quality of studies submitted for regulatory purposes and to enable comparison of studies utilising the same methodology in different laboratories, a number of internationally accepted test guidelines have been developed for various toxicological studies. The testing guidelines produced by the OECD are commonly used throughout the world and provide quality standards for different types of studies. Guidance is provided regarding test species and strain, the number of animals to be used, choice of chemical doses and duration of exposure, as well as parameters to be measured, observed and reported. By comparing studies that were conducted using equivalent test guidelines, regulators can identify potential human health hazards and set appropriate endpoints for risk assessment and management.

When assessing toxicology studies, consistency with international test guidelines is not the only measure of scientific quality. For some types of studies, guidelines have not yet been developed while for studies that were never intended for regulatory or risk assessment purposes (eg most studies published in scientific journals) some criteria may rarely be met. However, depending on how the study design, interpretation or reporting differs from the guidelines, the discrepancies may not affect the validity of the results. Specifically, data for individual animals is rarely reported in scientific publications; instead the data is presented as group means along with a measure for variance between control and treatment groups. This omission would not be considered a serious flaw and invalidate the study results. However, other elements of the testing guidelines may be considered more critical and omission may invalidate the study findings. For example, failure to independently code slides (or failure to report independent coding) used to visually score assay results would be considered as a potentially critical flaw, as it

would not be clear that the scoring was performed by an independent observer who was not aware of the treatment or control group being scored. In other cases, test guidelines may stipulate a maximum dose that is associated with minimal toxicity, for determining a specific carcinogenic or genotoxic end-point. In some experimental studies, that maximum dose may be exceeded up to ten-fold. In the absence of appropriate cytotoxicity tests, it may not be possible to determine whether any positive effects are indeed indicative of genotoxicity.

3.2 Statistical significance and biological or toxicological relevance

Statistical analysis is a useful tool for detecting differences between groups exposed to a test compound or not. Biologically this difference may be real or a chance or incidental finding. That is why a statistically significant result on its own without an evaluation of its biological and ultimately toxicological relevance provides only limited insight into the possible effects of a chemical. As described above, there are a range of other criteria that must be met in order to conclude that an effect is truly treatment-related and adverse.

Epidemiological data is often presented using an Odds Ratio (OR) with an associated confidence interval (CI; usually 95%). An OR is a relative measure of effect and is used in this context to compare the incidence of cancer (or some other health outcome) in individuals exposed to glyphosate with those who have not been exposed. If the OR is 1, the statistical analysis implies that there is no difference between the incidences of cancer in either group. The CI is used to determine the level of uncertainty around the OR, because the sample population used in the study is only a representative group of the overall population. The statistical test infers that the true population effect lies between the upper and lower CI. Therefore, a very narrow CI infers that the true effect is very close to the estimated OR, while a wide CI infers that the OR is less reliable. In addition, if the CI crosses 1 (eg 0.5–1.5), the statistical test is inferring that there is no difference between the two groups, in terms of cancer incidence. Therefore, the APVMA considered studies reporting positive associations between glyphosate exposure and cancer incidence that presented an OR greater than 1 and a narrow CI range that did not cross 1 to be more powerful than studies that had a wide CI range that crossed 1.

3.3 Historical control data and spontaneous tumour incidence

Consideration of historical control data is an important aspect of interpreting toxicology studies. Historical control data is a compilation of the findings from strain-, age- and sex-matched control animals from all the studies undertaken by the performing laboratory and provides an indication of the background frequency of tumours that occur in that species/strain of animals by chance. A statistically significant increase in tumour frequency may be observed in treated animals when a lower than normal tumour frequency is observed in control animals in that study. Conversely, a non-significant result may be observed when a higher than normal tumour frequency is observed in the control group. Therefore, historical control data is used to determine whether an increase in tumours is within the realms of normal biological variation or is in fact truly treatment related. For some common tumours
(eg liver, pituitary or adrenal), the historical control ranges are so wide that the incidences of tumours in both the concurrent control and treated groups often fit within their bounds. In these cases, the mean value or distribution of historical control data may be more useful than the range only.

3.4 Test species and route of administration

Data obtained from humans is preferable to data obtained from experimental animals because it increases the certainty that an observed effect is relevant to humans. Volunteer studies and human clinical trials provide accurate exposure metrics that can be directly linked with adverse outcomes. However, the extent of exposure can be difficult to determine in human observational studies (such as epidemiological studies), because subjects are often expected to rely on memory recall to provide exposure details and subjects are frequently exposed to more than one chemical. When evaluating studies conducted using animal models, those that use mammals are considered more relevant to human outcomes than non-mammalian species or *in vitro* cell culture studies.

When evaluating the toxicological effects of pesticides, such as glyphosate, studies in which the chemical was administered via the oral (gavage, diet, drinking water), dermal or inhalational routes are highly relevant because these are the only possible routes of exposure for humans. Subcutaneous (skin injection), intravenous (vein injection) and intraperitoneal (stomach cavity injection) administration are generally not directly relevant for chemical risk assessment purposes because humans would not be exposed via these routes. In addition, these routes of exposure bypass normal metabolic processes.

4 SUMMARY OF ASSESSMENTS AND CONCLUSIONS

4.1 The IARC glyphosate monograph

The IARC is a specialist cancer agency of the WHO and, as such, follows the general governing rules of the United Nations. However, IARC has its own Governing Council and Scientific Council. Currently, 25 countries are IARC members, including Australia.

The IARC assessment process

The IARC appoints a Working Group to evaluate carcinogenic risks to humans, which is guided by the Preamble (IARC 2006). The Preamble is a statement of scientific principles; however, the procedures that each Working Group use to implement those scientific principles are not specified and are the prerogative of each individual Working Group. The Monographs produced by the Working Groups assess the strength of available evidence that an agent could alter the age-specific incidence of cancer in humans. Working Group members have usually published significant research related to the carcinogenicity of the agents being reviewed.

The IARC Monographs evaluate cancer hazards and the Preamble emphasises the distinction between a hazard and a risk. A cancer hazard is defined in the Preamble as ‘an agent that is capable of causing cancer under some circumstances’ while a cancer risk is defined as ‘an estimate of the carcinogenic effects expected from exposure to a cancer hazard’. The Preamble cautions that the Monographs identify cancer hazards even when the risks are very low at current exposure levels (IARC 2006).

The IARC assessments also utilise a ‘strength-of-evidence’ approach, rather than the ‘weight-of-evidence approach’ more common in regulatory assessments. The weight-of-evidence approach assesses the predictive validity of a hypothesis, while the strength-of-evidence determines its level of extremeness (Simon 2014). Predictive validity is dependent on factors such as study design, sample size, background rates etc. A strength-of-evidence assessment may be based on a single study where the effect was easily noticeable or was apparent in a large population, even though the predictive value of the study was weak.

The IARC Preamble states that while the Monographs are used by regulatory authorities worldwide to make risk assessments and formulate regulatory decisions, they represent only one part of the body of information that informs regulatory decisions (IARC 2006). The Preamble acknowledges that public health options vary according to circumstance and geographical location and relate to a multitude of factors. As a result, the IARC does not regard regulation or legislation while developing Monographs, as it acknowledges that this is the responsibility of individual governments or other international organisations.

When assessing an agent for a Monograph, the Working Group reviews epidemiological studies, cancer bioassays in experimental animals, as well as exposure, mechanistic and other relevant data. In each case, the Working Group only considers data that has been determined by them to be relevant to the evaluation. Only reports that have been published or accepted for publication in the openly available scientific literature and data from government agency reports that are publicly available are reviewed (IARC 2006). Unlike regulatory authorities, IARC does not consider the often large number of unpublished studies submitted for regulatory assessment.

The outcome of the Working Group's assessment is a categorisation of an agent that reflects the strength-of-evidence from studies in humans and experimental animals and other relevant data. The classifications used by IARC and the circumstances that may lead to an agent being assigned to each group are listed below (IARC 2006):

- Group 1 – the agent is carcinogenic to humans
  - there is sufficient evidence of carcinogenicity in humans
  - evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence that the agent acts through a relevant mechanism of carcinogenicity in humans (exceptional circumstances)
- Group 2A – the agent is probably carcinogenic to humans
  - limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals
  - inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals and strong evidence that carcinogenesis is mediated by a mechanism that also operates in humans
  - limited evidence of carcinogenicity in humans but the agent clearly belongs to a class of agents for which one or more members have been classified in Group 1 or Group 2A (exceptional circumstances)
- Group 2B – the agent is possibly carcinogenic to humans
  - limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals
  - inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals
  - inadequate evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals, as well as supporting evidence from mechanistic and other relevant data
  - strong evidence from mechanistic and other relevant data.
- Group 3 – the agent is not classifiable as to its carcinogenicity to humans
  - inadequate evidence of carcinogenicity in humans and inadequate or limited evidence of carcinogenicity in experimental animals
  - inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans (exceptional circumstances)
  - agents that do not fall into any other group.
- Group 4 – the agent is probably not carcinogenic to humans
  - evidence suggesting lack of carcinogenicity in humans and experimental animals
  - inadequate evidence of carcinogenicity in humans but evidence suggesting lack of carcinogenicity in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data.

Assessment of glyphosate by IARC

In March 2015, IARC evaluated the potential carcinogenicity of five organophosphate pesticides and classified glyphosate (as well as malathion and diazinon) as 'probably carcinogenic to humans', Group 2A. The complete monograph was published in July 2015. Note that where the Working Group cited an unpublished study, it relied on the published summary report as the complete, original study report was not available.

The Working Group concluded that there was 'limited evidence of carcinogenicity' in humans, with a positive association observed between exposure to glyphosate and NHL (IARC 2015). The IARC preamble explains that 'limited evidence of carcinogenicity' in humans is concluded when the Working Group has determined that a credible causal link between the agent and cancer may have been identified 'but chance, bias or confounding could not be ruled out with reasonable confidence' (IARC 2006). The Working Group also concluded that there was 'sufficient evidence of carcinogenicity' in experimental animals (IARC 2015). The IARC Preamble describes that sufficient evidence of carcinogenicity is concluded when a causal relationship between the agent and an increased incidence of malignant neoplasms or an appropriate combination of benign and malignant neoplasms has been established in either two or more species of animals, or two or more independent studies in one species. Sufficient evidence is also considered to be established when an increased incidence of tumours is observed in both sexes of a single species in a well conducted study (preferably conducted according to GLP). Alternatively, sufficient evidence of carcinogenicity may be considered established in a single study in one species and sex when malignant tumours occur to an 'unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites' (IARC 2006).

The studies relied on by the Working Group for human carcinogenicity comprised reports of the Agricultural Health Study (AHS) and various case-control studies conducted in the US, Canada and Sweden. The Working Group concluded that these studies presented increased risks for the development of NHL associated with exposure to glyphosate (IARC 2015).

The AHS was a prospective cohort study of 54 315 licensed pesticide applicators from Iowa and North Carolina, which has produced data relating to the use of pesticides, such as glyphosate on the risk of cancer at various sites. Overall, the study concluded that exposure to glyphosate was not associated with all cancers combined (RR 1.0; 95% CI 0.90–1.2) or any cancer at a specific anatomical site (De Roos et al. 2005).

A study conducted in Canada reported an increased risk of NHL following more than 2 days per year of exposure to glyphosate in 51 exposed cases (OR 1.20; 95% CI 0.83–1.74 when adjusted for age, province and medical variables) (McDuffie et al. 2001); however, no adjustment for other pesticides was performed and the OR spans 1 (indicating that there was no difference between the incidence of cancer in either group). A study conducted in the US (De Roos et al. 2003) and two studies conducted in Sweden (Hardell & Eriksson 1999; Eriksson et al. 2008) reported an increased risk of NHL following glyphosate exposure, which persisted following adjustment for other pesticides. However, the results of Hardell & Eriksson (1999) should be treated with caution, as only 4 glyphosate-exposed cases and 3 controls were included and while an increased OR was reported (2.3), the 95% CI was wide (0.40–13.0), indicating poor precision and spans 1, indicating that there was no difference between the incidence of cancer in either group. Hardell et al. (2002) analysed pooled data that included the data presented in Hardell & Eriksson (1999)—a non-statistically significant elevated risk for NHL following glyphosate exposure with poor precision and an OR that spans 1 was identified (OR 1.86; 95% CI 0.55–6.20). In 29 exposed cases and 18 controls, Eriksson et al. (2008) reported an increased risk for NHL following more than 10 days/year exposure to glyphosate (OR 2.36; 95% CI 1.16–4.40) following adjustment for exposure to other pesticides. After pooling

data from three case-control studies of NHL conducted in the Midwest US in the 1980s, De Roos et al. (2003) reported an increased incidence of NHL following exposure to a number of individual pesticides, including glyphosate (OR 2.1; 95% CI, 1.1–4.0), based on 36 cases. However, while an increased risk was still identified following adjustment for exposure to other pesticides (OR 1.6, 95% CI 0.90–2.8), it was no longer significant. A case-control study also conducted among males in the Midwest US reported an increased risk of developing NHL for men who had ever farmed (OR 1.2; 95% CI, 1.0–1.5) and men who had ever handled glyphosate (OR 1.1; 95% CI, 0.7–1.9); however, no adjustment was made for other pesticides (Cantor et al. 1992). No association between glyphosate exposure and development of NHL was calculated in a hospital-based case-control study conducted in France (OR 1.0; 95% CI 0.5–2.2) (Orsi et al. 2009); however, only 12 exposed cases were assessed. One study conducted in Europe reported an elevated risk for B-cell lymphoma following glyphosate exposure (OR 3.1; 95% CI 0.6–17.1), but again, this study was based on few exposed cases (n=4) and controls (n=2), with a very wide CI (poor precision) that spans 1 and the authors of the paper concluded that no increased risk of either lymphoma overall, or B cell lymphoma was associated with glyphosate exposure (Cocco et al. 2013).

The Working Group also relied on three studies that reported an increased risk of multiple myeloma (a subtype of NHL) following more than 2 days glyphosate exposure per year (Brown et al. 1993; Orsi et al. 2009; Kachuri et al. 2013). However, none of these studies adjusted for the effect of other pesticides and in all three studies, the results were not statistically significant. Therefore, the variation observed in the results could be attributable to normal biological variation and not exposure to glyphosate or other pesticides. A report of data obtained by the AHS found no association between glyphosate exposure and NHL (OR 1.1; 95% CI 0.5–2.4; n=54 315) but saw an increased risk of multiple myeloma when the data were adjusted for multiple confounders, such as demographic and lifestyle factors, as well as other pesticides (OR 2.6; 95% CI 0.7–9.4; n=40 716) (De Roos et al. 2005). However, the number of myeloma cases included in the study was small (32 cases out of 2088 total cancer cases) and the wide CI spanning 1 indicates poor precision and a lack of difference between groups. Re-analysis of the data determined that the increased risk of multiple myeloma (OR 1.24; 95% CI 0.52–2.94) was only present in the subset of subjects for which there was no missing data (22 cases); however, again, the CI spans 1 (Sorahan 2015). This re-analysis of the data concluded that the observed increased risk of developing multiple myeloma following glyphosate exposure resulted from the use of an unrepresentative restricted dataset and that analysis of the full dataset provided no convincing evidence that glyphosate exposure is linked with the development of multiple myeloma (Sorahan 2015).

The studies relied on by the Working Group for animal carcinogenicity comprised two dietary studies in male and female mice, five dietary studies in male and female rats, as well as one drinking-water study of a glyphosate-based formulation in male and female rats.

In mice, one dietary study reported in summary form by the US EPA calculated a positive trend in the incidence of renal tubule carcinoma and renal tubule adenoma/carcinoma combined in male, but not female mice (IARC 2015). A second dietary study reported by the JMPR (2006) in mice observed a significant positive trend in the incidence of haemangiosarcoma incidence in male, but not female mice (IARC 2015). However, haemangiosarcomas were only observed at the highest dose tested in male mice (4/50; 8%). In females, haemangiosarcomas were reported at the lowest (2/50, 4%) and highest (1/50, 2%) doses tested.

Three dietary studies in rats evaluated by the JMPR found no significant increase in tumour incidence in any tissue (JMPR 2006). Of the remaining two studies (evaluated by the US EPA), one reported an increase in the incidence of pancreatic cell adenoma in male rats only; however, no statistically significant dose-response was evident and there was no progression to carcinomas (IARC 2015). In the final study, a significant increase in the incidence of

pancreatic islet cell adenoma and hepatocellular adenoma in males and thyroid C-cell adenoma in females was reported. However, again, there was no statistically significant dose-related trend in the incidence of pancreatic islet cell adenomas and no progression to carcinoma for any tumour type (IARC 2015). No significant increase in tumour incidence was observed following administration of a glyphosate formulation (13.85% solution, purity of glyphosate not reported) to rats in drinking water.

The Working Group concluded that there was strong evidence that glyphosate and glyphosate-based formulations are genotoxic and, along with the main metabolite, AMPA can act to induce oxidative stress. Two studies investigated genotoxicity following exposure of community residents to glyphosate-based formulations, reporting chromosomal damage (micronucleus formation) in blood (Paz-y-Miño et al. 2007) and significant increases in DNA damage (DNA strand breaks) (Bolognesi et al. 2009) four or two months following spraying, respectively. Other studies assessing the effects of either glyphosate or glyphosate-based formulations in human cells *in vitro* produced varied results (IARC 2015). The majority of the studies relied on by the Working Group that assessed genotoxicity in human cells *in vitro* reported DNA damage (DNA strand breaks), which can also be indicative of cytotoxicity and not just genotoxicity. Two studies were relied on by IARC as evidence of chromosomal damage in human lymphocytes *in vitro*. Both studies reported that glyphosate did not produce chromosomal damage without metabolic activation (Manas et al. 2009; Mladinic et al. 2009b). One study reported micronucleus formation following metabolic activation at the highest concentration tested only, but no concentration-related increase in micronucleus formation was evident (Mladinic et al. 2009b). Similarly, experiments utilising glyphosate or glyphosate-based formulations conducted in animals, both *in vivo* and *in vitro* produced varied results (IARC 2015). As for mammalian cells *in vitro*, many of the non-human mammalian genotoxicity studies utilised a DNA damage endpoint, which may be associated with cytotoxicity, rather than genotoxicity. One study assessing mutations in mouse uterine cells reported negative results. Four of the nine studies that assessed chromosomal damage (micronucleus formation) in mouse bone marrow cells produced negative results. Of the remaining five studies that reported positive results, three tested a single dose only, one reported a positive effect at the highest dose tested only and one reported a positive effect at the lowest dose tested only (IARC 2015). No chromosomal aberrations were reported following exposure to glyphosate (single ip dose) (Li & Long 1988) or a single oral dose of a glyphosate-based formulation in mouse bone marrow cells (Dimitrov et al. 2006); however, a single ip dose of a glyphosate-based formulation increased chromosomal aberration in a dose- and time-dependent manner (Prasad et al. 2009).

The Working Group concluded that there was weak evidence that glyphosate may affect the immune system and that glyphosate or glyphosate-based formulations induce receptor-mediated effects, such as aromatase activity. The Working Group also concluded that glyphosate-based formulations may affect cell proliferation or death, the latter via apoptosis; however, glyphosate alone either had no effect or had a weaker effect than the formulated products (JMPR 2006; IARC 2015).

4.2 Assessment of the IARC Monograph

The assessment of the IARC Monograph was undertaken by the Department of Health (OCS). The APVMA requested that OCS conduct a preliminary scoping review of the IARC Monograph to ascertain the relevance of the carcinogenicity classification of glyphosate and any implications that this may have to the registration of glyphosate and glyphosate-based formulations in Australia. In particular, the APVMA requested that OCS identify any relevant data not previously evaluated by Australia. This constituted Tier 1 of the OCS assessment (Supporting document 1).

Tier 2 of the OCS scoping assessment involved a detailed review of any studies that had been reviewed by IARC as part of its assessment of glyphosate and were identified by OCS as requiring further review during the Tier 1 assessment (Supporting document 2).

Previous OCS epidemiological review in 2005

An association between reported glyphosate use and an increased risk of NHL was reviewed by the OCS in 2005 (unpublished). Therefore, the OCS did not assess the epidemiological studies described in the IARC monograph published prior to 2005 and recommended that the APVMA rely on international assessments for any additional epidemiological information relating to glyphosate exposure. The OCS' unpublished 2005 assessment of epidemiological information relating to glyphosate exposure is summarised below.

The first report of an association of glyphosate exposure with NHL was from a case-control study conducted in Sweden; however, this estimate was based on only four exposed cases and three controls (Hardell & Eriksson 1999). A pooled analysis of this initial study with a study of hairy cell leukaemia (a rare subtype of NHL) suggested a relationship between glyphosate exposure and an increased risk of the disease (unadjusted analysis with an OR of 3 and 95% CI 1.1–8.5) (Hardell et al. 2002). A more extensive study across a large region of Canada found an increased risk of NHL associated with glyphosate use of 2 days or more per year, based on 23 exposed cases and 31 controls (OR = 2.1; 95% CI 1.2–3.7) (McDuffie et al. 2001). In a pooled analysis of case-control studies conducted in the US, De Roos et al. (2003) reported an association between glyphosate exposure and increased NHL risk in men after adjustment for other commonly used pesticides, based on 36 exposed cases and 61 controls (OR = 2.1; 95% CI 1.2–4.0).

By contrast, in another cohort study, De Roos et al. (2005) reported that glyphosate exposure was not associated with increased NHL risk in men after adjustment for other commonly used pesticides, based on 92 exposed cases. One plausible explanation for this conflicting result is that all previous studies had a lower number of exposed cases and were retrospective in design, and thereby susceptible to recall bias of exposure reporting. As information on exposures is obtained by questionnaires and interview of farmers or their next-of-kin, often years after the event, the quality of data on pesticide use obtained by recall is questionable (Blair et al. 2002). Indeed, recall bias is particularly problematic for widely used products such as Roundup and the potential for recall bias and for misclassification of pesticides were acknowledged as one of the limitations in all such studies. On the other hand, the study by De Roos et al. (2005) reported a higher number of exposed cases and was prospective in design, which should have largely eliminated the possibility of recall bias. On this basis and also based on the toxicity profile of glyphosate derived from animal studies, it is unlikely that exposure to this chemical is associated with an increased risk of NHL.

This is further supported by a recent epidemiological report showing that NHL incidence decreased between 1991–2000 in Sweden, Finland, Denmark and the US (Hardell & Eriksson 2003), a period in which glyphosate use increased very significantly. It is of interest to note that decreased NHL incidence during this period in Sweden also coincides with a decline in the prevalence of human immunodeficiency virus (HIV), which has been shown to be a risk factor for NHL (Pluda et al. 1993).

Tier 1 assessment of the IARC glyphosate monograph

Tier 1 assessment outcomes

REFERENCE LIST AND KEY STUDY REVIEW

The OCS examined the reference list from the IARC Monograph 112, which included 264 published papers. Publicly available papers were sourced and designated as either:

- relevant for the carcinogenicity classification for humans and requiring further analysis (Tier 2, Part 1)
  - studies previously reviewed by the EU or
  - studies not previously reviewed by the OCS or EU and
    - studies that used glyphosate technical
    - studies that investigated carcinogenicity, genotoxicity or oxidative stress
    - Studies that used relevant test animal models or cell lines, eg mouse, rat, human lymphocytes
- relevance for the carcinogenicity classification for humans unclear and to be determined internationally (the APVMA will rely on international assessment of these studies)
  - studies previously reviewed by the EU or
  - studies not previously reviewed by the OCS or EU and
    - studies that used a formulation of glyphosate
    - studies that were unclear as to the formulation or combination of active constituents used
    - Studies that do not fit the criteria for the other designations
- not relevant to the classification and excluded
  - studies previously reviewed by the OCS
  - studies undertaken using animal models or cell lines not relevant for assessing human toxicity; eg fish, frogs, bovine
  - studies investigating endpoints not relevant to a carcinogenicity classification; eg endocrine disruption, reproduction, immune function, neurotoxicity
  - environmental fate and residue studies
  - determination of glyphosate in air, soil, water or in vivo
  - market/industry summary publications
  - case studies regarding glyphosate poisoning
  - occupational exposure or biomonitoring studies.

Following analysis of the study abstracts, 174 references were excluded from requiring further review. The majority of these papers were excluded because the study utilised non-conventional species or methodology for evaluating human toxicity (eg fish). A total of 19 references were considered relevant to the carcinogenicity classification of glyphosate, requiring further in-depth revision. Of these 19 studies, 9 had been previously reviewed by the EU in

2013 and 10 had not previously been reviewed by either the OCS or the EU. The remaining 71 references were considered to require further review to determine their relevance to the carcinogenicity classification. Of these 71 references, 19 had been previously reviewed by the EU in 2013, five were referenced as US EPA papers (not referenced by the EU) and 47 had not been previously reviewed by either the OCS or EU. These studies will be assessed in detail by the JMPR in 2016.

RECOMMENDATIONS

Based on the Tier 1 assessment, the OCS recommended an evaluation of the studies listed in Table 4 (Appendix A) and an evaluation of the EU position for the key studies listed in Table 5 (Appendix B). This review constituted Tier 2 of the OCS scoping assessment of glyphosate. The studies referenced in the IARC Monograph that were not recommended for evaluation by the OCS are listed in Appendix C (Table 6).

The OCS noted that parallel reviews of the IARC Monograph were being planned or were in progress by independent expert international bodies (eg JMPR). Therefore, the OCS recommended that rather than undertaking a full review in isolation, the APVMA make use of this international assessment. This approach is consistent with the APVMA's policy on the use of international assessments.

Tier 2 assessment of the IARC glyphosate monograph

The Tier 2 assessment involved:

- Evaluation of 19 studies relevant to the carcinogenicity classification of glyphosate (Table 4, Appendix A). Of these, 16 were either considered or critically appraised by EFSA (2015).
  - 12 genotoxicity studies
  - 5 oxidative stress studies
  - 1 epidemiology study
  - 1 classification review report.

The Tier 2 assessment did not include a detailed review of the epidemiological studies or studies that evaluated the possible carcinogenicity of glyphosate-based formulations, as a number of international reviews of the IARC Monograph will be undertaken concurrently with the OCS assessment. A total of 47 studies that were not reviewed by the EU Renewal Assessment Report (RAR) and 19 studies that were reviewed by the EU RAR (Table 5, Appendix B) were not reviewed by the OCS in the Tier 2 assessment of glyphosate because their relevance to the carcinogenicity classification for humans was unclear. The APVMA will rely on international assessments of these studies.

Animal carcinogenicity studies

The OCS evaluated one published study that reviewed animal carcinogenicity studies to support regulatory requirements (Greim et al. 2015). The review paper included nine rat and five mouse studies in a weight-of-evidence assessment of the carcinogenicity of glyphosate that included a review of absorption, distribution, metabolism and excretion (ADME), acute toxicity, genotoxicity, epidemiology and animal chronic toxicity studies.

The authors refer to an article that qualitatively analysed the outcomes from seven cohort studies and 14 case-control studies that examined an association between glyphosate and cancers. No consistent pattern of positive statistical associations between total cancer or site-specific cancer in adults or children exposed to glyphosate was evident (Mink et al. 2012). All studies cited by Mink et al. (2012) were referenced in the IARC Monograph and five (Nordstrom et al. 1998; Hardell & Eriksson 1999; McDuffie et al. 2001; Hardell et al. 2002; De Roos et al. 2005) were included in a previous assessment of glyphosate by the OCS in 2005, which concluded that glyphosate is not mutagenic or carcinogenic and it is unlikely that exposure to glyphosate is associated with an increased risk of NHL. Of the remaining studies cited by Mink et al. (2012), four (Brown et al. 1990; Cantor et al. 1992; Carreon et al. 2005; Andreotti et al. 2009) were considered during the Tier 1 assessment as not appropriate for review because glyphosate was not referred to in the abstract and the remaining 12 were identified as requiring additional assessment in order to determine their relevance to the assessment. Therefore, a detailed appraisal of this paper was not conducted by the OCS as a part of the Tier 2 assessment.

Several one year toxicity studies in animals were reviewed by Greim et al. (2015) but not discussed in detail, as they were not designed to detect neoplasms. However, studies conducted in both rats and dogs indicated low toxicity of glyphosate following repeated daily exposure.

Greim et al. (2015) evaluated five chronic toxicity/carcinogenicity studies (conducted over a minimum duration of 18 months) in mice, four of which were considered reliable and were performed according to GLP following OECD testing guidelines (OECD TGs). In four of those studies, spontaneous tumours were observed at all doses. As no dose-response was observed, these were not considered to be treatment-related. One study observed evidence for an increase in the incidence of malignant melanomas at the highest dose tested; however, this tumour is known to be a common spontaneous tumour in the strain of mouse tested. Another study reported increased incidence of bronchio-aveolar adenocarcinoma and malignant lymphoma at the highest dose tested only; however, these were only observed in males and are known to be a common age-related neoplasm in the strain of mouse tested.

Greim et al. (2015) evaluated nine chronic toxicity/carcinogenicity (24 to 29 months) studies in rats submitted by industry, seven of which were conducted according to principles of GLP. Of the two non-GLP studies, one was conducted prior to the introduction of GLP. Some of the studies reported spontaneous and/or age-related neoplasms that did not exhibit a dose-response relationship and were therefore not considered treatment-related. In some cases, the tumours observed were known to be common age-related tumours in the particular strain of rat used. In addition, some studies reported the development of benign tumours that did not exhibit a dose-response relationship and did not progress to malignant neoplasms. Other studies reported no increase in tumour incidence following glyphosate exposure.

Greim et al. (2015) combined the results from the animal studies with results from human carcinogenicity epidemiology conclusions reported by Mink et al. (2012)[3] and concluded that glyphosate is not carcinogenic. They noted that while some studies reported an increase in a specific neoplasm at high dose, the pooled data did not identify any consistent pattern of neoplasm development or dose-response relationship. Therefore, the authors

[3] Mink et al (2012) concluded that there was no consistent evidence of an association between exposure to glyphosate and cancer in humans.

concluded that the observed effects were not consistent or reproducible and were not treatment related. The OCS agreed with the conclusion that the evidence indicates that glyphosate is not carcinogenic in animals.

Genotoxicity

The OCS appraised 11 studies and one review paper that assessed the genotoxicity of glyphosate.

DNA DAMAGE

Of these studies, six assessed genotoxicity via the comet assay (or single cell gel electrophoresis; SCGE) *in vitro*, using lymphocytes (Mladinic et al. 2009a; Mladinic et al. 2009b; Alvarez-Moya et al. 2014), HepG2 cells (liver carcinoma cells) (Gasnier et al. 2009), Hep-2 cells (epithelial carcinoma cells derived from a cervical cancer) (Manas et al. 2009), GM38 cells (diploid fibroblast cells) or HT1080 cells (fibrocarcinoma cells) (Monroy et al. 2005). All of these studies were considered by the EFSA RAR (2015). As previously described, DNA damage observed using sister chromatid exchange (SCE) or the comet assay is regarded as an indirect measure of genotoxicity and positive results using these endpoints may reflect induction of cytotoxicity, rather than genotoxicity, as DNA damage does not directly measure heritable events or effects that are closely associated with heritable events (Kier & Kirkland 2013).

The OECD TG 489 (2014) for comet assays specifies that exposure to the test substance should occur *in vivo* and cells subsequently isolated and analysed. In contrast, the study by Alvarez-Moya et al. (2014) exposed isolated human peripheral blood lymphocytes directly in vitro to the test substance. Therefore, it is difficult to compare these results with other studies as the exposed cells are likely to be more sensitive to direct exposure. Given this and other limitations in study design and reporting (including a lack of data relating to cytotoxicity), the OCS concluded that the genotoxic effects of glyphosate could not be determined from this study and that it was not reliable for regulatory purposes. Mladinic et al. (2009a) concluded that glyphosate technical is not genotoxic and does not cause oxidative stress at levels relevant to human exposure, and recommended further research utilising a larger sample population. The EFSA RAR (2015) noted that, while the study was a non-GLP, non-guideline study, it met broad scientific principles to determine genotoxicity; however, the positive results obtained at the highest dose tested may reflect cytotoxicity, rather than a true chromosome effect that would indicate genotoxicity. The OCS agreed with the assessment and concluded that the study demonstrated that glyphosate is not genotoxic and does not cause oxidative stress at concentrations relevant to human exposure, but that the results are only reliable as supporting evidence for regulatory purposes. In another study, the same research group concluded that glyphosate technical did not damage DNA at levels of expected human exposure (Mladinic et al. 2009b). However, the EFSA RAR noted a number of critical deficiencies in the study design and reporting (eg the study was not conducted according to GLP or international guidelines, and the proposed mechanism of genotoxicity is not relevant to human exposure levels). The OCS agreed with the conclusion of EFSA that the study is not suitable for regulatory (ie risk assessment) purposes.

Manas et al. (2009) concluded that glyphosate technical was genotoxic (as evidenced by DNA damage) in human Hep-2 cells between 3.00 and 7.50 mM (higher concentrations were cytotoxic) and Gasnier et al. (2009) concluded that exposure to a glyphosate-based formulation was genotoxic to human liver carcinoma (HepG2) cells. However, the study design and level of reporting detail of both studies was criticised by both EFSA and the OCS for a number of reasons. The positive results obtained by Gasnier et al. (2009) were observed only at exceedingly high concentrations that were above the limit dose limit, the potential for cytotoxicity due to membrane damage from surfactants is well known and was not controlled for, the results cannot be fully attributed to glyphosate technical

but may be related to the surfactants, no statistical analysis was performed, variation within the datasets were not reported (despite each experiment being conducted in triplicate) and there was an inadequate level of data reporting. Therefore, both EFSA and the OCS concluded that neither of the studies were suitable for regulatory purposes.

Monroy et al. (2005) reported a concentration-related increase in DNA migration in both normal human GM38 cells and human fibrosarcoma (HT1080) cells, which were statistically significant between 4 and 6.5 mM glyphosate and 4.75 and 6.5 mM glyphosate, respectively. At the highest dose (6.5 mM), DNA damage was approximately 5% and 30% for GM38 and HT1080 cells, respectively. Therefore, the authors concluded that glyphosate induces single-strand DNA breaks in mammalian cells. However, the EFSA RAR and OCS both identified a number of deficiencies in study design and reporting. The EFSA RAR (2015) suggested that the positive results seen may be secondary to cytotoxicity and the concentrations used may be at the threshold for cytotoxicity. When the cytotoxicity and genotoxicity results are combined, significant cytotoxicity (as defined by the authors as < 80% cell viability) was evident at 4.75 mM in HT1080 cells, at which genotoxicity results should therefore no longer be considered reliable. No negative control DNA migration results were reported for the HT1080 cells.
At concentrations at and below 5.5 mM, there was no significant change in the length of migration. The percentage of DNA that was not damaged remained higher than the 'DNA damage' scores combined until 5.5 mM.
In combination, these results suggest a lack of genotoxic potential at non-cytotoxic concentrations (4.75 mM).
For the GM38 cells, 80% of cells were viable at the highest concentration (6.5 mM) tested. Therefore, the data that reported significant DNA migration for the GM38 cells appear reliable. The DNA migration data support the DNA morphology data, with the percentage of cells with no DNA damage only remaining higher than the DNA damage combined up to 4 mM. Therefore, the OCS concluded that the results for HT1080 cells were not reliable for regulatory purposes and that the results for GM38 cells are reliable as supporting evidence only, due to a number of study design and reporting limitations.

One study utilised the SCE assay to assess genotoxicity in human lymphocytes, which was also considered by EFSA. Bolognesi et al. (1997) reported both glyphosate technical (purity not specified) and a glyphosate-based formulation induced a concentration-related increase in SCEs from 1 to 6 mg/mL and 0.1 to 0.33 mg/mL, respectively, and that a larger effect occurred with the formulated product than glyphosate technical. However, the EFSA and OCS identified a number of critical deficiencies in study design and reporting, including deviations from OECD guidelines: the experiment was conducted only in the absence of an exogenous source of metabolic activation; positive controls were not included and therefore the validity of the test system was not confirmed; only pooled data were provided (precluding assessment of the influence of inter-individual variation) and only two subjects were included, which does not allow a meaningful statistical analysis). Therefore, both EFSA and OCS concluded that the study was not reliable for regulatory purposes.

Bolognesi et al. (1997) investigated the potential for glyphosate (300 mg/kg) or Roundup® (900 mg/kg) to induce single-strand DNA breaks following ip administration, using the alkaline elution assay. EFSA concluded that the positive results of this assay may be secondary to cytotoxicity, as the doses of glyphosate were close to or in excess of the ip LD50 of glyphosate in mice. The OCS agreed with this assessment and concluded that the results of the alkaline elution assay are not reliable for regulatory purposes.

GENE MUTATION AND CHROMOSOMAL DAMAGE

Chromosomal effects, such as induction of chromosomal aberrations or micronuclei in cultured mammalian cells are considered direct measures of genotoxicity. Five studies assessed genotoxicity of glyphosate using the *in vivo*

micronucleus assay in various strains of mice, while one utilised the *in vitro* micronucleus assay in human lymphocytes. Significantly increased micronuclei, nuclear buds and nucleoplasmic bridges were reported following glyphosate treatment in the presence of metabolic activation at the highest concentration tested (580 µg/mL glyphosate) in human lymphocytes, but not at concentrations likely to be encountered by humans (Mladinic et al. 2009b). However, both the OCS and EFSA concluded that this study was not suitable for regulatory purposes: positive and negative control results were virtually indistinguishable, negative control data were not reported and despite the authors' claims that the concentrations of glyphosate tested correspond to acceptable safety levels based on evaluated *in vitro* endpoints, these findings need to be validated *in vivo*.

Four of the five reported *in vivo* micronucleus assays (Rank et al. 1993; Bolognesi et al. 1997; Manas et al. 2009; Prasad et al. 2009) utilised the ip administration route, which is not considered relevant for human exposure. Only one *in vivo* study (Chan & Mahler 1992) utilised a more appropriate dietary exposure model. A small but significant increase in micronucleus frequency was observed in male CD-1 mice, following ip exposure (two injections at a 24 hourly interval) to either 300 mg/kg glyphosate technical or 450 mg/kg Roundup® (equivalent of approximately 135 mg/kg glyphosate) (Bolognesi et al. 1997). However, positive controls were not used to validate the assay and the assay was not conducted according to international test guidelines, which specify that a minimum of three doses of the test substance be assessed in order to determine whether a dose-response relationship exists. In Balb-C mice, a significant increase in micronucleated erythrocytes was observed at high concentrations of glyphosate only (400 mg/kg) (Manas et al. 2009); however, this study was criticised by both EFSA and the OCS for major deviations from international test guidelines. In particular, erythrocytes (instead of immature, polychromatic erythrocytes) were scored for micronuclei and it did not appear that scoring was blinded. In Swiss albino mice, it was reported that glyphosate induced a significant dose- and time-dependent increase in bone marrow micronucleated polychromatic erythrocytes (Prasad et al. 2009). Again, this study was criticised by both EFSA and the OCS as the use of dimethyl sulphoxide (DMSO) as a solvent is highly unusual (glyphosate is soluble in water) and ip administration of DMSO has been shown to enhance the toxicity of glyphosate-based formulations. In contrast, no increase in micronucleus frequency was observed following dietary exposure in B6C3F1 mice (Chan & Mahler 1992) or ip exposure in NMRI-Bom mice (Rank et al. 1993). Positive control animals were treated for only 4 weeks (compared with 13 weeks for treated animals) in the dietary exposure study (Chan & Mahler 1992); therefore, the OCS concluded that the results were reliable only as supportive data for regulatory purposes. The other studies were not considered reliable for regulatory purposes, due to the limitations described above.

By applying centromere probes, Mladinic et al. (2009a) analysed micronuclei and nuclear instability in human lymphocytes exposed to glyphosate, with and without metabolic activation. The authors reported a significant increase in the proportion of micronuclei that contained centromeres only at the highest concentration of glyphosate tested (580 µg/mL) with metabolic activation, which the authors suggested could indicate aneugenic activity that is exhibited only above a threshold concentration. The number of early apoptotic and necrotic cells were significantly increased at 580 µg/mL, with and without metabolic activation. The authors concluded that glyphosate technical is not genotoxic at concentrations relevant to human exposure. The OCS agreed with the authors' conclusion and with EFSA's conclusion that the results are reliable as supporting evidence for regulatory purposes. Furthermore, the OCS agrees with EFSA that the positive results obtained at the highest dose tested indicated a possible threshold aneugenic effect associated with cytotoxicity, rather than a DNA-reactive clastogenic effect.

Three studies assessed genotoxicity using chromosome aberration studies in bone marrow cells obtained from Swiss albino mice (Prasad et al. 2009), SD mice (Li & Long 1988) and human lymphocytes (Manas et al. 2009).

The authors reported that glyphosate induced a significant dose- and time-dependent increase in aberrant cells compared with untreated cells in Swiss albino mouse bone marrow cells (Prasad et al. 2009), but not SD mice (Li & Long 1988) or human lymphocytes even at very high concentrations (up to 6 mM glyphosate) (Manas et al. 2009). However, as described above, the study by Prasad et al. (2009) was not considered suitable for regulatory purposes, as DMSO was used as the solvent (instead of water) and the glyphosate/DMSO solution was administered via ip injection. Li & Long (1988) deviated from international guidelines by testing only one concentration of glyphosate, examining only 50 cells per animal for aberrations and by administering glyphosate by ip injection. Manas et al. (2009) deviated from international guidelines by scoring 100 cells per treatment (instead of 200 cells), not reporting replicate data and not concurrently assessing cytotoxicity.

In addition to the chromosome aberration assay, Li & Long (1988) utilised a variety of other methods to assess genotoxicity, including prokaryotic genotoxicity tests (*Salmonella*/histidine plate incorporation reversion assay, *E. coli* WP2 reverse mutation assay, *B. subtilis* Rec-assay) and *in vitro* mammalian genotoxicity tests (Chinese hamster ovary hypoxanthine-guanine phosphoribosyl transferase or CHO-HGPRT gene mutation assay, unscheduled DNA synthesis). No positive responses were reported in any of the tests performed and the authors concluded that glyphosate is not genotoxic. Despite some deviations from international guidelines (only one positive control used and duplicate (rather than triplicate) plating was used in the *Salmonella*/histidine reversion assay and *E. coli* WP2 reverse mutation assay), the OCS and EFSA both concluded that the negative genotoxicity results of Li & Long (1988) were acceptable for regulatory purposes. Rank et al. (1993) also utilised the *Salmonella* plate incorporation reversion assay to assess genotoxicity; however, only Roundup® was tested and only two of the five recommended bacterial strains were used. The authors reported a weak mutagenic effect at 360 µg/plate in one strain (TA98) without metabolic activation and at 720 µg/plate in another strain (TA100) with metabolic activation. However, EFSA concluded that a reliable assessment was not possible due to marked cytotoxicity at and above 360 µg/plate and the lack of a concentration-response relationship. The OCS agreed with EFSA's assessment and concluded that the results were not reliable for regulatory purposes.

Overall, the OCS concluded that the weight-of-evidence indicates that glyphosate is not genotoxic in mammals at concentrations relevant to human exposure.

Oxidative stress

Overall, seven studies assessed the potential for glyphosate to induce oxidative stress. Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and their elimination. ROS are important for cell signalling and cycling and are normally physiologically-controlled to prevent cell damage.

Three studies assessed ROS production in response to in vitro treatment of human HepG2 cells with glyphosate (Chaufan et al. 2014), keratinocytes (HaCaT) (Elie-Caille et al. 2010) and erythrocytes (Kwiatkowska et al. 2014). In human HepG2 cells, a significant increase in ROS formation was observed in cells treated with a glyphosate-based formulation (140% of control), but not glyphosate technical or the glyphosate metabolite, AMPA (Chaufan et al. 2014). However, the OCS concluded that this study was of limited regulatory value, as: the product assessed is not registered for use in Australia; the concentration of glyphosate in the formulated product was unclear and cytotoxicity was higher than that observed for glyphosate technical. In addition, the LC_{50} for the formulation was used in the experiments on ROS formation, while the LC_{20} was used for the other treatments. In human keratinocytes, hydrogen peroxide (H_2O_2) was increased in cells treated with 50 mM glyphosate for 30 minutes (Elie-Caille et al. 2010). The concentrations of glyphosate used in this study were very high (between 10 and 70 mM). As the experiments were performed at the IC_{50}, cell responses due to osmotic stress rather than

glyphosate toxicity cannot be excluded. Furthermore, the EFSA RAR noted that the conclusion that treatment with glyphosate (50 mM) for 30 minutes resulted in overproduction of H_2O_2 was based on a qualitatively thicker and more intense fluorescent area in the cell cytosol, but no quantitative measurement was obtained. The OCS added that light microscopy images of the cells were not included. In human erythrocytes, significantly increased ROS production was observed following exposure to glyphosate, its metabolites and impurities at concentrations up to 5 mM (Kwiatkowska et al. 2014). However, the results were provided graphically without actual data, hence it is not possible to independently evaluate these results. Furthermore, no positive controls were tested, therefore the validity of the assays cannot be ascertained.

Chaufan et al. (2014) also investigated the enzymatic (catalase, CAT; glutathione-S-transferase, GST; superoxide dismutase, SOD) and non-enzymatic antioxidant activity (glutathione equivalents, GSH) in human HepG2 cells *in vitro* following exposure to either glyphosate, AMPA or a glyphosate-based formulation. Exposure to glyphosate did not increase the activity of any of the antioxidants evaluated. Exposure to a glyphosate-based formulation caused a significant increase in SOD and GSH activity, while exposure to AMPA also caused a significant increase in GSH. Tyrosine kinases are also important mediators of the cell signalling processes that are involved in various process such as cell proliferation and apoptosis, and have also been implicated in the development of cancer (Paul & Mukhopadhyay 2004). Chaufan et al. (2014) reported that exposure to the glyphosate-based formulation, but not glyphosate or AMPA increased tyrosine nitration compared with controls.

Overall, the OCS concluded that there was limited evidence for an increase in ROS production following exposure to glyphosate, its metabolites or impurities, or a glyphosate-based formulation in *in vitro* cell culture studies using high concentrations of the test substances; however, the weight-of-evidence indicates that exposure to glyphosate at concentrations relevant to human exposure is unlikely to result in increased ROS production in humans.

Caspases participate in the programmed cell death pathway. Some apoptotic cells display caspase 3/7 activity, in contrast to necrotic cells. Two studies investigated caspase activity *in vivo* in male Wistar rats, following ip administration of glyphosate (alone or in combination with other pesticides) (Astiz et al. 2009) and *in vitro* in human HepG2 cells (Chaufan et al. 2014). In rats, ip administration of glyphosate alone did not induce caspase 3 activity in liver or brain (Astiz et al. 2009). However, the sample size was small (n=4), the study was only conducted in males and the administration route (ip injection) is not directly relevant to human exposure scenarios. In human HepG2 cells, caspase 3/7 activity was indirectly measured in cell lysates. Caspase 3/7 activity was significantly increased by a glyphosate-based formulation, but not glyphosate technical. The OCS concluded that oxidative stress and apoptosis may be plausible mechanisms of action for the *in vitro* cytotoxicity of the glyphosate-based formulation; however, the concentrations of treatments were not specified, limiting the value of the study. Furthermore, the product assessed by Chaufan et al. (2014) is not registered for use in Australia, the concentration of glyphosate in the formulated product was unclear and the concentrations of treatments were not specified.

Calpains have also been implicated in apoptosis. In addition to investigating caspase activity, Astiz et al. (2009) also investigated calpain activity *in vivo* in male Wistar rats following exposure to glyphosate alone and in combination with dimethoate and/or zineb. In the liver, milli-calpain activity was not affected by glyphosate alone. In the brain, milli-calpain activity was significantly reduced in both the substantia nigra and cerebral cortex by glyphosate alone. The authors reported that similar data were obtained for μ-calpain activity, but the data were not presented in the publication. While the results presented by Astiz et al. (2009) were considered by IARC to be supportive of an oxidative stress mechanism of action for carcinogenicity by glyphosate, EFSA and the OCS both concluded that the results reported in brain tissue were not biologically plausible for humans, due to the

blood-brain barrier and rapid elimination of glyphosate via urine. Therefore, the OCS concluded that there was no reliable evidence that glyphosate exposure would be likely to increase caspase or calpain activity in humans following exposure via relevant administration routes.

Bolognesi et al. (1997) investigated oxidative stress in Swiss CD-1 male mice (n=3 per dose) following administration of either 300 mg/kg glyphosate technical or 900 mg/kg of Roundup® (~270 mg/kg glyphosate) via ip injection. Glyphosate technical increased 8-OhdG (8-hydroxy-2'-deoxyguanosine)—a marker of oxidative stress—in the liver 24 hours post-treatment, but did not stimulate a response in the kidney. In contrast, Roundup® increased 8-OhdG in the kidney at 8 and 24 hours post treatment, but did not induce a response in the liver. However, as no positive controls were used the validity of the assay cannot be confirmed.

Oxidative potential and impact on DNA was measured in human lymphocytes using Ferric-inducing ability of plasma (FRAP), thiobarbituric acid reactive substances (TBARS) and the human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) modified comet assay (Mladinic et al. 2009a). The authors reported significantly increased oxidative activity (increased frequency of micronuclei, nuclear buds, nucleoplasmic bridges, total antioxidant capacity (FRAP) and lipid peroxidation (TBARS)) at 580 µg/mL glyphosate. These effects were generally greater in the presence of an exogenous source of metabolic activation. However, no clear concentration-dependent effect was observed for any parameter. The number of early apoptotic and necrotic cells were significantly increased at 580 µg/mL, with and without metabolic activation. The authors concluded that glyphosate does not cause oxidative stress at concentrations relevant to human exposure. The OCS agreed with the conclusion by EFSA that as the study was not conducted according to international guidelines, it can only be used as supporting evidence for regulatory purposes and agrees with the authors' conclusions that the lack of a clear dose-response relationship coupled with positive effects only being apparent at the highest concentration of glyphosate tested indicate that glyphosate is not likely to cause oxidative stress at levels relevant to human exposure.

Three studies assessed various aspects of cell morphology and structural integrity *in vitro* in various human cell lines: HepG2 cells (Chaufan et al. 2014), keratinocyte HaCaT cells (Elie-Caille et al. 2010) and erythrocytes (Kwiatkowska et al. 2014). Human HepG2 cells treated with a glyphosate-based formulation exhibited a higher percentage of condensed and fragmented nuclei (23.5%) indicative of apoptotic cell death compared with negative controls, but positive control data was not provided (Chaufan et al. 2014). Although the OCS concluded that the glyphosate-based formulation was likely to be a stimulator of apoptosis, based on the changes in nuclear morphology and increased caspase 3/7 activity *in vitro*, they also concluded that this study was considered to be of limited regulatory value, for the reasons stated above. In human keratinocytes, exposure to glyphosate resulted in shrunken, elongated cells with significantly affected cell adhesion potential, indicative of apoptosis (Elie-Caille et al. 2010). However, the authors cautioned that the cell line used (HaCaT) exhibits possible distinct functional deficiencies compared with normal human keratinocytes and the results cannot be directly extrapolated to *in vivo* keratinocyte behaviour. Furthermore, a two-fold reduction in cell numbers was also observed. The OCS concluded that it was not possible, based on the information provided in the paper, to determine whether glyphosate induced structural cellular changes or whether sub-confluent cells may inherently develop abnormal morphology due to the reduction in cell numbers. In human erythrocytes, glyphosate exposure did not induce morphological changes (Kwiatkowska et al. 2014). In addition, Astiz et al. (2009) investigated the integrity of the inner and outer mitochondrial membranes and peroxidation of mitochondrial membrane lipids *in vivo* in male Wistar rats, again in both liver and brain cells. As the OCS concluded that the results in brain tissue were not biologically plausible in humans, only the results obtained from liver tissue are considered here. Glyphosate alone did not significantly reduce either inner or outer mitochondrial membrane potential and did not affect mitochondrial cardiolipin content in liver (Astiz et al. 2009). Nevertheless, the OCS and EFSA concluded that the study by Astiz et al. (2009) was

not reliable for regulatory purposes. Although the OCS concluded that there was limited evidence that a glyphosate-based formulation may be capable of stimulating apoptosis, there was not sufficient reliable information indicating that glyphosate is involved in apoptosis in humans, at realistic exposure concentrations and administration routes.

Overall, the OCS concluded that no definitive conclusions could be drawn on the ability of glyphosate products and their associated impurities to induce oxidative stress, as there is limited reliable information available regarding the involvement of an oxidative stress mechanism for inducing cytotoxicity.

4.3 Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

The JMPR is an expert scientific body that was established in 1963 and meets annually to scientifically evaluate pesticide residues in food. The JMPR provides expert scientific advice to the Codex Alimentarius Commission and its specialist committee on pesticide residues, the Codex Committee on Pesticide Residues. The Codex Alimentarius develops international food standards and guidelines, with the aim of protecting consumer health, ensuring fair trade practices and promoting coordination of all food standards work undertaken by government and non-government organisations.

There are two expert panels that meet in parallel (hence the term ‘Joint Meeting’), the Toxicology Panel (the WHO’s Core Assessment Group on pesticides), and the Residues Panel (Organised by the Food and Agricultural Organisation of the United Nations). The Toxicology Panel of the JMPR is responsible for evaluating the adverse effects of pesticides on human health (including carcinogenicity) and establishing health-based guidance values which in turn are important for establishing MRLs used in international trade. The Residues Panel are responsible for evaluating the dietary risks from residues present on food commodities and for setting MRLs. The JMPR is also at the forefront of developing new risk assessment methodologies for pesticides and setting international scientific policy on the interpretation of toxicological studies. Participation in the JMPR is not representational but based on expertise in toxicology and pesticide risk assessment.

The relationship between the WHO, JMPR and IARC

The WHO was established in 1948 to direct and coordinate international health within the UN’s system. The IARC is the specialised cancer agency of the WHO, but has its own Governing Council and Scientific Council. While the JMPR also works under the banner of the WHO, its role is to conduct risk assessments for pesticide residues in food, which includes the potential for pesticide residues in food to adversely affect human health in many ways, not just the potential to cause cancer.

The IARC classifies various chemicals, substances and situations in terms of their carcinogenic hazard, which indicates that some level of exposure could increase the risk to cancer. On the basis of this hazard identification and classification process, the JMPR may determine that it is necessary to evaluate or re-evaluate the safety of residues of that chemical in food, following its use in agriculture. Therefore, the two processes are complementary: the IARC determines whether a chemical may potentially cause cancer, while the JMPR determines whether it is likely humans will develop cancer following exposure to realistic residues of that chemical in food.

Assessment process

The process used by JMPR to assess potential risks associated with pesticide residues in food is described in detail in the International Programme on Chemical Safety (IPCS) Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food, which is a joint publication of the FAO and WHO. The IPCS has developed definitions of hazard and risk, which are adopted by JMPR for its risk analyses (IPCS 2009):

- hazard—inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent
- risk—the probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent.

Therefore, a risk assessment of food chemicals involves characterising the potential hazards associated with the chemical, as well as the potential risks to life and health resulting from exposure to those chemicals present in food over a specified period of time. This means that as well as looking at the potential for a chemical to cause harm, a risk assessment also considers the probability of that harm occurring as a result of realistic exposure scenarios. A risk assessment conducted by JMPR comprises four steps (IPCS 2009):

- Hazard identification—identification of the type and nature of adverse effects that a chemical is able to cause, taking into account the nature of the health hazard and the circumstances under which a hazard may be expressed.
- Hazard characterisation—assessment of the relationship between the administered dose of or exposure to a chemical and the incidence of the observed adverse health effect, including where possible, a dose-response relationship between increasing dose and health hazard incidence.
- Exposure assessment—evaluation of the exposure of for example, a human to a chemical and its derivatives, taking into account the occurrence and concentrations of the chemical in the diet, consumption patterns of foods containing the chemical, the likelihood of people consuming large amounts of those foods and the likelihood of high concentrations of the chemical being present in those foods. There are usually a range of intake or exposure estimates, which may be broken down by subgroups of the population.
- Risk characterisation—the information from the hazard characterisation and exposure assessment is integrated into suitable advice for risk-based decision making, by providing estimates of the potential risk to human health under various exposure scenarios, as well as the nature, relevance and magnitude of these risks.

The information generated from a risk characterisation may be either qualitative or quantitative, as defined by IPCS (2009) (Table 3). Any areas of uncertainty that result from gaps in the scientific evidence or any information on particularly susceptible subpopulations (eg young children, people with predisposing physiological conditions or people using the chemical as part of their occupation etc.) should be clearly outlined in the risk characterisation.

Table 3: Examples of qualitative and quantitative information outlined by the International Programme on Chemical Safety

Qualitative information	Quantitative information
Statements or evidence that demonstrates an absence	A comparison of dietary exposures with health-based

of toxicity even at high exposure levels	guidance values
Statements or evidence of safety in the context of specified uses	Estimates of risks at different levels of dietary exposure
Recommendations to avoid, minimise or reduce exposure	Risks at minimum and maximum dietary intakes
	Margins of exposure

The IPCS describes the general principles of toxicological study design, which should include compliance with GLP and adherence to internationally recognised organisations that provide guidance for standards of design and conduct of toxicological studies, such as the OECD. The IPCS outlines acceptable study design principles for determining absorption, distribution, metabolism and excretion, as well as general systemic toxicity, acute toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, neurotoxicity, immunotoxicity, food allergies/hypersensitivities and effects on the gastrointestinal tract and gut flora. There are also specific guidelines on designing and conducting studies in humans.

The IPCS goes on to provide guidance on the conduct of dose-response assessments, stating that where there is 'sufficient plausibility' for the presence of a cause-effect relationship, dose-response data are essential (IPCS 2009). Guidance is provided for setting health-based guidance values for substances present in food and drinking water, which are used to quantitate the range of acute or chronic oral exposure that presents no appreciable health risk. The ADI is generally set on the basis of the lowest NOAEL in the most sensitive species; however, a benchmark dose may also be used to determine the ADI. Where appropriate, an ARfD is also developed. Generally, a 100-fold uncertainty factor is used to convert the NOAEL obtained from a study using experimental animals into a health-based guidance value in humans; however, additional uncertainty factors may also be applied in certain circumstances (described by IPCS) (IPCS 2009). The default 100-fold uncertainty factor represents two 10-fold factors that allow for:

- differences between average responses in animals and average responses in humans
- variability in responses between average humans and highly sensitive humans.

Guidance is provided by IPCS on how to perform and interpret acute and chronic dietary exposure assessments for chemicals present in food. This assessment combines data about food consumption patterns with data about the concentration of chemicals in food to provide a dietary exposure estimate, which can be compared with the relevant health-based guidance value available for that chemical. The assessment should include the general population, as well as more vulnerable groups, or people expected to have different exposures from the general public, such as infants, pregnant women etc (IPCS 2009).

Pesticide residue data is evaluated by JMPR according to the IPCS guidelines, using data generated from pesticide use that was conducted according to Good Agricultural Practice, which stipulates that effective pest control be achieved while leaving the smallest residue amount practicable. National legislation stipules MRLs, which are the maximum concentrations of pesticide (or veterinary drug) residues permitted in or on a food.

Importantly, the IPCS provides guidance on how to perform a risk characterisation as a part of the risk assessment process, which integrates the information obtained during the hazard characterisation process and the exposure assessment to provide advice to risk managers (IPCS 2009).

Assessment of glyphosate

Glyphosate has been assessed by JMPR in 2003, 2006 and most recently, in 2011. Following the IARC decision in March 2015 to reclassify glyphosate as 'probably carcinogenic to humans' and noting that new data may have been generated since the JMPR's most previous assessment of glyphosate in 2011, the WHO established an ad hoc expert taskforce to evaluate the available data relating to glyphosate and report its findings to JMPR. The task force completed its assessment of the IARC monograph in September 2015 and recommended that JMPR conduct a full re-evaluation of glyphosate, as the IARC assessment included a number of peer reviewed scientific publications that had not been available during the JMPR's 2011 assessment (WHO 2015).

In October 2015, the WHO issued a data call for a number of substances, including glyphosate. This evaluation of glyphosate was discussed at an extraordinary meeting of the JMPR at WHO headquarters in Geneva, Switzerland on 9 to 13 May 2016. The Meeting summary report was published online in May 2016.

The summary report contained a description of how the Meeting evaluated genotoxicity and epidemiological evidence for the active constituent glyphosate, glyphosate-based formulated products and metabolites (JMPR 2016). The Meeting evaluated a large number of genotoxicity studies that were identified via various means: direct submission to JMPR, searches of publicly available literature, requests to the IARC Monographs Secretariat, or requests to industry groups. The Meeting also searched databases for any relevant articles published after the studies cited in the IARC Monograph, using defined search terms. These studies were either unpublished studies that had been submitted by a sponsor to support an application for registration (the majority of which adhered to internationally accepted guidelines) or peer-reviewed studies published in the scientific literature. The studies were separated into categories that reflected their phylogenetic relevance and the significance of the genetic end-point measured: human biomonitoring studies, *in vivo* mammalian studies, *in vitro* mammalian cell culture models, *in vitro* bacterial models, phylogenetically distant organisms, metabolites *in vivo* and finally, metabolites *in vitro*. Overall, mammalian *in vivo* studies were given more weight than *in vitro* cell culture studies or studies using phylogenetically distant organisms, and studies of gene mutations and chromosomal alterations were given more weight than studies measuring less serious or transient types of genotoxic damage. Studies that measured the effects of oral exposure were considered to be more relevant for determining dietary exposure. Human biomonitoring studies were most likely to be confounded by exposure to other pesticides or other limitations. An overall weight-of-evidence assessment approach was used to reach conclusions about the genotoxicity of glyphosate, based on an evaluation of the studies using the criteria described above as well as an assessment of the overall quality of each study.

The meeting used a pre-agreed evaluation process, as described in the JMPR (2016) Meeting summary, to:

- select glyphosate/cancer site combinations for inclusion in the evaluation
- screen papers for inclusion or exclusion in the evaluation
- evaluate the information for risk assessment.

Glyphosate/cancer site combinations were included if IARC identified positive associations from the evidence it assessed and all studies cited by IARC, published since the IARC assessment was completed or identified from reference lists of already identified papers were screened for inclusion in the evaluation. Papers were included if they were the most recent publication with the longest follow-up period for that glyphosate/cancer site combination and/or the most complete analysis of that glyphosate/cancer site combination with the largest sample size/number

of participants, providing that the exposure assessment was specific to glyphosate and quantitative (ie exposure was expressed on a ratio scale), and that the paper was relevant and could contribute to a quantitative risk assessment for that glyphosate/cancer site combination.

As described in the JMPR (2016) Meeting summary, for each paper that was included in the assessment:

- the quantitative exposure units were determined
- the magnitude of effect or uncertainty was described
- the quality of the study was reviewed
- the exposure assessment was described
- the manner in which exposure levels compared or translated to glyphosate residue levels or pathways was described.

As described in the JMPR (2016) Meeting summary, for each glyphosate/cancer site included in the assessment:

- the hazard from all studies contributing to the quantitative risk assessment was characterised
- the strength-of-evidence was summarised.

When evaluating the evidence for glyphosate/cancer site associations, the Meeting considered factors that would decrease the level of confidence in the body of evidence (including the risk of bias, unexplained inconsistencies and imprecision) as well as factors that would increase the level of confidence in the body of evidence (including a large magnitude of effect, dose-response and consistency) (JMPR 2016). When evaluating the information available for risk assessment and hazard characterisation, the Meeting evaluated the overall evidence for dose-response relationships, by comparing risk estimates with quantitative exposure measures (eg days of use per year) (JMPR 2016).

The Meeting considered prospective cohort studies to be a more powerful study design than case-control studies, as case-control studies are usually retrospective and are therefore more prone to recall and selection biases (JMPR 2016). The one large, prospective cohort study (the AHS cohort) found no evidence of a positive association between glyphosate exposure and NHL incidence. Various case-control studies reported varying results, with some reporting elevated risks (both significant and non-significant) and others not observing an association. The Meeting concluded that there was some evidence of a positive association between glyphosate exposure and the risk of NHL; however, the AHS—a large, high-quality prospective cohort study found no evidence of an association at any exposure level (JMPR 2016).

The Meeting identified nine carcinogenicity studies in mice, two of which were considered to be of insufficient quality for inclusion in the assessment (JMPR 2016). Equivocal evidence of lymphoma induction was apparent in 3/7 studies in male mice and 1/7 studies in female mice at high doses (5000–40 000 ppm or 814–4348 mg/kg bw/day). In contrast, higher doses (up to 50 000 ppm or 7470 mg/kg bw/day) in the remaining three studies did not cause an effect. In 4/7 studies, there was a trend for a marginal increase in induction of kidney adenomas in male mice at the highest dose tested; however, again, higher doses failed to illicit a response.

The Meeting identified 11 combined chronic toxicity and carcinogenicity studies in rats; however, one was considered inadequate for carcinogenicity assessment (short exposure duration of only 12 months) (JMPR 2016).

An increased incidence of various tumours (interstitial cell tumours of the testes, pancreatic islet cell adenoma, thyroid C-cell tumours, skin keratoma) was observed in 1/10 or (in one case) 2/10 studies. However, in all cases, higher doses used in other studies did not illicit a response. The Meeting also reported a lack of dose-response relationship for some tumour types. There was no evidence for spleen or kidney lymphoma induction in any of the studies. Therefore, the Meeting concluded that there was no reliable evidence for treatment-related tumours in rats at doses of up to 32 000 ppm (or 1750 mg/kg bw/day).

The Meeting concluded that glyphosate is not carcinogenic in rats, but was unable to exclude the possibility that glyphosate is carcinogenic in mice at very high doses (JMPR 2016).

The overall weight-of-evidence suggested that oral doses of up to 2000 mg/kg bw/day glyphosate (either alone or in a formulated product) are not associated with genotoxic effects in the majority of studies in mammals. In cell culture models and organisms that are phylogenetically different to humans, DNA damage and chromosomal effects have been observed following exposure to glyphosate. However, these effects have not been replicated in oral *in vivo* mammalian model studies. Therefore, the Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures (JMPR 2016).

The Meeting's overall conclusion relating to the carcinogenic potential of glyphosate was that, the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity in mammals following oral exposure, along with the epidemiological evidence from occupational exposure indicated that glyphosate is unlikely to pose a carcinogenic risk to humans via exposure from the diet (JMPR 2016).

The Meeting also concluded that there was no evidence from seven studies in rats that up to 30 000 ppm (or 1983 mg/kg bw/day) glyphosate resulted in reproductive toxicity. There was also no evidence for teratogenicity or developmental toxicity in rats (up to 3500 mg/kg bw/day; four studies) or rabbits (low-incidence fetal effects were observed in 3/7 studies at doses that exceeded maternal toxicity). There was no evidence of endocrine disruption, with a range of *in vitro* and *in vivo* assays demonstrating no interaction with oestrogen or androgen receptor pathways or thyroid pathways. There was no evidence of neurotoxicity in rats (up to 2000 mg/kg bw/day) or immunotoxicity in female mice (up to 500 ppm, or 1448 mg/kg bw/day) (JMPR 2016).

Finally, the Meeting concluded that the extent to which glyphosate adversely effects the microbiota of the human or mammalian GIT is unclear, as this is an emerging area of scientific research. However, the available information on minimum inhibitory concentration values suggest that it is unlikely that dietary glyphosate residues would be capable of adverse effects on normal GIT microbiota function (JMPR 2016).

The Meeting further concluded that the glyphosate metabolite, AMPA, is unlikely to be genotoxic following oral exposure in mammals and there was no evidence for embryo or fetal toxicity. Similarly, two other metabolites, *N*-Acetyl-glyphosate and *N*-Acetyl-AMPA are unlikely to be genotoxic in mammals (JMPR 2016).

4.4 European Food Safety Authority (EFSA)

Assessment process

The European Food Safety Authority requires scientific information that has adhered to OECD guidelines on toxicological testing of chemicals and the EU Test Method Regulation No. 440/2008, which stipulates in detail how the studies must be conducted. By European law, all required studies must be conducted according to the

principles of GLP. Scientific information that does not meet these standards but has been published in peer-reviewed journals are also included in the assessment.

When evaluating the carcinogenic effects of a chemical, the RMS delegated to conduct the assessment must follow the classification criteria outlined in EU Regulation (EC) No 1272/2008 on CLP criteria. The CLP criteria for establishing the level of evidence (eg sufficient, limited evidence etc.) for a carcinogenic effect are similar to those used by IARC; however, additional factors that influence the overall likelihood that a substance may be carcinogenic to humans must be taken into account. The emphasis placed on each individual factor is dependent on the amount and coherence of available evidence. Generally, more complete evidence is required to decrease the level of concern than is required to increase the level of concern. Some examples of factors to be taken into account include:

- tumour type and background incidence
- multi-site responses
- progression of lesions to malignancy
- reduced tumour latency
- whether responses are in single or both sexes
- whether responses are in single or multiple species
- structural similarity of the chemical to another substance for which there is good evidence of carcinogenicity
- routes of exposure
- comparison of absorption, distribution, metabolism and excretion between experimental animals and humans
- the possibility of a confounding effect of excessive toxicity at experimental doses
- mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression or mutagenicity.

Assessment of glyphosate

Glyphosate is registered for use throughout Europe and the UK and in 2010 was subjected to a re-assessment by the RMS, Germany, as mandated by the EC and coordinated by EFSA (See Section 2.3).

The BfR concluded that glyphosate was ‘unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential’ (EFSA 2015).

During the re-evaluation process, the BfR evaluated more than 150 new toxicology studies and re-assessed nearly 300 toxicological studies, as well as considering around 900 scientific publications and reviewing more than 200 in detail. The BfR concluded that the available data do not demonstrate that glyphosate exhibits carcinogenic or mutagenic properties or that it has adverse effects on fertility, reproduction or embryonal/fetal development in laboratory animals. The BfR concluded that there was convincing evidence that the toxicity associated with some glyphosate-containing products was attributable to co-formulants, such as tallowamines used as surfactants.

In July 2015, the BfR was commissioned to review the IARC monograph on the re-classification of glyphosate.

The BfR agreed with the conclusion that there is 'limited evidence in humans for the carcinogenicity of glyphosate' and its assessment of the epidemiological studies was comparable to that of the IARC Working Group. However, the BfR also noted that no consistent positive association between glyphosate exposure and the development of cancer was demonstrated and the most statistically highly-powered study detected no effect. The BfR further noted that it was not possible to differentiate between the effects of glyphosate and the co-formulants from the epidemiology studies discussed in the IACR monograph (Germany 2015).

The BfR disagreed with the conclusion by the IARC Working Group that there is 'sufficient evidence in animals for the carcinogenicity of glyphosate', which was based on a positive trend in the incidence of rare renal tumours, a positive trend for haemangiosarcoma in male mice and increased pancreatic islet-cell adenoma in male rats. The BfR assessed the studies relied on by the IARC Working Group and concluded that the weight-of-evidence suggests that there is no carcinogenic risk related to the use of glyphosate and that no hazard classification for carcinogenicity is warranted according to the CLP criteria (Germany 2015). Three studies conducted in mice reported a significant positive trend for renal tumours following glyphosate exposure, when data were analysed using the Cochran-Armitage test for linear trend; however, the analysis by pair-wise comparisons did not demonstrate a significant difference between the groups and the incidences of tumours were within the historical control range (up to 6% for adenoma and carcinoma combined). Similarly, two studies conducted in mice reported a significant positive trend for haemangiosarcoma following glyphosate exposure, when data were analysed using the Cochran-Armitage test for linear trend; however, analysis by pair-wise comparisons did not demonstrate a significant difference between the groups. Furthermore, the background incidence for haemangiosarcoma in male mice is up to 12%. Two of three studies conducted in mice reported a significant positive trend for malignant lymphoma following glyphosate exposure, when data were analysed using the Cochran-Armitage test for linear trend; however, the analysis by pair-wise comparisons did not demonstrate a significant difference between the groups in all three studies. Again, the incidences of malignant lymphoma were within the historical control range (up to 12%). The BfR determined that a significant difference to the incidence of pancreatic islet cell adenomas in rats occurred in the low dose group only, therefore was considered incidental (ie there was no dose-response effect). Therefore, the BfR concluded that the observed incidences of renal tumours, haemangiosarcoma and malignant lymphoma were spontaneous and not related to glyphosate exposure.

The BfR also disagreed with the IARC's conclusion that there 'is mechanistic evidence for genotoxicity, oxidative stress, inflammation, immunosuppression, receptor-mediated effects, and cell proliferation or death of glyphosate'. The BfR concluded that a weight-of-evidence assessment approach indicates that neither glyphosate nor AMPA induce mutations *in vivo* and no hazard classification for mutagenicity was warranted according to CLP criteria (Germany 2015). It further concluded that the mechanistic and other studies do not provide evidence for a carcinogenic mechanism. Consistently negative results were observed in *in vitro* bacterial assays and mammalian cell gene mutation assays and the majority (all of the GLP-compliant studies) of the *in vitro* chromosomal aberration tests and micronucleus tests were also negative. *In vitro* studies produced negative results for induction of DNA repair but positive results for induction of SCE and DNA strand breaks. *In vivo*, 14 somatic cell tests for induction of chromosomal aberrations or micronuclei were negative even at extremely high intraperitoneal doses and there was no evidence for mutagenic activity in germ cells. Two publications reported significant increases in micronuclei following ip administration; however, in both studies the dose tested was in the range of the ip LD_{50} of glyphosate in mice and one study was fundamentally flawed in design. Two publications reported induction of DNA strand breaks following exposure to very high ip doses or repeated oral doses, which were close to or exceeded the ip LD_{50} of glyphosate in mice; therefore, the observed positive results may be the result of secondary effects of cytotoxicity. However, the BfR noted that no firm conclusions can be drawn with regard to a need for classification according to the CLP criteria, regarding specific glyphosate-based formulations, for which there was some

evidence for *in vivo* mammalian chromosomal damage. The BfR recommended that further genotoxicity studies be conducted according to OECD test guidelines.

The BfR agreed with the IARC Working Group that glyphosate does not appear to exhibit endocrine disrupting properties (Germany 2015).

The BfR agreed with the IARC Working Group that there is some indication of induction of oxidative stress, based on *in vitro* studies using human cells and *in vivo* mammalian studies, particularly in blood plasma, liver, brain and kidney of rats; however, it was not indicative of genotoxic or carcinogenic activity in humans. Furthermore, the majority of this work was conducted using a glyphosate-based formulation rather than glyphosate alone. There was no indication of induction of oxidative stress by AMPA.

While the IARC Working Group concluded that there was 'weak evidence that glyphosate may affect the immune system, both the humoral and cellular response', the BfR concluded that the available data do not indicate that glyphosate or glyphosate formulations adversely affect the immune system (Germany 2015). However, it noted that the small number of available studies had methodological limitations and therefore no robust information was available to conclusively determine the possible immunomodulatory action of glyphosate. The BfR mostly agreed with the reporting of the studies relied on by IARC; however expanded on a number of points. For example, the IARC Working Group concluded that one study demonstrated 'pathological effects of glyphosate on the immune system' in rats (Chan & Mahler 1992). However, the only finding reported was a reduction in absolute/relative thymus weight in male rats at the highest dose of glyphosate tested. The BfR concluded that this reduction in thymus weight in male rats was likely related to non-specific toxicity, as evidenced by a lower weight gain and a lower final bodyweight (18%) in male rats, which was not observed in females.

4.5 The European Chemicals Agency (ECHA)

The ECHA is responsible for managing the harmonised classification (CLH) process for active constituent chemicals within plant protection products in the EU. The CLH is based solely on the hazardous properties (ie toxicity) of the chemical and does not take into account exposure; therefore, the CLH procedure conducted by ECHA is not a risk assessment. In that respect, the CLH procedure undertaken by ECHA is similar to the scope of the IARC assessment process.

As a part of the procedure for the renewal of the glyphosate registration in the EU, Germany submitted a proposal for CLH to ECHA. The ECHA launched a 45 day public consultation of the CLH proposal for glyphosate on 2 June 2016 (deadline for comment 18 July 2016). In addition to the existing CLH (eye irritation and aquatic toxicity), a new classification was proposed (ECHA 2016):

- STOT RE 2: May cause damage to organs through prolonged or repeated exposure.

This proposed classification was based solely on the results obtained from developmental studies conducted in rabbits (which appear to be the most sensitive laboratory animal species), where adverse effects (maternal toxicity; NOAEL = 50 mg/kg bw/day) occurred at doses lower than those occurring in the very large number of studies conducted in mice, rats and dogs over longer durations of exposure. Based on CLP hazard criteria, the NOAEL of 50 mg/kg bw/day is lower than the 28-day guidance value in rats (< 300 mg/kg bw/day) and therefore glyphosate technically qualifies for this statement.

The ECHA concluded that a weight-of-evidence approach indicated that glyphosate is not mutagenic and that no hazard classification for mutagenicity was warranted according to the CLP criteria (ECHA 2016). The ECHA considered that standard mutagenicity tests (eg cytogenetic tests or micronucleus assays) were more reliable and carried greater weight than 'indicator tests' (eg comet assays or DNA damage assessed via sister chromatid exchange or DNA strand breaks). Generally, these indicator tests are regarded as useful follow-up tests for confirmation of positive or equivocal standard *in vitro* test results.

Consistently negative results were obtained from *in vitro* bacterial assays and mammalian cell gene mutation assays. Guideline *in vitro* mammalian chromosome aberration tests and micronucleus tests also produced negative results. In contrast, positive results were reported in *in vitro* indicator tests for SCE and DNA strand breaks. Negative results were reported from 11 *in vivo* micronucleus tests or cytogenetic studies in somatic cells that followed international guidelines, while one study reported a weak positive effect in female mice receiving a very high (likely cytotoxic) dose. Inconsistent results were obtained in a number of published studies that did not adhere to international guidelines and generally tested low doses via the ip route. As for *in vitro* studies, positive results for DNA damage (eg strand breaks) were observed in a number of published indicator tests following high ip or repeated oral (via drinking water) administration, while a study assessing unscheduled DNA synthesis produced negative results. There was no evidence of mutagenic activity in germ cells of mice and rats following oral doses of up to 2000 mg/kg bw.

The ECHA concluded that a weight-of-evidence assessment of epidemiological data and data from long-term studies in both rats and mice indicate that no hazard classification for carcinogenicity was warranted for glyphosate according to the CLP criteria (ECHA 2016). In the discussion relating to carcinogenicity, the ECHA addressed the differing assessments of the available information by IARC and EFSA. The ECHA also noted that glyphosate differed from most other pesticides in that a number of comprehensive and high quality studies are available for nearly all toxicological endpoints.

A total of 5/8 long-term, guideline-compliant studies conducted in mice were considered by ECHA. The ECHA took into account the known very large variability of the incidence of spontaneous malignant lymphoma in both Swiss and CD-1 mice, the consistent lack of any dose-response relationship between tumour incidence and glyphosate exposure and the excessively high concentrations that elicited increased incidences of tumours in some studies and concluded that, overall, there was inconsistent evidence for the occurrence of malignant lymphoma, renal tumours and haemangiosarcoma in males but not females.

The ECHA evaluated a total of 7/11 studies conducted in rats, the majority of which (6/7) were guideline-compliant. The non-guideline study (Lankas 1981) was not considered suitable for regulatory purposes due to study design and reporting limitations. The ECHA took into consideration the consistent lack of statistical significance using pairwise analyses, the consistent lack of any dose-response relationships and the lack of reproducibility across multiple studies and concluded that there was no evidence for an association between glyphosate exposure and pancreatic islet cell adenomas, hepatocellular adenomas, C-cell thyroid adenomas or interstitial testicular tumours.

The ECHA also assessed human data on the potential carcinogenicity of glyphosate noting that the value of this data had limitations for regulatory assessments, as it was exclusively derived from epidemiological studies. Firstly, it is difficult to distinguish between the effects of the active constituent and co-formulants, because humans are never exposed to the active constituent alone. As the co-formulants are not only contained in glyphosate-based products, but are also contained within other formulated products, an assessment of the entire formulated product is not indicative of the safety of the active constituent or glyphosate-based products specifically. Secondly, humans

are exposed to a great number of environmental chemicals, making it difficult to attribute health effects to one specific chemical.

The ECHA described the results of the AHS study that analysed data from approximately 57 000 pesticide applicators. Analysis of this data did not identify an association between glyphosate and various forms of cancer, including leukaemia, melanoma, all lymphohaematopoietic cancers, NHL, or cancer of the lung, prostate, breast, colon, rectum, oral cavity, pancreas, kidney or bladder (De Roos et al. 2005; Blair & Freeman 2009). Some papers relied on by the IARC assessment reported positive associations between glyphosate exposure and NHL; however, this association was based on very small sample populations with low numbers of exposed subjects, relied on reported use (and was therefore susceptible to recall bias) by either primary or secondary (eg relatives) sources and was not statistically significant in one study (Nordstrom et al. 1998; Hardell & Eriksson 1999; McDuffie et al. 2001; De Roos et al. 2003; Hardell & Eriksson 2003; Eriksson et al. 2008). In contrast, the ECHA also described 18 papers that did not identify a risk between glyphosate exposure and various specific cancer types (Alavanja & Bonner 2012): prostate cancer (Alavanja et al. 2003; Band et al. 2011; Koutros et al. 2011), stomach and oesophageal adenocarcinomas (Lee et al. 2004), gliomas (Carreon et al. 2005), breast cancer (Engel et al. 2005; El-Zaemey et al. 2013), childhood cancer (following parental exposure) (Flower et al. 2004), pancreatic cancer (Andreotti et al. 2009), monoclonal gammopathy (Landgren et al. 2009), Hodgkin's lymphoma (Karunanayake et al. 2012), multiple myeloma (Pahwa et al. 2012; Kachuri et al. 2013), NHL (Schinasi & Leon 2014), lymphomas in general (including B cell lymphoma) (Cocco et al. 2013) or soft tissue sarcoma (Pahwa et al. 2011).

The ECHA concluded that, while epidemiological data is of limited value for detecting the carcinogenic potential of a pesticide, the data do not provide convincing evidence for an association between glyphosate exposure in humans and any cancer type and no hazard classification for carcinogenicity is warranted for glyphosate according the CLP criteria (ECHA 2016).

Following the public consultation, any received comments will be provided to the Committee for Risk Assessment (RAC), which will form an opinion on the hazard classes that were open for consultation only. For glyphosate, these include: all health hazards except respiratory sensitisation and aspiration hazard (carcinogenicity, germ cell mutagenicity and reproductive toxicity) and all environmental hazards except ozone layer hazards. In addition, ECHA may request further clarification and contact some of those who commented to discuss specific issues. From there, any opinion of the CLH proposal must be adopted by RAC within 18 months from the receipt of that proposal by ECHA and the 'background document', which contains the CLH report with RAC evaluations inserted will be published on the ECHA website. The ECHA will then forward the RAC opinion to the EC, which will determine whether the CLH is appropriate.

4.6 Health Canada

In 2010, Health Canada's PMRA commenced a re-evaluation of glyphosate in collaboration with the US EPA's re-evaluation of glyphosate. In April 2015, the PMRA published its Proposed Re-evaluation Decision (PRVD2015-01) for glyphosate, as discussed above in Section 2.2. In conducting re-evaluations of registered products, the PMRA utilises data from holders of product registrations, as well as published scientific reports, information from other regulatory agencies and any other information considered relevant to the evaluation. The PMRA evaluation of the available scientific information concluded that there were no unacceptable risks to human health or the

environment as a result of using glyphosate according to the proposed label directions and no additional data were requested.

The re-evaluation report describes how the potential risks to human health are assessed, which is similar to the method employed by the APVMA. The PMRA re-evaluation of glyphosate determined that adverse effects observed in animals occurred at doses more than 100 times higher than levels to which humans are normally exposed when using glyphosate according to label directions. The re-evaluation reported that glyphosate has low acute oral, dermal and inhalational toxicity, does not irritate the skin or cause allergic skin reactions in laboratory animals; however, it was a severe eye irritant.

The PMRA determined that acute dietary exposure represented between 12% and 45% of the ARfD for all of the population subgroups. The chronic dietary exposure estimate for the general population represented 30% of the ADI, with a range of 20% to 70% of the ADI for the various population subgroups. As a result, the PMRA concluded that acute and chronic dietary risks were not of concern when glyphosate is used according to the label directions.

The re-evaluation also assessed residential handler exposure from mixing, loading and applying glyphosate product to residential lawns and turf (primarily dermal) as well as incidental oral exposure of children playing in treated areas. Bystander exposure was estimated for scenarios where people enter non-cropland areas, such as parks or hiking areas that had recently been treated with glyphosate. For all of these assessments, assessed either alone or in combination with background chronic dietary exposure (discussed above), no evidence of health risk was determined. Similarly, the risk estimates associated with mixing, loading and applying glyphosate in an agricultural scenario or re-entering treated agricultural sites did not demonstrate any health risks, based on the current directions for use and agricultural use patterns.

The PMRA re-evaluation report addressed the IARC conclusions, emphasising that a hazard classification is not a health risk assessment. They also stressed that the level of human exposure is the factor that determines the risk and that this was not taken into account in the IARC classification of glyphosate. The PMRA considered the epidemiological information included in the IARC assessment and concluded that the majority lacked adequate characterisation of glyphosate exposure, which limited their suitability for assessing the hazard of glyphosate.

The PMRA concluded that the available *in vitro* and *in vivo* tests demonstrated that glyphosate is not genotoxic in rats or mice and that glyphosate is not carcinogenic in rats. While there was some evidence for a marginal increase in the incidence of ovarian tumours in mice, no dose-response was evident and the increased incidence was only observed at the highest tested doses and historical control data were not available. Therefore, the PMRA concluded that these results were of low concern for human health risk assessment.

Overall, the PMRA concluded that the weight-of-evidence obtained from both acute and chronic animal toxicity studies, genotoxicity assays and epidemiology studies indicates that glyphosate is unlikely to pose a human cancer risk.

4.7 New Zealand Environmental Protection Authority

The New Zealand Environmental Protection Authority commissioned a review of the evidence relating to the carcinogenicity of glyphosate. The scope of the review covered the basis on which the IARC Working Group classified glyphosate as a probable human carcinogen, which involved reviewing the quality of the evidence for

carcinogenicity in humans and animal models, as well as the data used to support mechanistic arguments (Temple 2016).

The review concluded that a possible dose-response relationship in humans could not be evaluated, as the epidemiological evidence did not indicate whether any internal exposure was measured or, if there was, the extent of that exposure. The review also agreed with conclusions by WHO in 2006, which reported that weak, rarely statistically significant associations between glyphosate exposure and lymphopoietic cancers do not generally meet the criteria for determining causal relationships from epidemiology data.

The review discussed each epidemiological study relied on by the IARC Working Group in its assessment that there was 'limited evidence' for carcinogenicity in humans, following exposure to glyphosate, as well as a review conducted by Mink et al. (2012) and the assessment conducted by the BfR for EFSA. As with other assessments, the review placed more weight on the prospective AHS cohort study, which did not identify an association between glyphosate and NHL, or a number of other cancer types, even though exposure was higher than that presented in the case-control studies. The review highlighted the fact that only two of the case-control cohort studies cited by the IARC Working Group reported statistically significant increased ORs at the 95% confidence level (Temple 2016).

The review noted that a small, non-significant increased risk of multiple myeloma was identified in the AHS cohort (De Roos et al. 2005), but described in detail the reassessment of that data, which questioned that result (Temple 2016). This re-assessment argued that the reported elevated risk ratio (RR) for multiple myeloma were not relevant, as they resulted from a restricted data set that (most likely by chance) were not actually representative of the population (Sorahan 2015). That is, a number of cases of multiple myeloma in the group of pesticide applicators who had never used glyphosate were excluded from the original analysis because they did not have data about the use of alcohol, smoking etc. This resulted in a false impression of increased risk in ever users, compared with those who had never used glyphosate. The re-analysis resulted in a RR of 1.1 (Sorahan 2015), compared with the original estimated rate ratio of 2.6, reported by De Roos et al. (2005).

One Swedish case-control study reported an association between glyphosate exposure and cancer risk after more than 10 years of exposure (OR 2.26, 95% CI 1.16–4.4) using 29 exposed cases and 18 unexposed controls (Eriksson et al. 2008) and was considered by the IARC Working Group to be a large study. In contrast, Temple (2016) concluded that 29 cases and 18 controls could not be considered a large study and had limited power to detect an effect. The significant effect reported in this study was only significant using a univariate evaluation and there was the possibility that results could have been confounded by earlier exposure to MCPA (2-methyl-4-chlorophenoxyacetic acid), which is associated with an increased risk of NHL.

The review highlighted that the key studies cited in support of 'sufficient evidence' for carcinogenicity in experimental animals consisted of three studies in mice: a positive trend for increased renal tubule carcinoma in one oral study; a positive trend for increased incidence of haemangiosarcoma in one oral study; and tumour promotion in a skin study. The review also highlighted that the IARC Working Group used different statistical tests (trend analysis) to assess the data in those studies, compared with the original analysis (pairwise comparisons). In the original pairwise comparisons, none of the studies produced positive associations. The IARC Working Group also did not take into account historical incidence data or the presence of a viral infection which may have affected survival rates and lymphoma incidence in one study. In addition, a number of studies that have been used by other regulators (which did not support an association between glyphosate and carcinogenicity) were not considered by the IARC Working Group noting that this is consistent with the scope of IARC. The New Zealand

review concluded that the total database of long-term carcinogenicity bioassays were consistently negative and the positive findings reported by the IARC Working Group are not considered supportive of carcinogenicity by other reputable scientific bodies, therefore the overall weight-of-evidence does not indicate that glyphosate is carcinogenic (Temple 2016).

The review concluded that the studies relied on by the IARC Working Group as 'strong evidence' for genotoxicity and oxidative stress primarily utilised *in vitro* mammalian cell studies, in which mammalian cells are directly exposed to glyphosate (or a formulated product) at high concentrations that are not realistic to *in vivo* exposure in animals or humans. The review highlighted that all studies that followed internationally accepted guidelines produced negative results, while all positive associations were achieved in studies that used unvalidated test methods or species, glyphosate formulations, or high intraperitoneal doses that are widely considered inappropriate for assessing genotoxicity in humans (Temple 2016).

The overall conclusion of the review was that, based on a weight-of-evidence approach that considered the quality and reliability of the available data, glyphosate is unlikely to be genotoxic or carcinogenic to humans and does not require classification as either a carcinogen or a mutagen (Temple 2016).

4.8 Adverse Experience Reporting Program (AERP)

The AERP is a post-registration program that assesses reports of adverse experiences associated with the use of agricultural and veterinary products, when the product has been used according to the approved label instructions.

Between 1996 and 2013, a total of four AERs relating to human safety were submitted to the AERP. All were classified as 'possible' or 'probable' by the AERP. Of the four AERs, one related to skin irritation while the remaining three were reports of eye irritation.

5 ASSESSMENT OUTCOMES

In the Tier 1 assessment, the OCS examined the reference list from the IARC Monograph 112 for glyphosate, which included 264 publisher papers. Following analysis of the study abstracts, 174 references were excluded from requiring further review (Table 6), mostly because the study utilised non-conventional species or methodology for evaluating human toxicity (eg fish). A total of 19 references were considered relevant to the carcinogenicity classification of glyphosate, requiring further in-depth revision (Table 4). The remaining 71 references were considered to require further review to determine their relevance to the carcinogenicity classification (Table 5). The APVMA will rely on international assessments of these papers.

The OCS concluded that, based on the results of the critical appraisal and the limited number of studies reviewed by the OCS in the Tier 2 assessment, there did not appear to be any additional information to indicate that glyphosate poses a carcinogenic risk to humans, on the basis of the following:

- a carcinogenic mechanism of action via genotoxicity or oxidative stress is not evident
- the level of cytotoxicity associated with *in vitro* genotoxicity testing of glyphosate was significant, limiting the ability of *in vitro* tests to determine the genotoxicity potential of glyphosate.

The OCS noted that there is some evidence that *in vitro*, glyphosate-based formulated products are more toxic to cells than glyphosate; however, this effect has not been confirmed *in vivo*. Furthermore, many of the studies exhibited significant methodological limitations, reducing the usefulness of the data.

No definitive conclusions could be drawn on the ability of glyphosate-based formulations to induce oxidative stress as there is limited information regarding the involvement of an oxidative stress mechanism for inducing cytotoxicity.

The OCS concluded that glyphosate was unlikely to pose a carcinogenic or genotoxic risk to humans.

The APVMA evaluated a number of recent assessments of glyphosate conducted by international organisations and regulatory agencies (JMPR, EFSA, ECHA, Health Canada and the NZ Environmental Protection Authority), which considered the publicly available data that was considered in the IARC monograph, as well as other published and unpublished data using a weight-of-evidence approach.

The APVMA agreed with the international assessments of the available epidemiological data that, while epidemiological data is of limited value for detecting carcinogenic potential of a pesticide, the weight-of-evidence does not provide convincing evidence for an association between glyphosate exposure in humans and any cancer type, as there was no consistent pattern of statistical associations that would suggest a causal relationship between glyphosate exposure and the development of cancer in adults or children (total or site-specific).

The APVMA agreed with the international assessments that the weight-of-evidence in experimental animals indicates that glyphosate does not pose a carcinogenic risk at realistic exposure levels, as no consistent dose-response relationship was evident in mice or rats and many of the reported tumours are common age-related tumours in rats and mice.

The APVMA agreed with the international assessments that glyphosate is not likely to be genotoxic, as well-designed *in vitro* tests consistently reported negative results. While some *in vitro* studies reported positive

results for, these were generally observed following very high intraperitoneal doses and most likely a secondary effect of cytotoxicity.

Between 1996 and 2013, a total of four ‘possible’ or probable’ AERs relating to human safety (skin or eye irritation) were submitted to the AERP. The APVMA is confident that the current safety and use directions included on approved labels for products containing glyphosate are sufficient to mitigate these known adverse effects.

6 PROPOSED REGULATORY POSITION

On the basis of the evaluation of the scientific information and assessments, the APVMA concludes that the scientific weight-of-evidence indicates that:

- exposure to glyphosate does not pose a carcinogenic risk to humans
- there is no scientific basis for revising the APVMA's satisfaction that glyphosate or products containing glyphosate:
  - would not be an undue hazard to the safety of people exposed to it during its handling or people using anything containing its residues
  - would not be likely to have an effect that is harmful to human beings
  - would not be likely to have an unintended effect that is harmful to animals, plants or things or to the environment
  - would be effective according to criteria determined by the APVMA by legislative instrument, and
  - would not unduly prejudice trade or commerce between Australia and places outside Australia.
- **there are no scientific grounds for placing glyphosate and products containing glyphosate under formal reconsideration**
- the APVMA will continue to maintain a close focus on any new assessment reports or studies that indicate that any of the above conclusions may need revising.

APPENDIX A – LIST OF KEY STUDIES REFERENCED IN THE IARC MONOGRAPH 112 REQUIRING FURTHER REVIEW BY OCS (TIER 2, PART 1)

The studies referenced in the IARC monograph that the OCS recommended for review are presented below in Table 4. These studies were selected according to the criteria outlined in Section 0 to be assessed in Tier 2, Part 1 of the OCS evaluation to determine whether glyphosate should be placed under formal reconsideration.

Table 4: List of studies relevant to the carcinogenicity classification of glyphosate that require evaluation

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/publication details	Comments	Website
Alvarez-Moya, C, Silva, MR, Valdez Ramírez, CV, Gallardo, DG, Sánchez, RL, Aguirre, AC, & Velasco, AF	2014	genotoxicity	glyphosate isopropylamine	human (lymphocyte cell line)	Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms. Genetics and molecular biology, 37(1), 105–10	Comet assay; glyphosate isopropylamine; human lymphocytes; positive results	http://www.scielo.br/scielo.php?pid=S1415-47572014000100016&script=sci_arttext
*Astiz, M, de Alaniz, MJ & Marra, CA	2009a	oxidative stress	glyphosate	rat (unknown strain)	Effect of pesticides on cell survival in liver and brain rat tissues. Ecotoxicology and environmental safety,72(7), 2025–32	Liver and brain rat cell survival; MOA for oxidative stress seen in previous study	http://www.sciencedirect.com/science/article/pii/S0147651309001018
*Bolognesi, C, Bonatti, S, Degan, P, Gallerani, E, Peluso, M, Rabboni, R, Roggieri, P & Abbondandolo, A	1997	genotoxicity	glyphosate and Roundup	swiss CD-1 mice; human (lymphocyte cell line)	Genotoxic activity of glyphosate and its technical formulation Roundup. Journal of Agricultural and food chemistry, 45(5), 1957–62	Uses roundup and glyphosate alone; positive results seen in both	http://pubs.acs.org/doi/abs/10.1021/jf9606518
Chan, P & Mahler, J	1992	genotoxicity	glyphosate	F344/N rats and B6C3F1	NTP technical report on the toxicity studies of Glyphosate (CAS No.	Effects in rats and mice; no mutagenicity in	http://europepmc.org/abstract/med/12209170

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/publication details	Comments	Website
				mice	1071-83-6) Administered In Dosed Feed To F344/N Rats And B6C3F1 Mice. Toxicity report series, 16, 1-D3	salmonella; negative for LLNA	
*Chaufan, G, Coalova, I & Rios de Molina Mdel, C	2014	oxidative stress	glyphosate, AMPA and glyphosate formulation	human (HepG2 cell line)	Glyphosate Commercial Formulation Causes Cytotoxicity, Oxidative Effects, and Apoptosis on Human Cells Differences With its Active Ingredient. International journal of toxicology, 33(1), 29–38	Shows formulation increases ROS and has toxic effects not seen in glyphosate alone	http://ijt.sagepub.com/content/33/1/29.short
*Elie-Caille, C, Heu, C, Guyon, C & Nicod, L	2010	oxidative stress	glyphosate	human keratinocyte (HaCaT cell line)	Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro-to nanoscale microscopic investigation. Cell biology and toxicology, 26(4), 331–39	Shows the timeline of membrane damage and ROS production in human keratinocytes	http://www.ncbi.nlm.nih.gov/pubmed/20043237
*Gasnier, C, Dumont, C, Benachour, N, Clair, E, Chagnon, MC & Seralini, GE	2009	genotoxicity	glyphosate and glyphosate formulations	human (HepG2 cell line)	Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology, 262(3), 184–91	Shows effects are dependent on formulation not glyphosate concentration	http://www.sciencedirect.com/science/article/pii/S0300483X09003047
*Gehin, A, Guillaume, YC, Millet, J, Guyon, C & Nicod, L	2005	oxidative stress	glyphosate and round-up	human keratinocyte (HaCaT cell line)	Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. International	Shows effects are due to formulation; uses human keratinocyte cell	http://www.sciencedirect.com/science/article/pii/S0378517304005733

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/publication details	Comments	Website
					journal of pharmaceutics,288(2), 219–26	line	
Greim, H, Saltmiras, D, Mostert, V & Strupp, C	2015	carcinogenicity/ epidemiology	glyphosate and glyphosate formulations	human, rat, mouse	Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. Critical reviews in toxicology, 45(3), 185–208	Shows no carcinogenic effect	http://www.tandfonline.com/doi/abs/10.3109/10408444.2014.1003423#.Vf9hMvk0VcY
JMPR	2006	classification					http://apps.who.int/iris/bitstream/10665/43624/1/9241665203_eng.pdf?ua=1
*Kier, LD & Kirkland, DJ	2013	genotoxicity	glyphosate and glyphosate formulations	in vitro and in vivo	Review of genotoxicity studies of glyphosate and glyphosate-based formulations. Critical reviews in toxicology,43(4), 283–315	Review of genotoxicity tesing for glyphosate and formulations	http://www.ncbi.nlm.nih.gov/pubmed/23480780
*Kwiatkowska, M, Huras, B & Bukowska, B	2014	oxidative stress	glyphosate, glyphosate metabolites and glyphosate impurities	human (erythrocyte cell line)	The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro). Pesticide biochemistry and physiology, 109, 34–43	Uses human erythrocytes; shows that ROS and damage only occurs at levels seen in acute poisoning	http://www.sciencedirect.com/science/article/pii/S0048357514000200
*Li, AP & Long, TJ	1998	genotoxicity	glyphosate	in vitro and in vivo	An evaluation of the genotoxic potential of glyphosate. Toxicological Sciences, 10(3), 537–46	Multiple genotoxicity tests; shows no genotoxic	http://toxsci.oxfordjournals.org/content/10/3/537.short

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/publication details	Comments	Website
						potential	
*Manas, F, Peralta, L, Raviolo, J, Ovando, HG, Weyers, A, Ugnia, L, Cid, MG, Larripa, I & Gorla, N	2009a	genotoxicity	glyphosate	human (Hep-2 cell line); mouse micronucleus	Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. Environmental Toxicology and Pharmacology, 28(1), 37–41	Shows positive genotoxicity results in Hep-2 cells and micronucleus mouse test at 400 mg/kg	http://www.sciencedirect.com/science/article/pii/S1382668909000258
*Mladinic, M, Berend, S, Vrdoljak, AL, Kopjar, N, Radic, B & Zeljezic, D	2009a	genotoxicity	glyphosate	human (lymphocyte cell line)	Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. Environmental and molecular mutagenesis, 50(9), 800–7	Shows no clear dose dependent effect	http://onlinelibrary.wiley.com/doi/10.1002/em.20495/abstract
*Mladinic, M, Perkovic, P & Zeljezic, D	2009b	genotoxicity	glyphosate	human (lymphocyte cell line)	Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. Toxicology letters, 189(2), 130–7	Cytome FISH assay; shows no hazardous effect on DNA at low concentrations	http://www.sciencedirect.com/science/article/pii/S0378427409002616
*Monroy, CM, Cortes, AC, Sicard, DM & de Restrepo, HG	2005	genotoxicity	glyphosate	human (GM38 and fibrosarcoma HT1080 cell lines)	Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate.Biomedica, 25 (3), 335–45	Suggests MOA not limited to plants	http://www.scielo.org.co/scielo.php?pid=S0120-41572005000300009&script=sci_arttext&tlng=pt
Prasad, S, Srivastava, S, Singh, M &	2009	genotoxicity	glyphosate	swiss albino mice	Clastogenic effects of glyphosate in bone marrow cells of Swiss albino mice. Journal of toxicology,	Shows positive clastogenic and cytotoxic effects in mouse bone	http://www.hindawi.com/journals/jt/2009/308985/abs/

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/publication details	Comments	Website
Shukla, Y					2009	marrow	
*Rank, J, Jensen, AG, Skov, B, Pedersen, LH & Jensen, K	1993	genotoxicity	glyphosate isopropylamine salt and Roundup	in vitro and in vivo	Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, Salmonella mutagenicity test, and Allium anaphase-telophase test. Mutation Research/Genetic Toxicology, 300(1), 29–36	Shows negative effects for glyphosate in three genotoxicity tests	http://www.sciencedirect.com/science/article/pii/0165121893901362

*Considered by EFSA (2015)

APPENDIX B – LIST OF KEY STUDIES REFERENCED IN THE IARC MONOGRAPH 112 THAT REQUIRE FURTHER REVIEW TO DETERMINE RELEVANCE TO THE CARCINOGENICITY CLASSIFICATION

The studies that were referenced in the IARC monograph that the OCS concluded required further assessment to determine their relevance to the carcinogenicity classification of glyphosate are presented below in Table 5. These studies were selected according to the criteria outlined in Section 0. The APVMA will rely on international assessments of these studies to determine whether glyphosate should be placed under formal reconsideration.

Table 5: List of studies recommended by the OCS for further assessment to determine if relevant to carcinogenicity classification of glyphosate

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/Publication details	Comments	weblink
*Alavanja, MC, Samanic, C, Dosemeci, M, Lubin, J, Tarone, R, Lynch, CF, Knott, C, Thomas, K, Hoppin, JA, Barker, J, Coble, J, Sandler, DP & Blair, A.	2003	Carcinogenicity/ epidemiology	unknown formulation	human	Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. American Journal of Epidemiology,157(9), 800–14	No direct reference to glyphosate in abstract, increased risk to 'other pesticides' only seen in subjects with a FHx of prostate cancer	http://aje.oxfordjournals.org/content/157/9/800.short
*Astiz, M, de Alaniz, MJ, & Marra, CA.	2009b	oxidative stress	glyphosate	rat	Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. Environmental toxicology and pharmacology, 28(3), 465–73	Glyphosate administered alone and in combo with other a.i.'s; unclear if results are for combo; in vivo rat model	http://www.sciencedirect.com/science/article/pii/S1382668909001392
Astiz, M, Hurtado de Catalfo, GE., García, MN, Galletti, SM,	2013	oxidative stress	glyphosate	wistar rat	Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by	Oxidative stress seen in testicular cells; investigates	http://www.sciencedirect.com/science/article/pii/S0147651313000389

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/Publication details	Comments	weblink
Errecalde, AL, de Alaniz, MJ, & Marra, CA.					lipoate and tocopherol. Ecotoxicology and environmental safety, 91, 129–38	antioxidant treatment after administration; unclear if administered in combo	
Benachour, N, & Séralini, GE.	2009	MOA	Roundup	human (umbilical, embryonic, placental cell lines)	Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells.Chemical research in toxicology, 22(1), 97–105	Uses glyphosate formulations, investigates metabolites	http://pubs.acs.org/doi/abs/10.1021/tx800218n
Benachour, N, Sipahutar, H, Moslemi, S, Gasnier, C, Travert, C, & Séralini, GE.	2007	MOA	Roundup (bioforce)	human (embryonic and placental cell lines)	Time-and dose-dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contamination and Toxicology,53(1), 126–33	Uses glyphosate formulations, investigates toxicity and endocrine-disruption	http://link.springer.com/article/10.1007/s00244-006-0154-8
*Bolognesi, C, Carrasquilla, G, Volpi, S, Solomon, KR, & Marshall, EJP.	2009	genotoxicity/ epidemiology	glyphosate + cosmo-flux	human	Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate.Journal of Toxicology and Environmental Health, Part A, 72(15-16), 986–97	Columbian aerial spray program; uses formulation as exposure to glyphosate; measurement of binucleated lymphocytes with micronuclei as DNA damage	http://www.tandfonline.com/doi/abs/10.1080/15287390902929741#.Ve0iNfk0VcY
Brewster, DW, Warren, J, & Hopkjns, WE.	1991	metabolism	glyphosate	SD rat	Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and	Tissue distribution study, shows no persistence in	http://toxsci.oxfordjournals.org/content/17/1/43.short

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/Publication details	Comments	weblink
					quantitation of glyphosate-derived materials following a single oral dose.Toxicological Sciences, 17(1), 43–51	body after single oral dose	
Brown, LM, Burmeister, LF, Everett, GD, & Blair, A.	1993	carcinogenicity/epidemiology	unknown formulation	human	Pesticide exposures and multiple myeloma in Iowa men. Cancer Causes & Control, 4(2), 153–56	No direct reference to glyphosate or roundup; shows little evidence of association between pesticides and multiple myeloma	http://link.springer.com/article/10.1007/BF00053156
Cattani, D, Cavalli, VLDLO, Rieg, CEH, Domingues, JT, Dal-Cim, T, Tasca, CI, & Zamoner, A.	2014	oxidative stress	Roundup	rat	Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. Toxicology, 320, 34–45	Uses formulation; neurotoxic effects on rat hippocampus	http://www.sciencedirect.com/science/article/pii/S0300483X14000493
Çavuşoğlu, K, Yapar, K, Oruç, E, & Yalçın, E.	2011	oxidative stress	Roundup	SA mouse	Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. Journal of medicinal food, 14(10), 1263–72	Uses formulation; ip to mice; studies the effect of Ginkgo against effects seen	http://online.liebertpub.com/doi/abs/10.1089/jmf.2010.0202
Chruscielska, K, Brzezinski, J, Kita, K, Kalhorn, D, Kita, I, Graffstein, B, & Korzeniowski, P.	2000	toxicity			Glyphosate. Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity. Pestycydy, 3	Chronic toxicity study review	

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/Publication details	Comments	weblink
Coalova, I, de Molina, MDCR, & Chaufan, G.	2014	oxidative stress	atanor + impacto (adjuvant)	human (Hep-2 cell line)	Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. Toxicology in Vitro,28(7), 1306–11	Uses formulation and adjuvant on Hep-2 cell line; shows toxicity and ROS	http://www.sciencedirect.com/science/article/pii/S0887233314001295
Cocco, P, Satta, G, Dubois, S, Pili, C, Pilleri, M, Zucca, M, 't Mannetje AM, Becker, N, Benavente, Y, de Sanjose, S, Foretova, L, Staines, A, Maynadie, M, Nieters, A, Brennan, P, Miligi L, Enna, MG & Boffetta, P.	2012	carcinogenicity/epidemiology	unknown formulation	human	Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. Occupational and environmental medicine, oemed-2012	No direct reference to glyphosate; based on pesticide exposure determined via survey	http://oem.bmj.com/content/early/2012/10/31/oemed-2012-100845.short
Culbreth, ME, Harrill, JA, Freudenrich, TM, Mundy, WR, & Shafer, TJ.	2012	MOA	glyphosate	human; mouse	Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. Neurotoxicology, 33 (6), 1499–510	Apoptosis induced by glyphosate, neurodevelopmental study; uses human and mouse neural cells	http://www.sciencedirect.com/science/article/pii/S0161813X12001271
Dennis, LK, Lynch, CF, Sandler, DP, & Alavanja, MC.	2010	carcinogenicity/epidemiology	unknown formulation	human	Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural heath study. Environmental Health Perspectives, 118(6), 812–	Uses formulation; no results relating to glyphosate	http://www.ladep.es/ficheros/documentos/10(35).pdf

Author(s)	Year	Endpoint	Formulation type (if stated)	Species	Title/Publication details	Comments	weblink
					17		
*De Roos, A, Zahm, SH, Cantor, KP, Weisenburger, DD, Holmes, FF, Burmeister, LF, & Blair, A.	2003	carcinogenicity/epidemiology	unknown formulation	human	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men.Occupational and Environmental Medicine, 60(9), e11–e11	Uses formulation; shows positive trend with NHL	http://oem.bmj.com/content/60/9/e11.short
*Dimitrov, BD, Gadeva, PG, Benova, DK, & Bineva, MV.	2006	genotoxicity	Roundup	mouse (bone marrow)	Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. Mutagenesis, 21 (6), 375–82	Comparative study using glyphosate formulation; negative results	http://mutage.oxfordjournals.org/content/21/6/375.short
*Engel, LS, Hill, DA, Hoppin, JA, Lubin, JH, Lynch, CF, Pierce, J, Samanic, C, Sandler, DP, Blair, A & Alavanja, MC.	2005	carcinogenicity/epidemiology	unknown formulation	human	Pesticide use and breast cancer risk among farmers' wives in the agricultural health study. American Journal of Epidemiology,161(2), 121–35	Uses formulation; glyphosate not directly referenced in the abstract; no clear association with breast cancer	http://aje.oxfordjournals.org/content/161/2/121.short
*Eriksson, M, Hardell, L, Carlberg, M, & Åkerman, M.	2008	carcinogenicity/epidemiology	unknown formulation	human	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. International Journal of Cancer, 123(7), 1657–63	Uses formulation; results were not adjusted for multiple exposures; shows increased risk of NHL for glyphosate	http://onlinelibrary.wiley.com/doi/10.1002/ijc.23589/pdf

*Considered by EFSA (2015)

APPENDIX C – LIST OF KEY STUDIES REFERENCED IN THE IARC MONOGRAPH 112 REVIEWED BY THE EU IN 2013 THAT WERE NOT CONSIDERED BY THE OCS

Table 6 below lists the studies referenced in the IARC Monograph 112 for glyphosate that were not considered to require further evaluation by the OCS, as well as the reasons for exclusion.

Table 6: List of excluded studies based on criteria outlined in Section 4.2

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Abraxis	2005			Plate kit	No	No
Acquavella	2004			Biomonitoring	No	No
Akcha	2012	genotoxicity		Not a relevant human model – oyster	No	No
Alavanja	1996	N/A	Yes	Outline of agricultural health study	No	No
Alvarez-Moya	2011	genotoxicity		Not a relevant human model	No	No
Andreotti	2009	carcinogenicity		No direct reference to glyphosate	No	Yes
Aris	2011			Maternal and fetal exposure to pesticides associated with GM foods	No	No
Band	2011	carcinogenicity		No direct reference to glyphosate, refrence to malathion	No	Yes
Battaglin	2005			Transformation products in streams	No	No
Bernal	2010			Liquid chromatography	No	No
Blair	2011			Exposure misclassification in AHS	No	No
Blakley	1997	immune function		Not relevant to carcinogenicity classification	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Bonini	2006			Oxidation of dye in antioxidant activity assay	No	No
Borggaard	2008			Fate of glyphosate in soil	No	No
Botero-Coy	2013a			Improvements in analytical assay	No	No
Botero-Coy	2013b			Liquid chromatography of glyphosate in rice, maize, soybeans	No	No
Brown	1990	carcinogenicity	Yes	No reference to glyphosate	No	No
Bruch	2013			Leaching assessment programme	No	No
Cantor	1992	carcinogenicity	Yes	No direct reference to glyphosate, reference to malathion	No	No
Carreon	2005	carcinogenicity	Yes	No direct reference to glyphosate	No	Yes
Cattaneo	2011	oxidative stress		Not a relevant human model – fish	No	No
Cavalcante	2008	genotoxicity		Not a relevant human model – fish	No	No
Cavas	2007	genotoxicity		Not a relevant human model – goldfish	No	No
CCM International	2011			Outlook for Chinese glyphosate industry	No	No
Centre de Toxicologie du Quebec	1988			Exposure of forestry workers	No	No
Chandra	1994			Spontaneous renal lesions in strains of mice	No	No
Chang	2011			Fate of glyphosate in the environment	No	No
Chen	2012			DNA damage in cyanobacteria	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Chen	2013			Residues on fruit and vegetables	No	No
Chen	2009			Glyphosate poisoning in Taiwan	No	No
Clair	2012	endocrine disruption		Not relevant to carcinogenicity classification	No	No
Clements	1997	genotoxicity		Not a relevant human model – tadpoles	No	No
ColomboPage News Desk	2014			Media—Sri Lanka lifts ban on sale of glyphosate	No	No
Connors	2004	genotoxicity		Not a relevant human model—mussel	No	No
Costa	2008	oxidative stress		Not a relevant human model—tadpoles	No	No
Curwin	2005			Pesticide contamination inside farm and non-farm homes	No	No
Curwin	2007			Uurinary pesticide conc.	No	No
de Castilhos	2013	genotoxicity		Not a relevant human model—fish	No	No
de Marco	1992			Soil breakdown of glyphosate	No	No
de Menezes	2011	oxidative stress		Not a relevant human model—fish	No	No
de Roos	2005a	carcinogenicity	Yes	Already reviewed by OCS	Yes	Yes
de Roos	2005b	carcinogenicity	Yes	Response to criticism	No	No
de Souza	2013	genotoxicity		Not a relevant human model—fish, used roundup, concluded the results seen could have been due to excipients	No	No
Dill	2010			Glyphosate development, applications and properties	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
dos Santos	2014	genotoxicity		Not a relevant human model—clam, uses atrazine and glyphosate formulation	No	No
Duke	2009			Glyphosate resistant crops	No	No
EC	2002			EU report on glyphosate	No	No
EFSA	2008			Residues report	No	No
el-Gendy	1998	immune response		Not relevant to carcinogenicity classification, not a relevant human model—fish	No	No
US EPA	1980a	teratology		Not relevant to carcinogenicity endpoint	No	No
US EPA	1980b	teratology		Not relevant to carcinogenicity endpoint	No	No
US EPA	1992			Glyphosate in drinking water	No	No
US EPA	1997			Pesticides sales and usage	No	No
US EPA	2015			Tox database	No	No
US EPA	1991c			Peer review of glyphosate	No	No
US EPA	1993a			Glyphosate RED	No	No
US EPA	1993b			Glyphosate RED factsheet	No	No
US EPA	2011			Pesticides sales and usage	No	No
Eustis	1994			Multiple-section histo sampling	No	No
FAO	2000			Review	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Farm Chemicals International	2015			Crop protection database	No	No
Ferreira	2010	oxidative stress		Not a relevant human model—fish	No	No
Forgacs	2012			Model for evaluation of reproductive and developmental toxicants	No	No
Freedonia	2012			Industry forecast	No	No
Frescura	2013			Not a relevant human model—fish, glyphosate used as a positive control	No	No
Geret	2013	genotoxicity		Not a relevant human model—oyster	No	No
Gholami-Seyedkolaei	2013	genotoxicity		Not a relevant human model—fish	No	No
Glusczuk	2011	oxidative stress		Not a relevant human model—fish	No	No
Glyphosate Task Force	2014			Glyphosate use	No	No
Granby	2001			Development of a method to measure glyphosate in cereal	No	No
Guha	2013			Residential pesticide use	No	No
Gui	2012			Neurotoxic effects, parkinsonism	No	No
Guilherme	2010	genotoxicity		Not a relevant human model—eel	No	No
Guilherme	2012a	oxidative stress		Not a relevant human model—fish	No	No
Guilherme	2012b	oxidative stress		Not a relevant human model—fish	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Guilherme	2014a	oxidative stress		Not a relevant human model—fish	No	No
Guilherme	2014b	genotoxicity		Not a relevant human mode—fish	No	No
Hardell	1999	carcinogenicity	Yes	Already reviewed by OCS	Yes	Yes
Hardell	2002	carcinogenicity	Yes	Already reviewed by OCS	Yes	Yes
HaYes	1991			Handbook of pesticide toxicology	No	No
Hidalgo	2004			Liquid chromatographic method in water	No	No
Hilton	2012			Global glyphosate market	No	No
Humphries	2005			Residues in atmosphere, soil and water	No	No
IARC	2006			Data for the monographs	No	No
IARC	2014			Key characteristics of carcinogens	No	No
IPCS	1994			Glyphosate environmental health criteria	No	No
IPCS	1996			Glyphosate data sheet	No	No
IPCS	2005			Glyphosate safety card	No	No
Jacob	1988			Metabolism of glyphosate in pseudomonas	No	No
Jan	2009			Residues measured by spectrophotometric method	No	No
Jauhaianen	1991			Occupational exposure	No	No
Johnson	2005			Occupational exposure	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Kalyanaraman	2012			Measuring reactive oxygen and nitrogen species method	No	No
Kavlock	2012			EPA toxcast program	No	No
Kojima	2004	endocrine disruption		Not relevant to carcinogenicity classification	No	No
Kojima	2010	endocrine disruption		Not relevant to carcinogenicity classification	No	No
Kolpin	2006			Glyphosate and AMPA in US streams	No	No
Kreutz	2011			Not a relevant human model—catfish	No	No
Kuang	2011			Analytical methods for determination of herbicides in food	No	No
Kumar	2014			Not relevant to carcinogenicity classification	No	No
Lavy	1992			Occupational exposure	No	No
Lee	2001			Methods of determination in water	No	No
Lopes	2014			Not relevant to carcinogenicity classification, not a relevant human model—fish	No	No
Lubick	2009			Environmental impact of the cocaine strategy	No	No
Lushchak	2009	oxidative stress		Not a relevant human model—goldfish	No	No
Mahendrakar	2014			Effects and treatment of poisoning	No	No
Malatesta	2008	cytotoxicity		Uses round-up formulation	No	No
Mance	2012			Magazine article, not relevant to carcinogenicity classification	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Mariager	2013			Acute effects, not relevant to carcinogenicity classification	No	No
Marques	2014	genotoxicity		Not a relevant human model—fish	No	No
Marques	2015	genotoxicity		Not a relevant human model—fish	No	No
Maza-Joya	2013	genotoxicity		Not a relevant human model—frogs	No	No
McDuffie	2001	carcinogenicity	Yes	Already reviewed by OCS	Yes	Yes
McQueen	2012			Maternal and prenatal exposure in communities	No	No
Ministry of Chemicals & Fertilizers	2008			Industry performance report	No	No
MLHB	2013			Measurement of glyphosate in human urine samples	No	No
Modesto	2010a	oxidative stress		Not a relevant human model—fish	No	No
Modesto	2010b	oxidative stress		Not a relevant human model—fish	No	No
Mohamed	2011	immune response		Not a relevant human model—freshwater snail	No	No
Moreno	2014	genotoxicity		Not a relevant human model—fish	No	No
Mortensen	2000			Effects and treatment of poisoning	No	No
Motojyuku	2008			Measurement of glyphosate in human serum by GC-MS	No	No
Muangphra	2014	genotoxicity		Not a relevant human model—earthworm	No	No
Nakashima	2002	immune		Not relevant to carcinogenicity classification	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
		response				
NCBI	2015			Open chemistry database	No	No
Nedelkoska	2004			HPLC of glyphosate in water	No	No
Nordstrom	1998	carcinogenicity		Already reviewed by OCS	Yes	No
NPIC	2010			Fact sheet	No	No
Nwani	2013	oxidative stress		Not a relevant human model—fish	No	No
Omran	2013	endocrine disruption		Not relevant for carcinogenicity classification	No	No
Ortiz-Ordonez	2011			Not a relevant human model—fish	No	No
Paganelli	2010	teratology		Not a relevant human model—frogs	No	No
Park	2013			Effects and treatment of poisoning	No	No
Perry	2014			Reporting of exposures to pesticides in the UK	No	No
Pesticides Residues Committee	2007			Pesticide monitoring report	No	No
Pesticides Residues Committee	2008			Pesticide monitoring report	No	No
Pesticides Residues Committee	2010			Pesticide monitoring report	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Piola	2013	toxicity		Not a relevant human model—earthworm	No	No
Poletta	2009	genotoxicity		Not a relevant human model—caiman	No	No
Poletta	2011	genotoxicity		Not a relevant human model—caiman	No	No
Republica de El Salvador	2013			Notice on prohibited pesticides	No	No
Roberts	2010			Effects and treatment of poisoning	No	No
Rueppel	1977			Metabolism of glyphosate in soil and water	No	No
Rumack	2015			Effects and treatment of poisoning	No	No
Sanchis	2012			Glyphosate in groundwater	No	No
Siddiqui	2012	genotoxicity		Not a relevant human model—fenugreek	No	No
Simonsen	2008			Glyphosate and AMPA in soil	No	No
Sinhorin	2014	oxidative stress		Not a relevant human model—fish	No	No
Slaninova	2009	oxidative stress		Not a relevant human model—fish	No	No
Sorensen	1999			Effects and treatment of poisoning	No	No
Sribanditmongkol	2012			Effects and treatment of poisoning	No	No
Stella	2004			Effects and treatment of poisoning	No	No
Szekacs	2012			Book about control of weeds	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
Temple	1992			Effects and treatment of poisoning	No	No
Thongprakaisang	2013	endocrine disruption		Not relevant for carcinogenicity classification	No	No
Tian	2012			Synthetic alternative to glyphosate	No	No
Tice	2013			Human hazard characterisation of chemicals	No	No
Tomlin	2000			Pesticide manual	No	No
Transparency Market Research	2014			Global glyphosate market	No	No
Truta	2011	genotoxicity		Not a relevant human model—barley	No	No
Tu	2001			Weed control handbook	No	No
Uren Webster	2014	reproductive/ developmental		Not a relevant human model—fish	No	No
Vasiluk	2005			Oral bioavailability of glyphosate in vitro	No	No
Vera-Candioti	2013	genotoxicity		Not a relevant human model—fish	No	No
Walsh	2000	reproductive/ developmental		Not relevant to carcinogenicity classification	No	No
Wang	2012	genotoxicity		Not a relevant human model—cyanobacterium	No	No
Wester	1991			Not relevant to carcinogenicity classification, dermal absorption	No	No
Xie	2005	endocrine		Not relevant to carcinogenicity classification, not a relevant human	No	No

Author	Year	Endpoint	Epidemiology study?	Reason for exclusion	Evaluated by	
					OCS	EU (2013)
		disruption		model—fish		
Yadav	2013	genotoxicity		Not a relevant human model—tadpoles	No	No
Yin	2011			Glyphosate use review	No	No
Yoshioka	2011			Measurement of glyphosate by liquid chromatography	No	No
Zahm	1990	carcinogenicity	Yes	2,4-D study	No	No
Zhao	2013	endocrine disruption		Not relevant to carcinogenicity classification	No	No
Zouaoui	2013			Effects and treatment of poisoning	No	No

ABBREVIATIONS

ADI	Acceptable daily intake (for humans)
ADME	Absorption, distribution, metabolism and excretion
AER	Adverse Experience Report
AERP	Adverse Experience Reporting Program
Agvet Code	Agricultural and Veterinary Chemicals Code, Schedule to the *Agricultural and Veterinary Chemicals Code Act 1994*
AHS	Agricultural Health Survey
AMPA	Aminomethylphosphonic acid
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARfD	Acute reference dose
ATDS	Australian Total Diet Survey
BfR	Federal Institute for Risk Assessment
CAT	Catalase
CHO-HGPRT	Chinese Hamster Ovary-Hypoxanthine-Guanine Phosphoribosyl Transferase
CLH	Harmonised classification
CI	Confidence Interval
CLP criteria	Classification, Labelling and Packaging of Substances and Mixtures
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EOS	Earth Open Source
EP	European Parliament
EPSPS	Enzyme 5-enolpyruvylshikimate-3-phosphate synthase
EU	European Union
FAO	Food and Agriculture Organisation

FRAP	Ferric-inducing ability of plasma
FSANZ	Food Standards Australia New Zealand
GLP	Good laboratory practice
GSH	Glutathione
GST	Glutathione-S-transferase
HIV	human immunodeficiency virus
hOGG1	Human 8-oxoguanine DNA N-glycosylase 1
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	Kilogram
L	Litre
LD_{50}	Lethal dose
MCPA	2-methyl-4-chlorophenoxyacetic acid
MEPs	Members of the European Parliament
mg/kg bw/day	Milligrams per kilogram of bodyweight per day
mg/L	Milligrams per litre
MRL	Maximum residue limit
NHL	Non-Hodgkin's lymphoma
NHMRC	National Health and Medical Research Centre
NOAEL	No observed adverse effect level
NRA	National Registration Authority
NRS	National Residue Survey
OCS	Office of Chemical Safety
OECD	The Organisation for Economic Co-operation and Development
OECD TGs	OECD Testing guidelines
8-OHdG	8-hydroxy-2'-deoxyguanosine

OR	Odds Ratio
PMRA	Pest Management Regulatory Agency
POEA	Polyethoxylated tallow amine (or polyoxyethylated tallow amine and various synonyms)
RAR	Renewal assessment rapport
RMS	Rapporteur member state
ROS	Reactive oxygen species
RR	Risk ratio
SCE	Sister chromatic exchange
SCGE	single cell gel electrophoresis
SOD	Superoxide dismutase
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
SWA	Safe Work Australia
TBARS	Thiobarbituric acid reactive substances
TGA	Therapeutic Goods Administration
UK	United Kingdom
US	United States
US EPA	US Environmental Protection Agency
US FDA	US Food and Drug Administration
WHO	World Health Organization

GLOSSARY

Acceptable daily intake	A level of intake of a chemical that can be ingested daily over an entire lifetime without any appreciable risk to health
Acute reference dose	The estimated amount of a substance in food or drinking-water, (expressed on a body weight basis), that can be ingested or absorbed over 24 hours or less, without appreciable health risk
Benchmark dose	A dose of a substance associated with a specified low incidence of risk, generally in the range of 1–10%, of a health effect; the dose associated with a specified measure or change of
Lethal dose	The amount of an ingested substance that kills 50 per cent of a test sample
Maximum residue limit	The highest concentration of a chemical residue that is legally permitted in a food
No observed adverse effect level	Greatest concentration or amount of a substance, found by experiment or observation, which causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organism under defined conditions of exposure

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Government of Japan Food Safety Commission

Glyphosate Risk Assessment Report: Pesticides

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Glyphosate

Summary

Food Safety Commission of Japan

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment of glyphosate (CAS No. 1071-83-6), an amino acid herbicide, based on results from various studies. Major adverse effects of glyphosate were observed on reduced gain of body weight, GI tract (diarrhea, increased cecum weight, bowel dilatation, thickening of intestinal mucosa), and liver (increased alkaline phosphatase (ALP), hepatocellular hypertrophy). Glyphosate had no neurotoxicity, carcinogenicity, reproductive toxicity, teratogenicity, and genotoxicity. As the whole, the lowest value among no-observed-adverse-effect levels (NOAELs) was 100 mg/kg bw/day obtained in the 90-days and one-year toxicity studies in dogs, and in the developmental toxicity studies of rabbits. FSCJ thus established an acceptable daily intake (ADI) for glyphosate at 1 mg/kg bw/day, applying a safety factor of 100 to the NOAEL. The lowest NOAEL for adverse effects elicited by a single oral administration of glyphosate was 1,000 mg/kg bw observed in an acute toxicity studies in rats and mice. It is thus unnecessary to specify an acute reference dose (ARfD), due to the exceeding of the cut off level (500 mg/kg bw).

Conclusion in Brief

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment of glyphosate (CAS No. 1071-83-6), an amino acid herbicide, based on results from various studies.

Several technical grades of glyphosate are currently available in Japan. Five-distinct assessment data sets were submitted from each manufacturer. Toxicological profiles were found to be largely consistent among them after the verification individually. The summary of the risk assessment of each technical grade of glyphosate (Glyphosate I to V) is shown in Appendix.

The active ingredient of glyphosate is distributed various salt form such as glyphosate ammonium salt (CAS No. 40465-66-5), glyphosate isopropylamine salt (CAS No. 38641-94-0) and glyphosate potassium salt (CAS No. 70901-12-1). Those salts are soluble in water. Whatever salt are applied to crops, the residue on the crops exists in the form of free acid. FSCJ established the unified acceptable daily intake (ADI) and acute reference dose (ARfD) of glyphosate through compiling these assessment results.

In general, ^{14}C-glyphosate orally administrated rapidly reached to the C_{max} value in plasma and then was eliminated in rats. At least 20% of the radioactivity was absorbed and excreted efficiently in feces. Unchanged glyphosate and aminomethyl phosphonic acid (AMPA) were found in urine and feces.

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The fates of ^{14}C-glyphosate in livestock (goats and chicken) were also examined. Unchanged glyphosate was found as the major radioactive substance in urine, feces, organs and tissues, and AMPA was also found as the minor component.

On the fate of ^{14}C-glyphosate, and isopropylamine, potassium, trimesium or sodium salt of ^{14}C-glyphosate in plants, AMPA was found more than 10% of the total radioactive residue (TRR). *N*-Acetylglyphosate and *N*-acetyl-AMPA were detected in the glyphosate tolerant soybean and corn as more than 10% of TRR.

Major adverse effects of glyphosate were observed on reduced gain of body weight, GI tract (diarrhea, increased cecum weight, bowel dilatation, thickening of intestinal mucosa), and liver (increased alkaline phosphatase (ALP), hepatocellular hypertrophy). Glyphosate had no neurotoxicity, carcinogenicity, reproductive toxicity, teratogenicity, and genotoxicity.

Among no-observed-adverse-effect levels (NOAELs) of each technical grade of glyphosate, the lowest value was 75 mg/kg bw/day on Glyphosate I derived from the maternal effects in the developmental toxicity study of rabbits. FSCJ, however, recognized it appropriate to set 100 mg/kg bw/day as the overall NOAEL in the developmental toxicity studies of rabbits, considering the dose settings and the toxicological effects observed in the four other corresponding studies.

As the whole, the lowest value among NOAELs was 100 mg/kg bw/day obtained in the 90-days and one-year toxicity studies in dogs, and in the developmental toxicity studies of rabbits. FSCJ thus established an ADI for glyphosate at 1 mg/kg bw/day, applying a safety factor of 100 to the NOAEL.

The lowest NOAEL for adverse effects elicited by a single oral administration of glyphosate was 1,000 mg/kg bw observed in an acute toxicity studies in rats and mice. It is thus unnecessary to specify an ARfD, due to the exceeding of the cut off level (500 mg/kg bw).

In plants, AMPA, *N*-acetyl-AMPA, and *N*-Acetylglyphosate were observed as exceeded 10% of TRR. *N*-acetyl-AMPA and *N*-Acetylglyphosate were not detected in rats. *N*-acetyl-AMPA had a very low acute toxicity (LD_{50} was beyond 5,000 mg/kg bw), and no genotoxicity. Thus the residue definition for the dietary risk assessment was identified to be glyphosate and *N*-Acetylglyphosate in agricultural products, and glyphosate (parent compound only) in livestock products.

Appendix

Glyphosate I

FSCJ conducted a risk assessment of glyphosate (CAS No. 1071-83-6) [glyphosate ammonium salt (CAS No. 40465-66-5), glyphosate isopropylamine salt (CAS No. 38641-94-0) and glyphosate potassium salt (CAS No. 70901-12-1)], an amino acid herbicide, based on results from various studies.

The data used in the assessment include fate in animals (rats and rabbits), fate in plants (soybeans, grapes and others), residues in crops, subacute toxicity (rats, mice and dogs), chronic toxicity (dogs), combined chronic toxicity/carcinogenicity (rats), carcinogenicity (mice), two-generation reproductive toxicity (rats), developmental toxicity (rats and rabbits), and genotoxicity.

Major adverse effects of glyphosate were observed on GI tract (diarrhea, loose feces) and reduced gain of body weight. None of carcinogenicity, reproductive toxicity, teratogenicity and genotoxicity was observed.

Based on the results from various studies, glyphosate (parent compound only) was identified as the relevant substance for the residue definition for dietary risk assessment in agricultural products.

The lowest NOAEL obtained in all the studies was 75 mg/kg bw/day in a developmental toxicity study in rabbits. FSCJ established ADI of 0.75 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

The lowest NOAEL for adverse effects elicited by a single oral administration of glyphosate was 1,000 mg/kg bw obtained in an acute toxicity study in mice. It is thus unnecessary to specify an ARfD, due to the exceeding of the cut off level (500 mg/kg bw).

Glyphosate II

FSCJ conducted a risk assessment of glyphosate (CAS No. 1071-83-6) [glyphosate potassium salt (CAS No. 70901-12-1)], an amino acid herbicide, based on results from various studies.

The data used in the assessment include fate in animals (rats), fate in plants (paddy rice, lemon and others), residues in crops, subacute toxicity (rats and dogs), subacute neurotoxicity (rats), chronic toxicity (rats and dogs), combined chronic toxicity/carcinogenicity (rats and mice), two-generation reproductive toxicity (rats), developmental toxicity (rats and rabbits), and genotoxicity.

Major adverse effects of glyphosate were observed on reduced gain of body weight and liver (increased alanine aminotransferase (ALT) and ALP). None of neurotoxicity, carcinogenicity, reproductive toxicity, teratogenicity and genotoxicity of glyphosate was observed.

Based on the results from various studies, glyphosate and N-acetylglyphosate were identified as the relevant substance for the residue definition for dietary risk assessment in agricultural products.

The lowest NOAEL obtained in all the studies was 100 mg/kg bw/day in a developmental toxicity study in rabbits. FSCJ established an ADI of 1 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

The lowest NOAEL for adverse effects elicited by a single oral administration of glyphosate was 1,000 mg/kg bw obtained in an acute neurotoxicity study in rats. It is thus unnecessary to specify an ARfD, due to the exceeding of the cut off level (500 mg/kg bw).

Glyphosate III

FSCJ conducted a risk assessment of glyphosate (CAS No. 1071-83-6) [glyphosate isopropylamine salt (CAS No. 38641-94-0)], an amino acid herbicide, based on results from various studies.

The data used in the assessment include fate in animals (rats), fate in plants (paddy rice, apple and others), residues in crops, subacute toxicity (rats, mice and dogs), subacute neurotoxicity (rats), chronic toxicity (dogs), combined chronic toxicity/carcinogenicity (rats), carcinogenicity (mice), two-generation reproductive toxicity (rats), developmental toxicity (rats and rabbits), and genotoxicity.

Major adverse effects of glyphosate were observed on GI tract (diarrhea, bowel dilatation, thickening of intestinal mucosa), kidney (nephrosis), liver (increased ALP, hepatocellular hypertrophy), and blood (decreased red blood cell (RBC)). None of neurotoxicity, carcinogenicity, reproductive toxicity, teratogenicity, and genotoxicity relevant to human health was observed.

Based on the results from various studies, glyphosate (parent compound only) was identified as the relevant substance for the residue definition for dietary risk assessment in agricultural products.

The lowest NOAEL obtained in all the studies was 100 mg/kg bw/day in a 90-day subacute toxicity study in rats and in dogs, and in a one-year chronic toxicity study in dogs. FSCJ established an ADI of 1 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

The lowest NOAEL for adverse effects elicited by a single oral administration of glyphosate was 5,000 mg/kg bw obtained in an acute toxicity study in rats and mice. It is thus unnecessary to specify an ARfD, due to the exceeding of the cut off level (500 mg/kg bw).

Glyphosate IV

FSCJ conducted a risk assessment of glyphosate (CAS No. 1071-83-6) [glyphosate isopropylamine salt (CAS No. 38641-94-0)], an amino acid herbicide, based on results from various studies.

The data used in the assessment include fate in animals (rats), fate in plants (paddy rice, apple and others), residues in crops, subacute toxicity (rats, mice and dogs), chronic toxicity (dogs), combined chronic toxicity/carcinogenicity (rats), carcinogenicity (mice), two-generation reproductive toxicity (rats), developmental toxicity (rats and rabbits), and genotoxicity.

Major adverse effects of glyphosate were observed on reduced gain of body weight, GI tract (loose feces, increased cecum weight), and blood (anemia). None of carcinogenicity, reproductive toxicity, teratogenicity and genotoxicity was observed.

Based on the results from various studies, glyphosate (parent compound only) was identified as the relevant substance for the residue definition for dietary risk assessment in agricultural products.

The lowest NOAEL obtained in all the studies was 100 mg/kg bw/day in a developmental toxicity study in rabbits. FSCJ established an ADI of 1 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

The lowest NOAEL for adverse effects elicited by a single oral administration of glyphosate was 5,000 mg/kg bw obtained in an acute toxicity study in rats and mice. It is thus unnecessary to specify an ARfD, due to the exceeding of the cut off level (500 mg/kg bw).

Glyphosate V

FSCJ conducted a risk assessment of glyphosate (CAS No. 1071-83-6) [glyphosate isopropylamine salt (CAS No. 38641-94-0)], an amino acid herbicide, based on results from various studies.

The data used in the assessment include fate in animals (rats), fate in plants (paddy rice, apple and others), residues in crops, subacute toxicity (rats and dogs), subacute neurotoxicity (rats), chronic toxicity (dogs), combined chronic toxicity/carcinogenicity (rats), carcinogenicity (mice), two-generation reproductive toxicity (rats), developmental toxicity (rats and rabbits), and genotoxicity.

Major adverse effects of glyphosate were observed on GI tract (loose feces and diarrhea). None of neurotoxicity, carcinogenicity, reproductive toxicity, teratogenicity and genotoxicity was observed.

Based on the results from various studies, glyphosate (parent compound only) was identified as the relevant substance for the residue definition for dietary risk assessment in agricultural products.

The lowest NOAEL obtained in all the studies was 200 mg/kg bw/day in a developmental toxicity study in rabbits. FSCJ established an ADI of 2 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

No adverse effects elicited by a single oral administration of glyphosate was observed. It is thus unnecessary to specify an ARfD.

Levels relevant to toxicological evaluation of glyphosate

Species	Study	Technical Grade No.	Dose (ppm)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical endpoints (Notes)
Rat	90-day toxicity study	I	0, 1,000, 5,000, 20,000[a]	M: 1,270[b] F: 1,620[b]	-	No toxicity
			0, 200, 2,000, 5,000, 12,500[a]	M: 339 F: 339	M: 839 F: 802	M/F: Reduced gain of body weight, etc
		II	0, 1,000, 5,000, 20,000[a]	M: 81.3 F: 90.4	M: 414 F: 447	M/F: Increased ALT, etc
		III	0, 100, 300, 1,000, 3,000[c] (mg/kg bw/day)	M: 100 F: 300	M: 300 F: 1,000	M/F: loose feces, diarrhea, etc
			0, 2,000, 10,000, 50,000[a]	M: 672 F: 736	M: 3,690 F: 3,790	M/F: Hepatocellular hypertrophy, etc
		IV	0, 3,000, 10,000, 30,000[a]	M: 168 F: 195	M: 569 F: 637	M/F: Increased cecum weight, etc
		V	0, 1,000, 10,000, 50,000[a]	M: 79 F: 90	M: 730 F: 844	M/F: Increased ALP, etc
	90-day neurotoxicity study	II	0, 2,000, 8,000, 20,000[a]	M: 617 F: 1,630[b]	M: 1,550 F: -	M: Reduced gain of body weight, etc F: No toxicity (No subacute neurotoxicity)
		III	0, 2,000, 10,000, 50,000[a]	M: 734 F: 858	M: 4,090 F: 5,010	M/F: Diarrhea, etc (No subacute neurotoxicity)
		V	0, 1,000, 5,000, 20,000[a]	M: 1,500[b] F: 1,560[b]	-	M/F: No toxicity (No subacute neurotoxicity)
	One-year toxicity study	II	0, 2,000, 8,000, 20,000[a]	M: 141 F: 167	M: 560 F: 671	M/F: Increased ALT/ALP, etc
	Two-year combined chronic toxicity/ carcinogenicity study	I	0, 2,000, 8,000, 20,000[a]	M: 362 F: 457	M: 940 F: 1,180	M: Cataract like change F: Reduced gain of body weight (Not carcinogenic)
		II	0, 2,000, 6,000, 20,000[a]	M: 121 F: 145	M: 361 F: 437	M/F: Increased ALP/ALT, etc (Not carcinogenic)
		III	0, 500, 4,000, 32,000[a]	M: 201 F: 239	M: 1,750 F: 2,000	M/F: Decreased RBC (Not carcinogenic)
		IV	0, 3,000, 10,000, 30,000[a]	M: 104 F: 115	M: 354 F: 393	M/F: Increased absolute cecum weight, etc (Not carcinogenic)
		V	0, 1,500, 5,000, 15,000[a]	M: 1,080[b] F: 349	M: - F: 1,380	M: No toxicity F: Mineralization of medullary-cortical zone in the kidney (Not carcinogenic)

(continued.)

Species	Study	Technical Grade No.	Dose (ppm)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical endpoints (Notes)
Rat (cont'd)	Two-generation of reproductive toxicity study	I	0, 2,000, 10,000, 30,000[a]	PM: 666 PF: 777 F_1M: 711 F_1F: 804	PM: 1,980 PF: 2,320 F_1M: 2,230 F_1F: 2,540	P/F_1: Reduced gain of body weight, etc (No effect on reproduction)
		II	0, 1,000, 3,000, 10,000[a]	Parent PM: 293 PF: 1,050[b] F_1M: 352 F_1F: 1,220[b] Offspring PM: 293 PF: 323 F_1M: 352 F_1F: 371	Parent PM: 985 PF: - F_1M: 1,160 F_1F: - Offspring PM: 293 PF: 1,050 F_1M: 352 F_1F: 1,220	Parent M: Reduced gain of body weight, etc F: No toxicity Offspring M/F: Reduced gain of body weight (No effect on reproduction)
		III	0, 400, 4,000, 40,000[a]	PM: 360 PF: 374 F_1M: 480 F_1F: 465	PM: 3,810 PF: 3,730 F_1M: 5,040 F_1F: 4,860	P/F_1: Reduced gain of body weight, etc (No effect on reproduction)
		IV	0, 1,200, 6,000, 30,000[a]	PM: 417 PF: 485 F_1M: 458 F_1F: 530	PM: 2,150 PF: 2,530 F_1M: 2,410 F_1F: 2,760	P: Loose feces, dilated caecum, etc F_1: Dilated caecum, etc (No effect on reproduction)
		V	0, 1,500, 5,000, 15,000[a]	PM: 959[b] PF: 1,170[b] F_1M: 1,170[b] F_1F: 1,380[b]	PM: - PF: - F_1M: - F_1F: -	P/F_1: No toxicity (No effect on reproduction)
	Developmental toxicity study	I	0, 300,1,000, 3,500[c] (mg/kg bw/day)	Maternal/Fetus: 1,000	Maternal/Fetus: 3,500	Maternal: Increased mortality rate, etc Fetus: Low body weight, etc (Not teratogenic)
		II	0, 250, 500, 1,000[c] (mg/kg bw/day)	Maternal/Fetus: 1,000[b]	Maternal/Fetus: -	No toxicity (Not teratogenic)
		III	0, 250, 500, 1,000[c] (mg/kg bw/day)	Maternal/Fetus: 1,000[b]	Maternal/Fetus: -	No toxicity (Not teratogenic)
		IV	0, 30, 300, 1,000[c] (mg/kg bw/day)	Maternal: 300 Fetus: 1,000[b]	Maternal: 1,000 Fetus: -	Maternal: Loose feces, etc Fetus: No toxicity (Not teratogenic)
		V	0, 100, 500, 1,000[c] (mg/kg bw/day)	Maternal/Fetus: 1,000	Maternal/Fetus: -	No toxicity (Not teratogenic)

(continued.)

Species	Study	Technical Grade No.	Dose (ppm)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical endpoints (Notes)
Mouse	90-day toxicity study	I	0, 5,000, 10,000, 50,000[a]	M: 1,870 F: 2,740	M: 9,700 F: 14,800	M/F: Reduced gain of body weight
		III	0, 2,000, 10,000, 50,000[a]	M: 1,630 F: 423	M: 7,990 F: 1,960	M: Loose feces, bloody feces, etc F: Decreased absolute/ relative kidney weight
		IV	0, 5,000, 10,000, 50,000[a]	M: 600 F: 765	M: 1,220 F: 1,490	M/F: Increase trend in cecum weight
	Two-year combined chronic toxicity/ carcinogenicity study	II	0, 100, 1,000, 8,000[a]	M: 118 F: 159	M: 991 F: 1,340	M/F: Reduced gain of body weight (Not carcinogenic)
	18-month carcinogenicity study	I	[2 year] 0, 1,000, 5,000, 30,000[a]	M: 830 F: 979	M: 4,930 F: 6,130	M/F: Reduced gain of body weight (Not carcinogenic)
		III	0, 500, 5,000, 50,000[a]	M: 685 F: 909	M: 7,470 F: 8,690	M/F: Loose feces, etc (Not carcinogenic)
		IV	0, 1,600, 8,000, 40,000[a]	M: 838 F: 153	M: 4,350 F: 787	M: Increased absolute/ relative cecum weight, etc F: Reduced gain of body weight, etc (Not carcinogenic)
		V	0, 500, 1,500, 5,000[a]	M: 810[b] F: 1,080[b]	M: - F: -	No toxicity (Not carcinogenic)
Rabbit	Developmental toxicity study	I	0, 75, 175, 350[c] (mg/kg bw/day)	Maternal: 75 Fetus: 350[b]	Maternal: 175 Fetus: -	Maternal: Diarrhea, loose feces Fetus: No toxicity (Not teratogenic)
		II	0, 100, 175, 300[c] (mg/kg bw/day)	Maternal: 100 Fetus: 175	Maternal: 175 Fetus: 300	Maternal: Reduced gain of body weight, etc Fetus: Low body weight, etc
		III	0, 87.5, 175, 350[c] (mg/kg bw/day)	Maternal/Fetus: 350	Maternal/Fetus: -	No toxicity (Not teratogenic)
		IV	0, 10, 100, 300[c] (mg/kg bw/day)	Maternal: 100 Fetus: 300[b]	Maternal: 300 Fetus: -	Maternal: Loose feces, abortion/premature birth (2 cases), reduce trend in body weight gain Fetus: No toxicity (Not teratogenic)
		V	0, 50, 200, 400[c] (mg/kg bw/day)	Maternal: 200 Fetus: 400[b]	Maternal: 400 Fetus: -	Maternal: Death, diarrhea, reduced gain of body weight, etc Fetus: No toxicity (Not teratogenic)

(continued.)

Species	Study	Technical Grade No.	Dose (ppm)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical endpoints (Notes)
Dog	90-day toxicity study	I	[6-month] 0, 10, 60, 300[c] (mg/kg bw/day)	M: 300[b] F: 300[b]	M: - F: -	No toxicity
		II	0, 2,000, 10,000, 50,000[a]	M: 323 F: 334	M: 1,680 F: 1,750	M: Decreased Alb, TP, etc F: Increased ALP
		III	0, 30, 100, 300[c] (mg/kg bw/day)	M: 100 F: 100	M: 300 F: 300	M/F: Reduce gain of body weight, etc
		IV	0, 1,600, 8,000, 40,000[a]	M: 1,020[b] F:1,010[b]	M: - F: -	No toxicity
		V	0, 30, 300, 1,000[c] (mg/kg bw/day)	M: 300 F: 300	M: 1,000 F: 1,000	M/F: Loose watery feces, etc
	One-year toxicity study	I	0, 20, 100, 500[c] (mg/kg bw/day)	M: 500[b] F: 500[b]	M: - F: -	No toxicity
		II	0, 3,000, 15,000, 30,000[a]	M: 907[b] F: 448	M: - F: 926	M: No toxicity F: Reduced gain of body weight
		III	0, 30, 100, 300[c] (mg/kg bw/day)	M: 100 F: 100	M: 300 F: 300	M/F: Diarrhea, bloody feces, etc
		IV	0, 1,600, 8,000, 50,000[a]	M: 182 F: 184	M: 1,200 F: 1,260	M/F: Loose feces, reduce trend in body weight gain, etc
		V	0, 30, 125, 500[c] (mg/kg bw/day)	M: 500[b] F: 500[b]	M: - F: -	No toxicity
Genotoxicity		I	No evidence of genotoxicity			
		II	No evidence of genotoxicity			
		III	No genotoxicity relevant for human health [Pseudo positive in *in vitro* chromosomal aberration test (+S9), but negative in *in vivo* micronucleus test up to the highest dose in accordance with OECD TG.]			
		IV	No evidence of genotoxicity			
		V	No evidence of genotoxicity			

M, Male; F, Female; M/F, both sexes; PM, Male in P (Parent) generation; PF, Female in P generation; F_1M, Male in F_1 generation; F_1F, Female in F_1 generation; -, No effect observed at the highest dose tested; [a], Dietary administration; [b], Highest dose tested; [c], Gavage administration; Alb, Albumin; TP, Total protein.

Absorption, distribution, excretion and metabolism of glyphosate in animals

Kinetics

Dose (mg/kg bw)	1		10				25		100	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
T_{max} (hr)	3.9	8	4	1.7	6	3	4	4	2	2
C_{max} (μg/g)	0.016	0.037	0.168	0.413	0.125	0.162	0.29	0.74	5.61	5.94
$T_{1/2}$ (hr)	10.9	8.07					18	12	-	-
α (hr)		-	-				-	-	2.3	2
β (day)		-	-				-	-	-	2.6
AUC	0.257[a] (hr μg/mL)	0.338[a] (hr μg/mL)	245 (min μg/mL)	226 (min μg/mL)			4.6[a] (hr μg/g)	9.5[a] (hr μg/g)	46.9[b] (hr μg/mL)	64.1b (hr μg/mL)
Rate of absorption (%)	28.1–32.5		30.2–36.2		19–30		39.9		22.9–36.2	

/: Not measured; -: Not indicated; [a]: $AUC_{0\text{-}24\ hr}$

Metabolism

In both urine and feces samples, the major radioactive component was unchanged glyphosate (urine: 15.2–31.0% of administrated dose, feces: 67.6–83.0% of administrated dose). Small amounts of aminomethyl phosphonic acid (AMPA), 0.06–0.66% of administrated dose in urine, trace-1.4% of administrated dose in feces) and methyl aminomethyl phosphonic acid (MAMPA) were detected.

Distribution in tissues

Dose (mg/kg bw)	Hours after administration (hrs[a])	Sex	Organ (μg/g)
1	72	Male	Bone (0.123), Gastro-intestinal tract (0.031), Kidney (0.020), Carcass[b] (0.016), Liver (0.012), Others (0.010>)
		Female	Bone (0.112), Gastro-intestinal tract (0.075), Carcass (0.024), Skin (0.014), Liver (0.012), Kidney (0.012), Others (0.010>)
10	72	Male	Bone (0.511), Gastro-intestinal tract (0.152), Kidney (0.068), Carcass (0.062), Liver (0.059), Others (0.05>)
		Female	Bone (0.395), Gastro-intestinal tract (0.152), Carcass (0.056), Kidney (0.049) Liver (0.044), Others (0.03>)
	168	Male	Bone (0.552), Carcass (0.106), Others (0.05>)
		Female	Bone (0.313), Carcass (0.087), Others (0.05>)
		Male	Bone(0.47), Bone marrow(0.044), Kidney(0.035), Carcass (0.034), Gastro-intestinal tract (0.030), Others (0.03>)
		Female	Bone (0.58), Bone marrow (0.093), Gastro-intestinal tract (0.032), Carcass (0.028), Lymph node (0.028), Others (0.025>)
25	120	Male	Bone (1.29), Large intestine (0.555), Carcass (0.294), Liver (0.216), Small intestine (0.206), Kidney (0.202), Others (0.2>)
		Female	Bone (2.31), Stomach (0.796), Liver (0.333), Kidney (0.320), Urinary bladder (0.282), Lung (0.234), Small intestine (0.221), Carcass (0.201), Others (0.2>)
100	168	Male	Liver (0.518), Kidney (0.483), Stomach (0.424), Others (0.4>)
		Female	Stomach (0.600), Kidney (0.440), Liver (0.416), Others (0.4>)

[a], After single oral administration; [b], Remaining without organs/tissues.

Excretion

Dose (mg/kg bw)	1[a]		10[b]		10[a]				25[c]		100[a]	
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urine (%)	18.4	27.2	13.3	11.0	28.6	22.5	22.5	19.4	42.9	61.4	55.5	36.2
Feces (%)	72.6	62.4	88.5	88.7	62.4	69.4	74.6	84.3	47.3	32.0	43.5	62.9

Dose (mg/kg bw)	100[a]		250[c]		600[a]		1,000[b]		1,000[a]			
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urine (%)	39.4	43.1	42.0	39.9	30.3	29.5	16.8	17.7	17.8	14.3	23.0	22.9
Feces (%)	41.2	42.4	49.2	55.6	74.7	74.2	89.6	84.5	68.9	69.4	75.6	76.6

[a], During 168 hrs after single oral administration; [b], During 72 hrs after single oral administration; [c], During 48 hrs after single oral administration.

U.S. Environmental Protection Agency
(EPA)

Glyphosate Issue Paper:
Evaluation of Carcinogenic Potential

September 2016

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EPA's Office of Pesticide Programs
September 12, 2016

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List of Acronyms

ADME: Absorption, Distribution, Metabolism, and Excretion
AHS: Agricultural Health Study
AOP: Adverse Outcome Pathway
AMPA: Aminomethylphosphonic Acid
BrdU: Bromodeoxyuridine
CA: Chromosomal Aberration
CARC: Cancer Assessment Review Committee
CBPI: Cytokinesis Block Proliferation Index
CHL: Chinese Hamster Lung
CHO: Chinese Hamster Ovary
CPRC: Carcinogenicity Peer Review Committee
EFSA: European Food Safety Authority
EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase
FAO: Food and Agriculture Organization
FIFRA: Federal Insecticide, Fungicide, and Rodenticide Act
FISH: Fluorescence *in situ* Hybridization
GC-MS: Gas Chromatography-Mass Spectrometry
HL: Hodgkin Lymphoma
HPLC: High-Performance Liquid Chromatography
HPRT: Hypoxanthine-Guanine Phosphoribosyl Transferase
IARC: International Agency for Research on Cancer
JMPR: Joint Meeting Pesticide Residues
MGUS: Monoclonal Gammopathy of Undetermined Significance
MN: Micronuclei
MOA: Mode of Action
MPCE: Micronucleated Polychromatic Erythrocytes
MRID: Master Record Identifier (a unique number assigned to each study submitted to EPA)
MTD: Maximum Tolerated Dose
NB: Nuclear Bud
NCR: National Research Council
NHL: Non-Hodgkin Lymphoma
NPB: Nucleoplasmic Bridges
NTP: National Toxicology Program
OCSPP: Office of Chemical Safety and Pollution Prevention
OECD: Organization for Economic Cooperation and Development
OPP: Office of Pesticides Program
PCE: Polychromatic Erythrocytes
PK: Pharmacokinetic
PPE: Personal Protective Equipment
PWG: Pathology Work Group
RED: Registration Eligibility Decision
ROS: Reactive Oxygen Species
SAP: Scientific Advisory Panel
SCE: Sister Chromatid Exchange

SCGE: Single Cell Gel Electrophoresis
TAC: Total Antioxidant Capacity
TK: Thymidine Kinase
UDS: Unscheduled DNA Synthesis
USGS: United States Geological Survey
UV: Ultraviolet
WHO: World Health Organization
XPRT: Xanthine-Guanine Phosphoribosyl Transferase

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1.0 Introduction

1.1 Background

Glyphosate is a non-selective, phosphonomethyl amino acid herbicide registered to control weeds in various agricultural and non-agricultural settings. The herbicide acts by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, which is not present in mammalian systems. Glyphosate was initially registered in 1974. Since then, several human health analyses have been completed for glyphosate. In 1986, EPA issued the Glyphosate Registration Standard which updated the agency's toxicity database for this compound. In 1993, EPA issued the registration eligibility decision (RED) that indicated that glyphosate was eligible for re-registration.

Currently, glyphosate is undergoing Registration Review[1], a program where all registered pesticides are reviewed at least every 15 years as mandated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The initial docket opening for glyphosate occurred in 2009 with the publication of the human health scoping document and preliminary work plan[2]. As part of this process, the hazard and exposure of glyphosate are reevaluated to determine its potential risk to human and environmental health. Risks are assessed using current practices and policies to ensure pesticide products can still be used safely. Registration Review also allows the agency to incorporate new science. For human health risk assessment, both non-cancer and cancer effects are evaluated for glyphosate and its metabolites, aminomethylphosphonic acid (AMPA) and *N*-acetyl-glyphosate; however, this document will focus on the cancer effects only. EPA expects to complete its complete human health risk assessment in 2017 that will include an assessment of risk from anticipated exposures resulting from registered uses of glyphosate in residential and occupational settings.

1.2 Evaluation of Carcinogenic Potential

Since its registration, the carcinogenic potential of glyphosate has been evaluated by EPA several times. In 1985, the initial peer review of glyphosate was conducted in accordance with the Proposed Guidelines for Carcinogen Risk Assessment. The agency classified glyphosate as a Group C chemical (Possible Human Carcinogen), based on the presence of kidney tumors in male mice. In 1986, the agency requested that the FIFRA Scientific Advisory Panel (SAP) evaluate the carcinogenic potential of glyphosate. The panel determined that the data on renal tumors in male mice were equivocal (only an increase in adenomas was observed and the increase did not reach statistical significance). As a result, the panel recommended a Group D chemical classification (Not Classifiable as to Human Carcinogenicity) for glyphosate and advised the agency to issue a data call-in notice for further studies in rats and/or mice to clarify the unresolved questions (FIFRA SAP Report, 1986)[3].

[1] Additional information on the Registration Review process can be found at: https://www.epa.gov/pesticide-reevaluation/registration-review-process

[2] Documents of the Registration Review can be found in the public docket at: EPA-HQ-OPP-2009-0361, accessible at www.regulations.gov.

[3] Review available at: http://www.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-103601_24-Feb-86_209.pdf

With the submission of two rat carcinogenicity studies following this data call-in, a second peer review was conducted in 1991 by the agency's Carcinogenicity Peer Review Committee (CPRC) to incorporate the new data. In accordance with the agency's 1986 Draft Guidelines for Carcinogen Risk Assessment, the CPRC classified glyphosate as a Group E Chemical: "Evidence of Non-Carcinogenicity for Humans" based upon lack of evidence for carcinogenicity in mice and rats and the lack of concern for mutagenicity (TXR# 0008897).

Most recently, in September 2015, a third review was done by the Cancer Assessment Review Committee (CARC). Relevant glyphosate data available to EPA at that time for glyphosate were reevaluated, including studies submitted by the registrant and studies published in the open literature. The agency performed this evaluation in support of Registration Review in accordance with the 2005 Guidelines for Carcinogen Risk Assessment, classified glyphosate as "Not Likely to be Carcinogenic to Humans" (CARC, 2015; TXR #0057299).

Recently, several international agencies have evaluated the carcinogenic potential of glyphosate. In March 2015, the International Agency for Research on Cancer (IARC), a subdivision of the World Health Organization (WHO), determined that glyphosate was a probable carcinogen (group 2A) (IARC, 2015). Later, in November 2015, the European Food Safety Authority (EFSA) determined that glyphosate was unlikely to pose a carcinogenic hazard to humans (EFSA, 2015). In May 2016, the Joint Food and Agriculture Organization (FAO)/WHO Meeting on Pesticide Residues (JMPR), another subdivision of the WHO, concluded that glyphosate was unlikely to pose a carcinogenic risk to humans from exposure through the diet (JMPR, 2016). Some individual countries (e.g., France, Sweden) have been moving to ban glyphosate based on the IARC decision, while other countries (e.g., Japan, Canada) have continued to support their conclusion that glyphosate is unlikely to pose a carcinogenic risk to humans.

The recent peer review performed by CARC served as an initial analysis to update the data evaluation for glyphosate at that time. Based on an evaluation of the studies included in the recent analyses by IARC, JMPR, and EFSA, the agency then became aware of additional relevant studies not available to EPA. As a result, EPA also requested information from registrants about studies that existed, but had never been submitted to the agency. The current evaluation incorporates these additional studies. In addition, the Agency conducted a systematic review of the open literature and toxicological databases for glyphosate by using a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment". As such, the current evaluation also provides a more thorough evaluation than the 2015 CARC review.

1.3 Overview of Draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment"

In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of scientific evidence in the context of understanding of the mode of action (MOA)/adverse outcome pathway (AOP) (U.S. EPA, 2010). The draft framework, which includes two key components, problem formulation and use of the MOA/AOP pathway frameworks, was reviewed

favorably by the FIFRA SAP in 2010 (FIFRA SAP, 2010). Recently, EPA has applied this framework to the evaluation of atrazine and chlorpyrifos[4].

OPP's draft framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety MOA/human relevance framework, which highlights the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek *et al.*, 2014). Consistent with recommendations by the National Research Council (NRC) in its 2009 report on *Science and Decisions*, OPP's draft framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage starts with planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern, and exposure pathways and duration along with key gaps in data or scientific information. Specific to glyphosate, the scoping document prepared for Registration Review (J. Langsdale *et al.*, 2009) along with the review conducted by the CARC (CARC, 2015) represent the problem formulation analyses for the weight-of-evidence evaluation for carcinogenic potential. A summary of the US exposure profile is provided in Section 1.4 below to provide context for interpreting the various lines of evidence.

One of the key components of the agency's draft framework is the use of the MOA framework/AOP concept (Figure 1.1) as a tool for organizing and integrating information from different sources to inform the causal nature of links observed in both experimental and observational studies. Specifically, the modified Bradford Hill Criteria (Hill, 1965) are used to evaluate strength, consistency, dose response, temporal concordance and biological plausibility of multiple lines of evidence in a weight-of-evidence analysis.

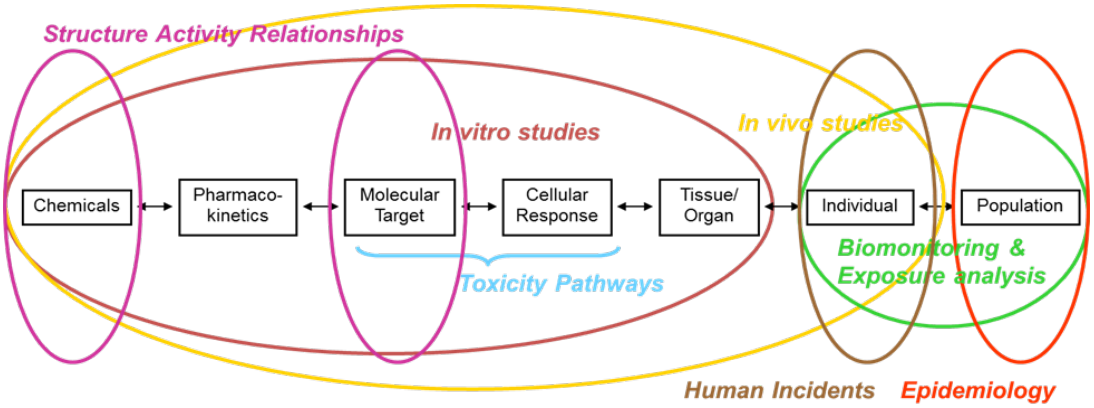

Figure 1.1. Source to outcome pathway (adapted from NRC, 2007).

[4] Chlorpyrifos Revised Human Health Risk Assessment for Registration Review; 29-DEC-2014; D424485.
U.S. EPA 2010 SAP Background White Paper – Re-evaluation of Human Health Effects of Atrazine: Review of Experimental Animal and In Vitro Studies and Drinking Water Monitoring Frequency. EPA-HQ-OPP-2010-0125.
U.S. EPA 2011 SAP Issue Paper – Re-evaluation of Human Health Effects of Atrazine: Review of Cancer Epidemiology, Non-cancer Experimental Animal and In Vitro Studies and Drinking Water Monitoring Frequency. EPA-HQ-OPP-2011-0399.

1.4 Summary of the Exposure Profile in the United States

All pesticide products provide critical information about how to safely and legally handle and use pesticide products. Pesticide labels are legally enforceable and all carry the statement "it is a violation of Federal law to use this product in a manner inconsistent with its labeling." In other words, the label is law. As a result, a key function of the pesticide product label is to manage the potential risk from pesticides.

Labeled uses of glyphosate include over 100 terrestrial food crops as well as other non-agricultural sites, such as greenhouses, aquatic areas, and residential areas. It is also registered for use on glyphosate-resistant (transgenic) crop varieties such as corn, soybean, canola, cotton, sugar beets, and wheat. Registered tolerances in the United States include residues of the parent compound glyphosate and *N*-acetyl-glyphosate, a metabolite found in/on glyphosate-tolerant crops[5].

Dietary (food and water) exposures are anticipated from applications to crops. Since there are registered uses of glyphosate that may be used in residential settings, residential handlers may be exposed to glyphosate during applications. Exposures may also occur from entering non-occupational areas that have been previously treated with glyphosate. Occupational/commercial workers may be exposed to glyphosate while handling the pesticide prior to application (mixing and/or loading), during application, or when entering treated sites. The agency considers all of the anticipated exposure pathways as part of their evaluation for human health.

Oral exposure is considered the primary route of concern for glyphosate. Oral absorption has been shown to be relatively low for glyphosate (~30% of administered doses) with negligible accumulation in tissues and rapid excretion (primarily unchanged parent) via the urine. Due to its low vapor pressure, inhalation exposure to glyphosate is expected to be minimal. Dermal penetration has also been shown to be relatively low for human skin (<1%) indicating dermal exposure will only contribute slightly to a systemic biological dose. Furthermore, in route-specific inhalation and dermal toxicity studies, no adverse effects were observed. This all suggests that there is low potential for a sustainable biological dose following glyphosate exposure.

In residential/non-occupational settings, children 1-2 years old are considered the most highly exposed subpopulation with oral exposures from dietary (food and water) ingestion and incidental oral ingestion (e.g., hand-to-mouth activities) in treated areas. There is also potential for dermal exposures in previously treated areas. Using HED's standard exposure assessment methodologies which are based on peer-reviewed and validated exposure data and models[6], a high-end estimate of combined exposure for children 1-2 years old is 0.47 mg/kg/day (see Appendix E).

[5] All currently registered tolerances for residues of glyphosate can be found in the Code of Federal Regulations (40 CFR §180.364).

[6] Available: http://www2.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedures-residential-pesticide

At the time of initial registration (1974), total use of glyphosate in the United States was approximately 1.4 million pounds (Benbrook, 2016). In 1995, total use of glyphosate increased to approximately 40 million pounds with agriculture accounting for 70% of use. With the introduction of transgenic crop varieties in the United States circa 1996, (such as soybean, cotton, and corn) use of glyphosate increased dramatically (Green and Owen, 2011), and in 2000 the total use of glyphosate in the United States was approximately 98.5 million pounds. By 2014, total annual use of glyphosate was approximately 280-290 million pounds (based on Benbrook, 2016 and industry proprietary data accessible to EPA) with agriculture accounting for 90% of use. Although glyphosate use has continuously increased up to 2012, the stabilization of glyphosate usage in recent years is due to the increase in a number of glyphosate-resistant weed species, starting with rigid ryegrass identified in California in 1998 and currently totaling 16 different weed species in the United States as of March 2016. Figure 1.2 below provides a visual representation of the increased agricultural use of glyphosate in the United States using proprietary market research data from 1987-2014.

The increased use of glyphosate may be partly attributed to an increase in the number of farmers using glyphosate; however, it is more likely that individuals already using glyphosate increased their use and subsequent exposure. With the introduction of transgenic crop varieties, glyphosate use shifted from pre-emergent to a combination of pre- and post-emergent applications. Additionally, application rates increased in some instances and more applications were allowed per year (2-3 times/year). Maps from the United States Geological Survey (USGS) displaying glyphosate use in the United States indicate that although use has drastically increased since 1994, areas treated with glyphosate for agricultural purposes appear to be approximately the same over time (Figures 1.3-1.4). The introduction of transgenic crops in some cases led to a shift in crops grown on individual farms, such that more acreage within the farm would be dedicated to growing the glyphosate-tolerant crops replacing other crops. In addition, during the 2000s there was also an increase in growing corn for ethanol production, which could also have resulted in increased acreage dedicated glyphosate-tolerant corn.

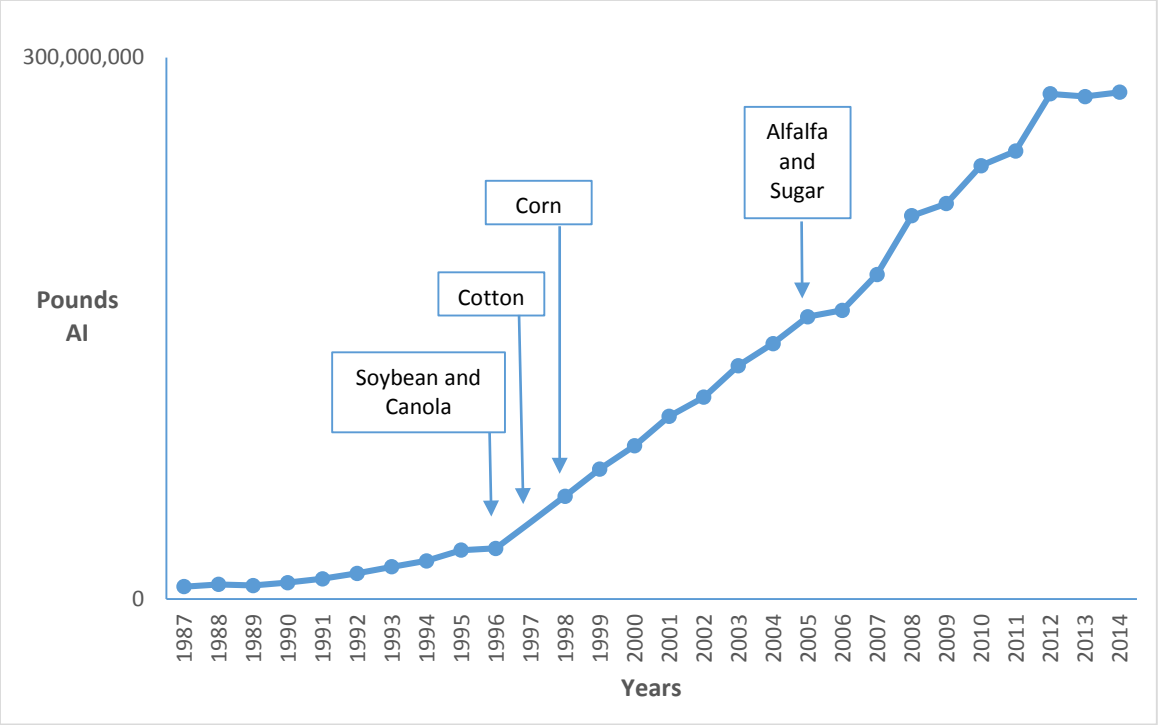

Figure 1.2. Glyphosate agricultural usage (pounds applied annually) from 1987- 2014. Boxes indicate years when glyphosate-resistant crops were introduced. Source: Proprietary Market Research Data (1987 – 2014).

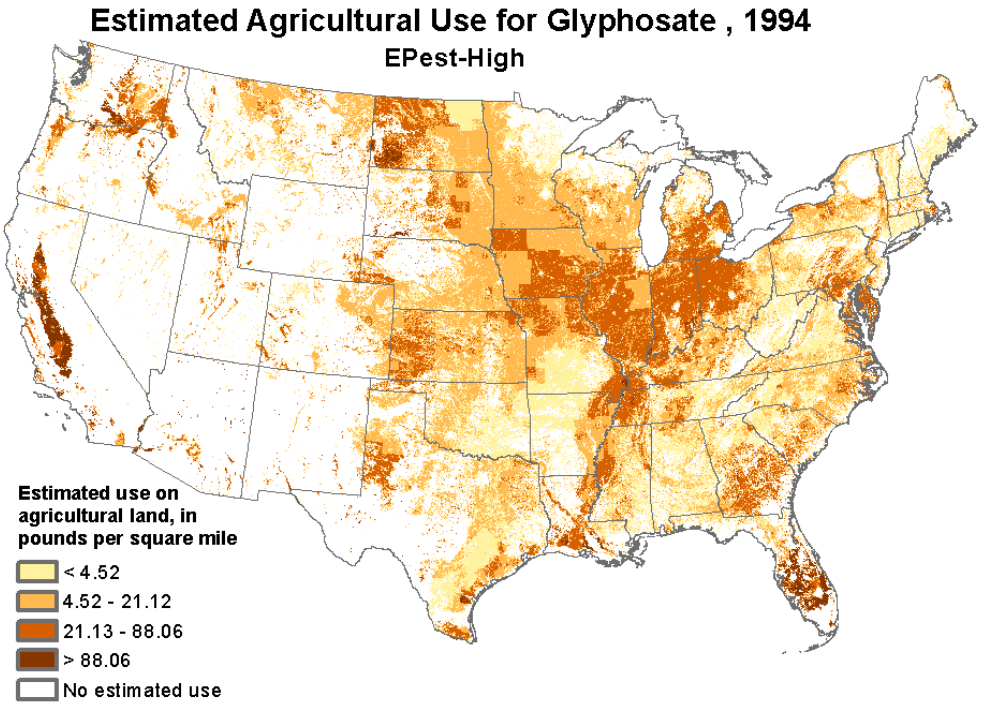

Figure 1.3. Map of estimated agricultural use for glyphosate in 1994 from USGS (http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=1994&map=GLYPHOSATE&hilo=H)

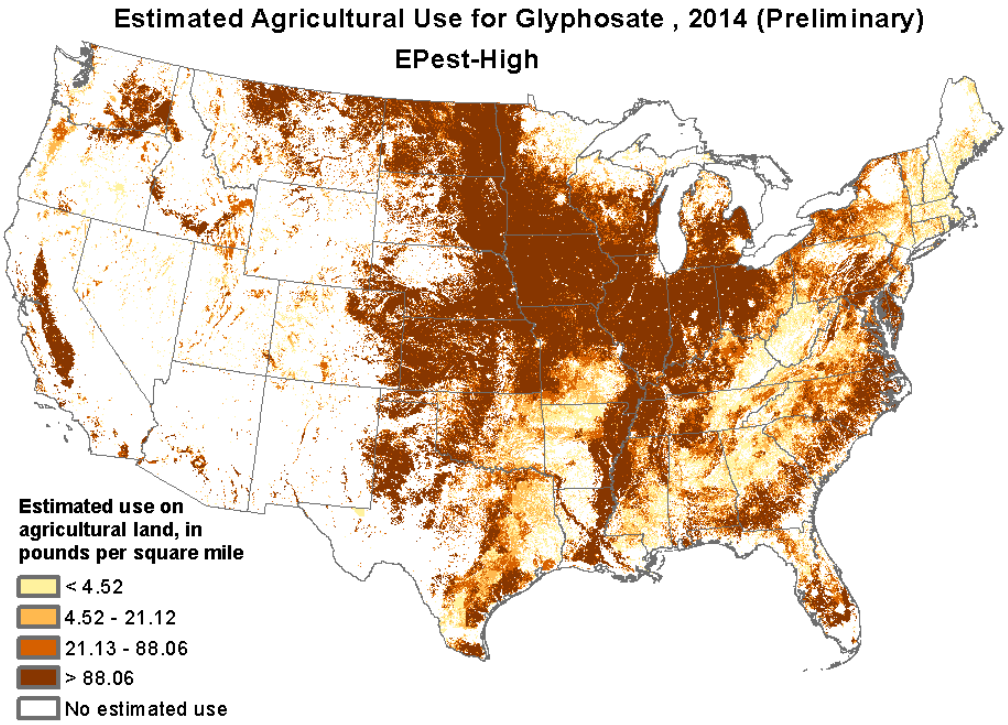

Figure 1.4. Map of estimated agricultural use for glyphosate in 2014 from USGS (http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2014&map=GLYPHOSATE&hilo=H)

The potential exposure to occupational handlers is dependent on the formulation, specific task (mixer, loader, and/or applicator), rate of application, and acreage treated. Using HED's standard occupational exposure assessment methodologies which are based on peer-reviewed and validated exposure data and models[7], mixer/loaders result in the highest potential exposure estimates. Assuming no personal protective equipment (PPE), exposure estimates for mixer/loaders range from 0.03-7 mg/kg/day using the maximum application rate for high acreage agricultural crops (6 lb ai/acre)[8]. For applicators, exposure would be lower with estimates ranging from 0.02-0.03 mg/kg/day using the same application rate and acreage.

The maximum potential exposures from currently registered uses of glyphosate in residential and occupational settings in the United States are used in the current evaluation to aid in the determination of whether findings in laboratory studies are relevant for human health risk assessment. In Sections 4.0 and 5.0, descriptions are provided for animal carcinogenicity and genotoxicity studies, respectively. Results from these studies, particularly those administering high doses, are put into context with the human exposure potential in the United States.

[7] Available: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/occupational-pesticide-handler-exposure-data

[8] Based on use information provided by the Joint Glyphosate Task Force for the following end-use products: EPA Registration Nos.: 100-1182, 228-713, 524-343, 524-475, 524-537, 524-549, 524-579, 4787-23, and 62719-556.

1.5 Organization of this Document

In this analysis of the human carcinogenic potential of the active ingredient glyphosate, the agency has performed a comprehensive analysis of available data from submitted guideline studies and the open literature. This includes epidemiological, animal carcinogenicity, and genotoxicity studies. Consistent with the 2010 draft framework, the agency has evaluated these multiple lines of evidence and conducted a weight-of-evidence analysis. Although there are studies available on glyphosate-based pesticide formulations, the agency is soliciting advice from the FIFRA SAP on this evaluation of human carcinogenic potential for the active ingredient glyphosate only at this time. The remainder of this document is organized by the following:

- *Section 2.0 Systematic Review & Data Collection Methods* provides a description of methods used to compile all relevant studies used in the current evaluation.
- *Section 3.0 Data Evaluation of Epidemiology* describes the available epidemiological studies, evaluates relevant studies for study quality, and discusses reported effect estimates.
- *Section 4.0 Data Evaluation of Animal Carcinogenicity Studies* provides a description and evaluation of the available animal carcinogenicity studies for glyphosate.
- *Section 5.0 Data Evaluation of Genetic Toxicity* summarizes and discusses the various genotoxicity assays that have been tested with glyphosate.
- *Section 6.0 Data Integration & Weight of Evidence Analysis Across Multiple Lines of Evidence* integrates available data discussed in Sections 3.0-5.0 to consider concepts, such as strength, consistency, dose response, temporal concordance and biological plausibility in a weight-of-evidence analysis. This section also provides discussion of the data in the context of cancer descriptors provided in the 2005 Guidelines for Carcinogen Risk Assessment.
- *Section 7.0 Collaborative Research Plan for Glyphosate and Glyphosate Formulations* provides a discussion of planned research that is intended to evaluate the role of glyphosate in product formulations and the differences in formulation toxicity.

2.0 Systematic Review & Data Collection

In recent years, the National Academy of Sciences National Research Council (NRC) has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific risk assessments to inform regulatory decision making (NRC, 2011). The NRC defines systematic review as “a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies" (NRC, 2014). Consistent with NRC’s recommendations, EPA’s Office of Chemical Safety and Pollution Prevention (OCSPP) is currently developing systematic review policies and procedures. In short, OCSPP employs “fit for purpose” systematic reviews that rely on standard methods for ***collecting, evaluating, and integrating*** the scientific data supporting the agency’s decisions. The concept of fit for purpose implies that a particular activity or method is suitable for its intended use. Inherent in this definition is the concept that one size does not fit all situations and thus flexibility is allowed. However, it is notable that with flexibility comes the

importance of transparency of documented processes; including the importance of transparency and clarity in approaches to data collection, evaluation, and integration. These are described throughout the document with data collection in Sections 2.1.1-2.1.2, evaluation in Sections 3-5, and integration in Section 6.

As a result, more recent evaluations are starting to reflect this progression in the agency's process. Similar to the draft framework for incorporating human epidemiologic and incident data, systematic review begins with a problem formulation to determine the scope and purpose of the search. Studies are considered based on their relevance to answer specific questions and those studies deemed relevant are then further considered for their usefulness in risk assessment.

The agency strives to use high-quality studies when evaluating the hazard potential of pesticidal chemicals and considers a broad set of data during this process. This includes registrant generated studies required under FIFRA, as well as peer-reviewed scientific journals and other sources, such as other governments and academia. A wide range of potential adverse effects are assessed using acute, subchronic, chronic, and route-specific studies; predominately from studies with laboratory animals, in addition to epidemiologic and human incident data. All studies are thoroughly reviewed to ensure appropriate conduct and methodologies are utilized, and that sufficient data and details are provided. In this way, hazards are identified and potential risks characterized to ensure that decisions are informed by the best science available.

2.1 Data Collection: Methods & Sources

Data were collected by searching the open literature (Section 2.1.1) and other publicly available sources (e.g., recent internal reviews, evaluations by other organizations) (Section 2.1.2). Internal databases were also searched for submitted studies conducted according to Organization for Economic Cooperation and Development (OECD) test guidelines, OCSPP harmonized test guidelines, and other pesticide test guidelines (OPP guidelines) (Section 2.1.2).

It should be noted that glyphosate is primarily manufactured as various salts with cations, such as isopropylamine, ammonium, or sodium. These salts are derivatives of the active substance glyphosate and increase the solubility of technical-grade glyphosate acid in water. All of these forms were considered for the current evaluation.

2.1.1 Open Literature Search

As part of the evaluation of the human carcinogenic potential of glyphosate, the literature review described here uses concepts consistent with fit for purpose systematic review, such as detailed tracking of search terms and which literature have been included or excluded. The primary goal of the literature search was to identify relevant and appropriate open literature studies that had the potential to inform the agency on the human carcinogenic potential of glyphosate. Therefore, non-mammalian studies were not considered, and several terms were used in the search string in an attempt to exclude non-mammalian studies.

To obtain literature studies, OPP worked with EPA librarians to conduct searches in PubMed, Web of Science, and Science Direct. A search was conducted on May 6, 2016 in PubMed and Web of Science using the following search string to yield 141 and 225 results, respectively:

((glyphosate OR "1071-83-6" OR roundup OR "N-(Phosphonomethyl)glycine") AND (aneuploid* OR chromosom* OR clastogenic* OR "DNA damag*" OR "DNA adduct*" OR genome* OR genotoxic* OR micronucle* OR cancer* OR carcinogen* OR oncogenic* OR mutagen* OR cytotoxic* OR tumor* OR tumour* OR malignanc* OR neoplasm* OR *oma)) NOT (fish* OR frog* OR tadpole* OR insect* OR eco* OR amphibian* OR reptil* OR invertebrate* OR fly OR flies OR aquatic OR bird* OR aqueous OR water OR yeast* OR worm* OR earthworm* OR bacteria* OR lichen OR resist* OR "herbicide resist")

Due to differences with using Science Direct, the search string was slightly changed. This search was also conducted on May 6, 2016 and yielded 459 results:

((glyphosate OR "1071-83-6" OR roundup OR "N-(Phosphonomethyl)glycine") AND (aneuploid* OR chromosom* OR clastogenic* OR (DNA pre/2 (damag* OR adduct*)) OR genome* OR genotoxic* OR micronucle* OR cancer* OR carcinogen* OR oncogenic* OR mutagen* OR cytotoxic* OR tumor* OR tumour* OR malignanc* OR neoplasm* OR *oma)) AND NOT (eco* OR fish* OR frog* OR tadpole* OR invertebrate* OR bird* OR insect* OR fly OR flies OR amphibian* OR reptil* OR yeast* OR aquatic OR aqueous OR water OR worm* OR earthworm* OR bacteria* OR lichen OR resist* OR "herbicide resist")

After cross-referencing the results obtained from the three open literature searches for duplicates, a total of 735 individual articles were obtained (Appendix A) and one additional study (Alvarez-Moya et al., 2014) not identified in the search was added to this list for a total of 736 individual articles. All of the studies were evaluated to determine if the study would be considered relevant to the issue of concern (i.e., human carcinogenic potential of glyphosate). Many of the articles were not considered to be within the scope of the search or not considered relevant in general (658 articles). Additionally, 27 articles were not appropriate due to the type of article (i.e., correspondence, abstract only, not available in English, retraction). Of the 51relevant articles, 42 were used in the current evaluation (31 genotoxicity, 9 epidemiological, and 2 animal carcinogenicity). Three articles also reported on the potential of glyphosate and its metabolites to be developed into therapeutic drugs for cancer treatment. The remaining 6 articles evaluated effects on glyphosate or glyphosate formulations on cellular processes, mostly focusing on epidermal cells, and were not considered informative for the current evaluation.

2.1.2 Studies Submitted to the Agency

For all pesticides, there are toxicology data requirements that must be submitted to the agency for registration. These studies, defined under the 40 CFR Part 158 Toxicology Data Requirements, provide information on a wide range of adverse health outcomes, routes of exposure, exposure durations, species, and lifestages. They typically follow OECD, OCSPP, or OPP accepted protocols and guidelines, which ease comparisons across studies and chemicals. The toxicological databases for glyphosate[9] were reviewed and all relevant animal, genotoxicity, and metabolism studies were collected for consideration.

[9] Glyphosate pesticide chemical (PC) codes: 103601, 103603, 103604, 103605, 103607, 103608, 103613, 128501, and 417300.

Several resources were used to ensure all relevant studies were included in the current evaluation. The list of studies obtained from the toxicological database and the open literature search were cross-referenced with recent internal reviews (CARC, 2015; S. Recore *et al.*, 2014). This list was also cross-referenced with review articles from the open literature [Chang and Delzell (2016), Greim et al. (2015), Kier and Kirkland (2013), Kier (2015), Mink *et al.* (2012), Schinasi and Leon (2014), and Williams *et al.* (2000)][10]. EPA requested studies from registrants that were not previously available to the EPA. As a result, numerous studies were subsequently submitted to the agency. Study reports for one animal carcinogenicity study and 17 genotoxicity studies, were not available to the agency and have been noted in the relevant sections below. For these studies, data and study summaries provided in Greim et al. (2015) and Kier and Kirkland (2013) were relied upon for the current evaluation.

2.2 Evaluation of Relevant Studies

Studies submitted to the agency are evaluating based on OECD, OCSPP, or OPP test guideline requirements to determine whether studies are acceptable for use in risk assessment. In the current evaluation, animal carcinogenicity, genotoxicity, and metabolism studies located in the internal databases with access to full study reports were evaluated in this manner. Those classified as unacceptable were noted and subsequently excluded from the current evaluation.

In order to evaluate open literature studies, criteria described in the OPP guidance for considering and using open literature toxicity studies to support human health risk assessment was utilized (U.S. EPA, 2012). This guidance assists OPP scientists in their judgement of the scientific quality of open literature publications. More specifically, the document discusses how to screen open literature studies for journal articles/publications that are relevant to risk assessment, how to review potentially useful journal articles/publications and categorize them as to their usefulness in risk assessment, and how the studies may be used in the risk assessment. As with submitted studies, those deemed unacceptable were noted and subsequently excluded from the current evaluation.

3.0 Data Evaluation of Epidemiology

3.1 Introduction

Epidemiological studies are valuable for risk assessment since they may provide direct evidence on whether human exposure to a chemical may cause cancer. Studies of high quality and adequate statistical power are preferable and remove the need to account for extrapolation from animals to humans or extrapolation from high to low doses. Epidemiological studies can also be integrated with experimental evidence when determining or clarifying the carcinogenic potential of a chemical for risk assessment. The key considerations in evaluating epidemiologic studies are study design, exposure assessment, outcome assessment, confounding control, statistical analyses, and risk of other bias.

[10] All review articles, except Schinasi and Leon (2014), were funded and/or linked to Monsanto Co. or other registrants.

OPP routinely evaluates the available epidemiological literature. As part of Registration Review of glyphosate, an evaluation was initially conducted in 2014 (S. Recore *et al.*, 2014) and subsequently another evaluation was performed in 2015 (CARC, 2015). The 2015 evaluation began with the epidemiological studies previously identified in the 2014 evaluation and included three additional studies that were not included in the 2014 evaluation. These studies were identified in review articles, included in the evaluation by IARC (2015), or were published since the 2014 OPP evaluation. Both the 2014 and 2015 OPP evaluations considered the design and overall quality of the epidemiological studies; however, formal study quality evaluations and rankings were not conducted. In the current review, all of the studies in the 2015 report, as well as additional epidemiological articles identified from a comprehensive search and cross-referencing with available resources as described under Section 2.0, were considered in the current evaluation, which totaled 58 epidemiological studies. The following sections provide a description of how epidemiological studies were evaluated for study quality and subsequent overall rankings, a summary of relevant studies, and a discussion of the overall results.

3.2 Considerations for Study Quality Evaluation and Scope of Assessment

This section summarizes how specific study characteristics were factored into the determination of a study's overall quality category. It should be noted that these study quality considerations are specific to the issue of concern (i.e., carcinogenic potential of glyphosate). These considerations are considered 'fit-for-purpose' under this context and could differ in another regulatory or scientific context. Although the basic concepts apply broadly, the study quality considerations are tailored specifically to studies investigating the association between glyphosate exposure and cancer outcomes. As with all research studies, the design elements of an epidemiological study have potential impacts on study quality and relevance to the research question under investigation. Each study was, therefore, judged to be of high, moderate, or low quality in each of the following six domains affecting study quality: study design, exposure assessment, outcome assessment, confounder control, statistical analysis, and susceptibility to bias (See Section 3.2.1 for general considerations under each domain). A similar approach was recently used by OPP for the evaluation of epidemiological studies for organophosphate pesticides (A. Lowit *et al.*, 2015).

Primary literature and associated meta-analyses evaluating the association between glyphosate exposure and a cancer outcome were the focus of this analysis. Reviews were only used to identify individual studies that should be considered for study evaluation. Commentaries, correspondence, and letters to the editor without original data were excluded. Of the relevant studies identified, studies with the most complete analyses utilizing the greatest number of cases and controls (e.g., pooled case-control studies) were evaluated for ranking (see Appendix B for visual representation of these studies). If studies did not collect exposure information on glyphosate from individual subjects, did not assess an outcome (e.g., biomonitoring studies), and/or did not provide a quantitative measure of an association between glyphosate and a cancer outcome, then these studies were assigned a low quality ranking and were not further evaluated in detail (see Figure 3.1). A similar process was used by JMPR for their identification of epidemiological studies for evaluating the carcinogenic potential of glyphosate and two other pesticides (JMPR, 2016).

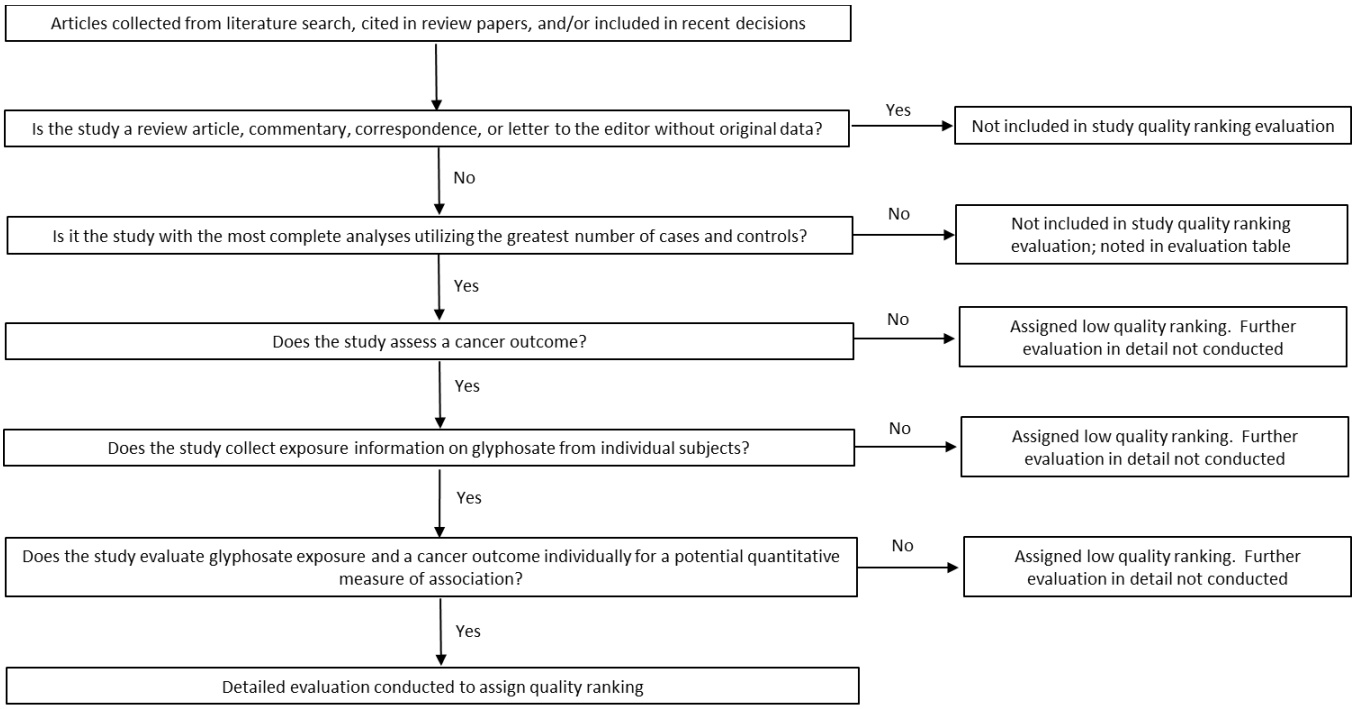

Figure 3.1. Study evaluation process for epidemiological studies.

3.2.1 Study Designs

In judging an individual study's contribution to the strength of evidence in the epidemiologic literature base, the following general hierarchy of observational study designs was considered (from most to least preferred): prospective cohort study (including nested case-control studies), case-control study, and cross-sectional study. It is important to note, however, that this hierarchy of study designs reflects the *potential* for the collection of high quality information (related to exposure, outcome, confounders, and effect modifiers) and *potential* for efficient and valid estimation of the true association. Thus, in deliberating on quality, care has been taken to consider the circumstances and particulars of each individual study to consider whether the study was well conducted independent of the type of study design.

The study designs used in the epidemiological literature reviewed were analytical and descriptive studies. Cohort and case-control study designs are analytical studies used to evaluate relative incidence of health and disease outcomes by exposure status. Cross-sectional and ecological studies are generally considered descriptive or hypothesis-generating study designs; however, they can also be used to test hypotheses regarding prevalence of health outcomes and, under certain conditions, incidence as well.

Table 3.1. Epidemiological Study Quality Considerations[a].

Parameter	High Score	Moderate Score	Low Score
Study Design	Cohort	Case-control	Cross-sectional/Ecological

Table 3.1. Epidemiological Study Quality Considerations[a].			
Parameter	**High Score**	**Moderate Score**	**Low Score**
Exposure Assessment	Questionnaire and/or interview answered by subjects for chemical-specific exposure	Questionnaire and/or interview for chemical-specific exposure answered by subjects or proxy individuals	Low-quality questionnaire and/or interview; information collected for groups of chemicals rather than chemical-specific; no chemical-specific exposure information collected; ever/never use of pesticides in general evaluated
Outcome Assessment	State or National registries, physicians, and/or special surveillance programs with cases verified by histopathological evaluation for tumors; appropriate consideration of prevalent vs. incident cases; analysis by valid method specific for biomarkers	State or National registries, physicians, and/or special surveillance programs without histopathological verification for tumors; analysis by assays that are less specific for biomarkers of interest	No outcome evaluated; unclear/no consideration for whether prevalent or incident cases are appropriate; biomarker methods not validated
Confounder Control	Good control for important confounders related to cancer, standard confounders, and known confounders for glyphosate and cancer outcomes (e.g., exposure to multiple pesticides) through study design or analytic control with well measured co-exposures (i.e., cumulative exposure)	Moderately good control for confounders related to cancer; standard variables accounted for and; attempt to control for known confounders via a less efficient measure of co-exposure (e.g., ever/never use)	No adjustments for confounders
Statistical Analyses	Appropriate to study question and design, supported by relatively adequate sample size, maximal use of data, reported well	Acceptable methods, lower/questionable study power	Minimal attention to statistical analyses, sample size evidently low, comparison not performed or described clearly
Risk of (Other) Bias	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of effect estimate, no/low potential of selection bias	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate, evidence of potential selection bias with low impact on effect estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanation for study findings, evidence of selection bias with high potential to impact effect estimate

[a] Overall study quality ranking based on comprehensive assessment across the parameters.

3.2.1.1 Analytical Studies

(1) Cohort Study

In a typical cohort study, such as the Agricultural Health Study (AHS), individuals are classified according to exposure status (i.e., presence, absence, or magnitude of exposure) and then followed over time to quantify and compare the development (i.e., incidence) of the health outcome of interest by exposure group. Conceptually, the non-exposed comparison group in a cohort study provides an estimate of the incidence of the outcome among the exposed, had they, counter-to-fact, not been exposed. Apart from chance variations, a valid cohort study comparing exposed individuals to non-exposed individuals provides an estimate of the relative risk (or rate) of the disease associated with exposure. Ideally, the exposed and non-exposed groups are exchangeable, in the sense that switching the exposed to non-exposed, and non-exposed to exposed would yield the same measure of association (e.g., relative risk). If this were the case then, apart from chance, a cohort study would yield a measure of association equivalent to that produced in a corresponding (intervention) study where exposure status was randomly assigned.

The chief advantage of the cohort study design is that it affords the investigator the opportunity to avoid and/or adjust for potential biases (i.e., selection bias, information bias, and confounding); however, these biases may also be avoided in other well-designed study designs, such as a case-control study. Cohort studies also allow for discernment of the chronological relationship between exposure and outcome, and can be particularly efficient for studying uncommon exposures. The primary disadvantage of the cohort study design is logistical inefficiency with respect to the necessary time, expense, and other resources needed to conduct them. Cohort studies are particularly inefficient for evaluating associations with rare outcomes and diseases with long induction or latency periods. Case-control studies that are nested within a cohort study (nested case-control studies) share the attributes of the cohort study and may be more efficient. However, when follow-up throughout the study period is incomplete, the potential for selection bias is increased, especially if follow-up rates are related to exposure status.

Two sub-categories of cohort studies – prospective and retrospective – are often applied to distinguish between studies in which the health outcome has occurred (retrospective study), or has not occurred (prospective study) at the time the investigators initiate the study. This distinction is important primarily as it pertains to the potential differences in the quality (e.g., completeness, accuracy, and precision) of information that can be ascertained by the investigators, and also as it relates to potential sources of bias. Although not always true, the prospective study design is considered the preferable of the two, as investigators can potentially have more choices in determining how exposure, outcome, and covariate information is collected. In a retrospective study conducted to evaluate the same hypothesis, by contrast, the investigators would have to rely on exposure information based on self-reporting or historical records. Such reporting is subject to (human) errors in recall, however when such errors are uncorrelated with disease state, there can be a bias towards the null due to random exposure measurement error (information bias) and only when such errors are correlated with the disease state can there be bias away from the null.

(2) Case-Control Study

In a typical case-control study, individuals are classified according to their outcome status (i.e., cases who have developed the outcome of interest, and controls who represent the population

from which the cases arise). The relative odds of exposure are then compared between cases and controls. The primary advantage of case-control studies is that they are logistically efficient relative to cohort studies, often being conducted at a fraction of the cost and in a fraction of the time as a corresponding cohort study. Case-control studies can be used to examine associations between multiple exposures and a given health outcome. They are particularly efficient for evaluating rare outcomes, but are inefficient for studying uncommon exposures. An important point to evaluate in each case-control study is the potential for selection bias, which arises if the exposure distribution among the control subjects is not representative of the exposure distribution among the population that gave rise to the cases. When participation rates between cases and controls are low or distinctly imbalanced, the potential for selection bias is increased, especially if participation rates are related to exposure status. Case-control studies that rely on self-reported exposure measures are also potentially susceptible to information bias which could result in bias towards the null or away from the null.

3.2.1.2 Descriptive Studies

Cross-sectional studies are used to evaluate associations between exposure and outcome prevalence in a population at a single point in (or period of) time. The primary advantage of a cross-sectional study is logistical efficiency. They are relatively quick and inexpensive to conduct, as a long period of follow-up is not required, and exposure and outcome assessments occur simultaneously. Cross-sectional studies have three primary *potential* disadvantages: 1) potential difficulty in discerning the temporal relationships (i.e., whether the exposure precedes the outcome); 2) estimating outcome prevalence rather than incidence of the outcome; and 3) the possible overrepresentation of cases of the outcome with long duration relative to the average in the population, and often with a better prognosis.

Ecological studies are used to evaluate associations between exposures and outcomes using population-level rather than individual-level data. The primary advantages of ecological studies are related to logistical efficiency, as they often rely on pre-existing data sources and require no individual-level exposure, outcome, or covariate assessments. The primary weakness of the ecologic study is the potential for confounding and resultant inappropriate extrapolation of associations observed on the aggregate-level to associations on an individual level. The discrepancy that associations observed at the population level are not observed at the individual level is referred to as the ecological fallacy. Semi-ecological studies are less susceptible to the ecological fallacy due to incorporation of individual-level data on outcomes and/or confounders. The quality of these studies depends on the ability of the group exposure data to represent individual exposure and the research question of interest.

3.2.2 Exposure Measures

As described in Section 3.2 and Figure 3.1, studies assigned a low quality ranking based on an initial evaluation were not further evaluated in detail. In all of the studies included in the analysis that were reviewed and ranked for study quality, exposure information was collected from subjects and/or proxy individuals via questionnaires and/or interviews. These exposure assessments typically include questions to determine the amount of direct pesticide use or to collect information on behaviors and conditions associated with pesticide use (e.g., occupation, tasks). This type of reporting likely misclassifies actual pesticide exposure. If conducted as part

of a prospective exposure assessment, these errors are likely to be non-differential with respect to the outcome(s) of interest. In a retrospective assessment, the subject or proxy has knowledge of the outcome; therefore, these errors may be differential or non-differential. Studies that exclusively used subjects rather than including proxy individuals were considered more reliable and given a higher weight given that the subjects would have a more accurate recollection of their own exposure.

3.2.3 Outcome Measures

All of the studies evaluated in detail, except one, utilized state or national cancer registries, physicians, and/or special surveillance programs to determine outcome status (i.e., subjects with or without a cancer of interest). In several studies, the cases were also verified by histopathological evaluation. Overall, outcome measures were relatively consistent across studies and these assessments are likely to have minimal errors. The remaining study evaluated in detail (Koureas et al., 2014) assessed oxidative DNA damage rather than a type of cancer. For this evaluation, the oxidation by-product 8-hydroxydeoxyguanosine (8-OHdG) was measured by enzyme immunoassay. This type of assay generally exhibits low specificity. More sensitive quantitative methods are available to analyze genomic DNA for 8-OHdG by high-performance liquid chromatography (HPLC) with electrochemical detection, gas chromatography-mass spectrometry (GC-MS), and HPLC tandem mass spectrometry. Consideration of incident or prevalent cases should also be carried out. By using only incident cases, there is greater confidence that exposures occurred prior to the development of the outcomes. Inclusion of prevalent cases can lead to an over-representation of cases with a long course of disease.

3.2.4 Confounding

The degree to which confounders were controlled varied across studies. Some studies adjusted for particular medical variables, while others did not. Some standard variables, such as age, geographical location, and sex, were either adjusted for analytically or by matching in case-control studies. Several studies collected information on potential confounders; however, not all of these variables were evaluated or results of the evaluation were not reported. The direction and magnitude for confounders are, in general, difficult to determine because they are dependent upon the relationship of each confounding factor with glyphosate and the cancer under investigation. Several studies considered the potential for confounding from co-exposure to other pesticides; however, only a few reported effect estimates between glyphosate exposure and cancer risk adjusted for the use of other pesticides. Given most people in the epidemiological studies who use pesticides occupationally will be exposed to multiple pesticides and, in some instances, those other pesticides were observed to be risk factors for the same cancer, this is a particularly important concern to address in either the study design or in the statistical analyses. Across numerous studies, co-exposures to other pesticides was found to be positively correlated with exposure to glyphosate and exposure to those other pesticides appear to increase the risk of some cancers. As a result, the direction of confounding would be to inflate any true effect of glyphosate in the absence of statistical control. This underlines the importance of controlling for co-exposures to other pesticides.

For NHL, other potential confounders, such as exposure to diesel exhaust fumes, solvents, ultraviolet radiation, livestock, and viruses, have been identified. Some of these are more

plausible than others. For example, occupational exposure to diesel exhaust fumes (e.g., McDuffie et al., 2002; Karunanayake et al. 2008; Baris et al. 2001; Maizlish et al. 1998) and solvents (Wang et al., 2009; Kato et al., 2005; Olsson and Brandt, 1988) are considered likely to increase the risk of NHL. Agricultural workers are exposed to diesel fumes when using agricultural vehicles when applying pesticides, such as glyphosate, and when using heavy equipment during mixing, loading, and/or applying pesticides. Agricultural workers are also exposed to solvents. Solvents are often used in pesticide products to aid the delivery of the active ingredient and enhance efficacy. Solvents are also used for cleaning and maintenance/repair of agricultural equipment used for mixing, loading, and/or applying pesticides. With an association between exposure and outcome of interest, it is reasonable to consider diesel exhaust fumes and solvents as probable confounders; however, neither of these factors were accounted for in any of the studies evaluated in detail. There is also evidence that ultraviolet (UV) radiation may increase the risk of NHL (Karipidis et al., 2007; Zhang et al., 2007). As a result, there is a support that UV radiation is also a potential confounder given the extended amount of time agricultural workers spend outside performing activities, including those associated with pesticide use. This was also not accounted for in any of the studies evaluated in detail.

3.2.5 Statistical Analyses

Statistical analyses that were appropriate to the study question and study design, supported by adequate sample size, maximized the use of available data, and were well characterized in the report were weighted most highly. Acceptable statistical methods, questionable study power, and analytical choices that resulted in the loss of information were given moderate weight. Reports with only minimal attention paid to the conduct and reporting of the statistical analyses were given the lowest weight.

3.2.6 Risk of Bias

The internal validity of the studies reviewed was judged by noting the design strategies and analytic methods used in each study to constrain or eliminate selection bias, information bias, and confounding. Selection bias can occur when the sampling of the population by the investigator yields a study population that is not representative of the exposure and outcome distributions in the population sampled. Put simply, selection bias occurs if selection of the study sample yields a different estimate of the measure of association than that which would have been obtained had the entire target population been evaluated. Although there are numerous sources of selection bias, there are several mechanisms that may have induced selection bias in the studies reviewed: low participation rates of eligible individuals due to non-responsiveness or refusal (self-selection bias); loss to follow-up (i.e., failure to retain all study participants initially enrolled in the study); and, in a case-control study, control selection bias arising because the exposure distribution in the control sample does not represent the exposure distribution of the study base (i.e., the population that gave rise to the cases or more formally, the person-time experience of that population).

Information bias (also referred to as observation bias) arises when study participants are incorrectly categorized with respect to their exposure or outcome status, or when errors arise in the measurement of exposure or outcome, in the case of continuously distributed measures.

Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random). Non-differential misclassification of exposure (or non-differential exposure measurement error) occurs when the probability or magnitude of error in the classification or measurement of exposure is independent of the outcome status of the study participants. Non-differential exposure measurement error typically results in a bias towards the null which may obscure any true effect of the exposure of interest. Similarly, non-differential misclassification of outcome (or outcome measurement error) occurs when the probability or magnitude of error in the assignment of outcome status or level is independent of exposure status. Non-differential outcome measurement error typically does not cause bias but does decrease the precision of effect estimates and therein inflates the width of confidence intervals. In contrast, differential exposure misclassification (or measurement error) occurs when the error in the exposure assignment is not independent of the outcome status. The mechanisms that cause non-differential misclassification in the currently reviewed literature include random errors in exposure recall from subjects or proxy respondents. The mechanisms that could induce differential misclassification include recall bias and interviewer/observer bias. Note that mismeasurement of confounders can result in residual confounding of the association of interest, even when adjustment for that confounder has been conducted in the analysis.

Studies in which major sources of potential biases were not likely to be present, studies in which potential sources of bias were present, but effectively addressed and analyzed to maximize the study validity, and studies in which sources of bias were unlikely to influence the magnitude and direction of the effect estimate were given more weight than studies where sources of bias may be present, but not addressed in the study.

3.3 Review of Quality Results

Each study was judged to be of high, moderate, or low quality in each of the six domains affecting study quality, as discussed above and in Table 3.1. The results of the quality assessment are presented separately for each group below. The quality rankings presented are specific to the current evaluation of the carcinogenic potential of glyphosate. As noted above and in Table 3.2, several studies were not included in the ranking evaluation because they did not represent the most complete analysis. Rather, the subjects were included in a larger analysis (e.g., pooled case-control study) to produce a greater number of cases and controls (see Appendix B for visual representation of these studies). For example, Cantor *et al.* (1992) was not individually evaluated for ranking because the data from this study were pooled with data from other studies in De Roos *et al.* (2003), which was included.

3.3.1 "High" Quality Group

Three studies were given a high quality ranking: De Roos *et al.* (2005), Eriksson *et al.* (2008), and Koutros *et al.* (2013).

De Roos *et al.* (2005) was the only cohort study available for ranking. This prospective cohort study evaluated associations between various pesticide exposures, including glyphosate, and cancer incidence for numerous solid and non-solid tumors in the AHS. The aim of the AHS is to evaluate the role of agricultural exposures in the development of cancer and other diseases in the

farming community. AHS recruited 52,934 licensed private pesticide applicators along with 32,345 of their spouses between 1993 and 1997. In the first two phases of the study, the cohort also included 4,916 commercial pesticide applicators from Iowa. As a prospective analysis of the AHS cohort, information was obtained from exposed subjects at enrollment and no proxies were necessary. Exposure was evaluated as ever/never use, cumulative lifetime exposure, and intensity-weighted cumulative exposure. Due to the study design, the potential for many biases were reduced. Additionally, the study adjusted and/or considered numerous factors, including use of other pesticides. Study participants provided detailed pesticide exposure information prior to enrollment in the study and this information has been incorporated into the study evaluation by determining tertile cut points and calculating effect estimates by comparing to the lowest tertile. Additional evaluations with quartiles and quintiles were performed for cancers with elevated effect estimates in the study and for NHL.

Eriksson *et al.* (2008) was a population-based case-control study that recruited a consecutive series of incident cases of NHL in several regions of Sweden from physicians treating lymphoma within specified health service areas. Cases were verified pathologically and matched to randomly selected controls from the national population registry by age, sex and health service area. Exposure information was collected from exposed individuals (i.e., no use of proxy respondents) using a comprehensive questionnaire including a total work history with in depth questions about exposures to pesticides, solvents, and other chemicals. Interviewers were blinded to case/control status. The study only reported minimal demographic information on subjects (age and sex) and a table with subject characteristics (e.g., smoking status, alcohol intake, physical activity, education) that could potentially be used to adjust effect estimates was not provided. Glyphosate exposure was reported in 29 cases and 18 controls during the study period. Multivariate analyses were adjusted for co-exposure to different agents, including MCPA, "2,4,5-Y and/or 2,4-D", mercurial seed dressing, arsenic, creosote, and tar. An analysis for a potential exposure-response relationship was also conducted; however, it was not clear whether this analysis controlled for co-exposure to other pesticides based on the statistical methods description. The number of cases and controls were also not reported for this analysis.

Koutros *et al.* (2013) was a nested case-control study within the AHS that evaluated the association between pesticide use and prostate cancer. Exposure information was collected from exposed subjects (no proxies necessary) through the enrollment questionnaires, as well as in a follow-up questionnaire administered 5 years after enrollment. This study evaluated the association between glyphosate and prostate cancer diagnoses from enrollment (1993-1997) through 2007 resulting in a longer follow-up time than many of the other case-control studies that utilized AHS subjects. The study used lifetime cumulative exposure and intensity-weighted cumulative exposure metrics. Analyses were also conducted using unlagged exposure and 15-year lagged exposure, which excluded the most recent 15 years of exposure for both exposure metrics. Although the effect estimate reported for glyphosate in this study was not adjusted for co-exposure to other pesticides, additional analyses were not considered necessary since there was no association observed.

3.3.2 "Moderate" Quality Group

Twenty-one case-control studies were assigned a moderate quality rating (Table 3.2). In general, these studies share many study design characteristics. Exposure information was collected from subjects and/or proxy individuals, the outcome measurement(s) utilized state/national registries and surveillance programs, appropriate statistical analyses were performed, some covariates but maybe not all relevant covariates were evaluated and/or considered, and risks of bias were minimized to some extent. Sample sizes varied across studies. Case-control studies investigating solid tumors included study populations in the United States and Canada. For non-solid tumors, study populations were located in the United States, Canada, Sweden, France, Germany, Italy, Ireland, Spain, and the Czech Republic. Although several nested case-control studies shared most of the characteristics of the AHS cohort study, these studies were primarily given a moderate quality ranking since co-exposure to other pesticides was not accounted for in the analyses.

3.3.3 "Low" Quality Group

Seven case-control and 27 cross-sectional/ecological studies were assigned a low quality ranking. All of these studies, except one case-control study (Cocco *et al.*, 2013) and one descriptive study (Koureas et al., 2014), were not subjected to a detailed evaluation because they did not report a quantitative measure of an association between glyphosate exposure and a cancer outcome, did not collect information on glyphosate exposure from all subjects, and/or did not evaluate risk to a cancer outcome (Appendix D). In many instances, effect estimates were reported only for total pesticide exposure. Additionally, exposure was assumed and glyphosate-specific exposure information was not collected. In other studies, the aim of the study was to assess exposure methods for epidemiological studies and/or to evaluate the impact of exposure misclassification; therefore, there was no evaluation of a cancer outcome.

It should be noted that some of the studies assigned a low quality ranking in the current evaluation were included in the recent evaluation by IARC. There were a number of descriptive studies that evaluated the genotoxicity in human populations; however, these studies did not meet the criteria for inclusion in the ranking as described in Section 3.2 and Figure 3.1. In most instances, these studies reported effect estimates for total pesticide exposure and/or assumed glyphosate exposure without collecting glyphosate-specific exposure information. For case-control studies, Cocco et al. (2013), Dennis et al. (2010) and Ruder et al. (2004) were included in the 2015 IARC evaluation, but were not considered informative in the current evaluation.

Detailed evaluations were not performed in the current evaluation for Dennis et al. (2010) and Ruder et al. (2004) because a quantitative measure of an association between glyphosate and a cancer outcome was not reported. Cocco et al. (2013) received a detailed evaluation and was assigned a low quality ranking. This case-control study, which evaluated lymphoma risk across six European countries, was not considered informative due to a combination of numerous limitations in the study. The power of the study was low with only four cases and two controls exposed to glyphosate. Control ascertainment was not consistent across countries, with a mix of hospital- and population-based controls used. The overall participation rate for population-based controls was found to be much lower than the overall participation rates of the cases or hospital-based controls. Lastly, the study was limited to ever/never use of glyphosate and did not control for confounders, in particular exposure to other pesticides. Although this study was included in

the IARC evaluation, IARC also stated that the study had very limited power to assess the effects of glyphosate on risk of NHL.

The other study subjected to a detailed evaluation and assigned a low quality ranking was Koureas *et al*. (2014). This cross-sectional study evaluated the association between glyphosate exposure and oxidative DNA damage in 80 Greek pesticide sprayers. Although the study reported a non-statistically significant effect estimate for glyphosate, it is limited in its ability to contribute to the overall evaluation of the carcinogenic potential of glyphosate. The effect estimate was not adjusted for any standard covariates or potential confounders, including co-exposure to other pesticides. The power of the study was questionable. There were 80 subjects, but the number exposed to glyphosate was not reported. The outcome is measured using an immunoassay that is less specific for measuring the biomarker of interest than other available analytical methods. Lastly, the study evaluates primary DNA damage, but does not measure the consequence of genetic damage. An increase in oxidative DNA damage may lead to cell death or initiate DNA repair rather than lead to a mutation.

Due to the limitations in the studies assigned a low quality ranking, they do not provide reliable information to evaluate associations between glyphosate exposure and cancer outcomes. Therefore, the remaining sections of this document do not further discuss these studies except to note when a study is included in meta-analyses.

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
Alavanja *et al.* (2003)	This study was not included in the study quality ranking because the data were used in the updated analysis by Koutros *et al.* (2013).						
Andreotti *et al.* (2009)	Nested Case-control	Questionnaire answered by subjects at study enrollment followed by take-home questionnaire; examined exposure for glyphosate as ever/never, and intensity-weighted cumulative exposure days; spouses either self-administered questionnaire (81%) or telephone interview (19%)	State cancer registries without histopathological verification; exclusion of subjects with prevalent cancer at enrollment; follow-up ~ 9 years	Adjusted for age, smoking, and diabetes for both exposure metrics as well as applicator type forever/never exposure metric No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional logistic regression to obtain OR and 95% CI	Exposure misclassification particularly for spouses, low response rate to take-home questionnaire (40%) but unclear if affected cases and controls differently, insufficient power for pesticide exposure interactions	Moderate
Band *et al.* (2011)	Population-based case-control Males only	Self-administered questionnaire answered by subjects or proxies for deceased subjects requesting work history and demographic information; use of a job exposure matrix to estimate exposure to pesticides	Cancer registry with histopathological verification; excluded farmers that worked all outside of British Columbia; included prostate cancer cases prior to the PSA era	Adjustment for alcohol consumption, cigarette years, education level, pipe years, and respondent type. Marital status and ethnicity not significant No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Conditional logistic regression to obtain ORs and 95% CIs	Recall bias, use of proxy for deceased, exposure misclassification, participation rates cited from another study, use of cancer patients as controls (excluding lung and unknown cancer)	Moderate
Brown *et al.* (1990)	Pooled population-based case-control Males only	In-person interviews using standardized questionnaire with subjects or proxies for deceased/incapacitated; supplementary questionnaire administered by telephone for Iowa subjects to obtain more	State cancer registry (Iowa) and special surveillance network including hospitals and pathology laboratories (Minnesota); cases ascertained retrospectively and prospectively (2 years after start of study);	Adjusted for vital status, age, state, ever used tobacco daily, close relative with lymphopoietic cancer, nonfarming job related to risk of leukemia in the study, exposure to substances related to risk in this study	Unconditional logistic models to obtain OR and 95% CI; questionable power (15 cases)	Recall bias; exposure misclassification, use of proxy respondents	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
		detailed information from those indicating pesticide use	~26% of cases deceased or too ill when identified and ~15% deceased or too ill at time of interview; histopathological verification by pathologists	(benzene, napthalene, hair dyes) No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)			
Brown *et al.* (1993)	Population-based case-control Males only	In person interviews with standardized questionnaire to obtain detailed information on farm activities and use of pesticides from subjects or proxies	State cancer registry (Iowa) ascertained retrospectively and prospectively (2 years after start of study); ~26% of cases deceased or too ill when identified and ~15% deceased or too ill at time of interview; histopathological verification by pathologists	Adjusted for vital status and age; smoking and education evaluated and not found to be significant No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Logistic models to obtain OR and 95% CI; questionable power (11 cases)	Recall bias; exposure misclassification, use of proxy respondents	Moderate
Cantor *et al*. (1992)	This study was not included in the study quality ranking because the data were used in the pooled analysis conducted by De Roos *et al.* (2003).						
Carreon *et al.* (2005)	This study was not included in the study quality ranking because the data were used in the pooled analysis conducted by Yiin *et al.* (2012).						
Cocco *et al*. (2013)	European multi-center case-control Hospital-based and population-based (mixed for 2 countries, only hospital-based for the rest)	Trained interviewers conducted in person interviews using structured questionnaire answered by subjects; those identified as agricultural worker on questionnaire given subsequent questions about pesticide use, crops, etc.	Surveillance centers, 20% of slides from each center reviewed by pathologist	Adjustment for age, sex, education, and center. No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional logistic regression to obtain ORs and 95% CIs; Low power (4 cases, 2 controls)	Recall bias, selection bias (low response rate for population-based controls and differed from cases), exposure misclassification, mix of hospital- and population-based controls,	Low
De Roos *et al*. (2003)	Population-based case-control Males only Pooled analysis of	Interviews with subjects or proxy for deceased subjects. Different interview techniques across states. One study collected information on	State cancer registries (one state chose a random sample, other states chose all cases), surveillance programs, and hospitals without	Adjustment for age, study site, and other pesticides. First degree relative with haematopoietic	Logistic regression and hierarchical regression to obtain ORs and 95% CIs	Recall bias, exposure misclassification, , use of proxy for deceased, , varying quality of questionnaire/interview techniques across studies	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
	Cantor *et al.*, 1992; Hoar *et al.*, 1986; Zahm *et al.*, 1990	pesticide use and then followed-up with questions on selected specific pesticides, another study had a direct question about a selected list of specific pesticides, and the last study used an open ended question without prompting for specific pesticides	histopathological verification	cancer, education, and smoking not found to be important confounders. No adjustment for other potential confounders (e.g., solvents, diesel fumes, UV radiation)			
De Roos *et al*. (2005)	Prospective cohort (licensed pesticide applicators)	Questionnaire answered by subjects at enrollment and with subsequent take-home questionnaire; examined exposure as ever/never, cumulative lifetime days, and intensity-weighted cumulative exposure days	State cancer registries without histopathological verification; follow-up ~7 years	Adjustment for state of residence, age, education, smoking history, alcohol consumption, family history of cancer, use of other common pesticides No adjustment for other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Poisson regression to obtain RRs and 95% CIs	Major sources of potential biases unlikely, potential exposure misclassification due to any changes in exposure since enrollment, follow-up period may be limited	High
Engel *et al.* (2005)	Nested case-control Females only	Take-home questionnaire from spouses of enrolled applicators used to obtain farm exposures, general health information, and reproductive health history; Information obtained from applicators used as measure of possible indirect exposure to spouses	State cancer registries identifying malignant breast cancer; ~5 years average follow-up time	Adjusted for age, race and state. Evaluated BMI, age at menarche, parity, age at first birth, menopausal status, age at menopause, family history of breast cancer, physical activity, smoking, alcohol consumption, fruit and vegetable consumption and education but none	Poisson regression to obtain RRs and 95% CIs	Exposure misclassification, exposure to other pesticides (however no association observed), lack of information on length of marriage could result in overestimating exposure based on husband	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
				found to be significant No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)			
Eriksson *et al*. (2008)	Population-based case-control	Questionnaire answered by subjects; follow-up by phone if incomplete answers; excluded exposures that occurred during the same calendar year and year before diagnosis (cases) or enrollment (controls); minimal demographic information reported	Physicians treating lymphoma within specified health service areas and verified by pathologists	Adjustment for age, sex, year of diagnosis/enrollment, as well as exposure to other pesticides in multivariate analyses. Not stated what adjustments were made for other pesticides in latency analyses. No adjustment other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional logistic regression and multivariate analyses to obtain ORs and 95% CIs; not clear how multivariate was performed; questionable power (29 cases, 18 controls); also included analysis of ≤10 vs. >10 years exposure	Recall bias, exposure misclassification, lack of subject demographics/ characteristics (e.g., smoking, alcohol consumption, race, etc)	High
Flower *et al.* (2004)	Nested case-control	Questionnaire answered by applicators at enrollment; spouses enrolled through a questionnaire brought home by applicator; females (applicators and spouses) were asked to complete a questionnaire on female and family health that collected information on children born during or after 1975	State cancer registry to identify childhood cancer cases (diagnosed from birth through 19 yrs of age) for children of parents enrolled; hybrid prospective/retrospective ascertainment; excluded female applicators	Child's age at parent's enrollment was included in model; parental age at child's birth, child's sex, child's birth weight, history of parental smoking, paternal history of cancer, and maternal history of miscarriage were evaluated but not found to be significant and not included in model No adjustment for co-	Logistic regression to obtain OR and 95% CI; calculated standardized incidence ratios to compare observed number of childhood cancer cases identified to the expected number; low/questionable power (6 parental cases, 13 maternal cases)	Exposure misclassification, lack of timing data to determine if exposure occurred prior to conception or during pregnancy, exposure to other pesticides (however no association observed and lack of power for adjustment)	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
				exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)			
Hardell and Eriksson (1999)	This study was not included in the study quality ranking because the data were used in the pooled analysis conducted by Hardell *et al.* (2002).						
Hardell *et al*. (2002)	Population-based case-control Males only Pooled analysis of Hardell and Eriksson 1999 and Nordstrom *et al.*, 1998	Questionnaire answered by subjects or proxy for deceased subjects to obtain complete working history and exposure to different chemicals; follow-up with interview for clarification	Registries with histopathological verification	Adjustment for age, vital status, and county (by matching). Exposure to other pesticides in multivariate analysis. No adjustment for other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Conditional logistic regression to obtain OR and 95% CI (univariate and multivariate analyses). Questionable power (8 cases/8 controls)	Recall bias, exposure misclassification, use of proxy for deceased	Moderate
Hohenadel *et al*. (2011)	This study was not included in the study quality ranking because a more complete analysis was conducted by McDuffie *et al.* (2001).						
Kachuri *et al.* (2013)	Population-based case-control Males only	Questionnaire answered by subjects or proxies; pesticide use collected via detailed telephone interview on all participants with 10+ hours of pesticide use during lifetime and 15% random sample of those who did not; exposure based on lifetime exposure to glyphosate	Cancer registries or hospitals in 6 Canadian provinces with histopathological verification for 36.55% of samples	Adjustment for age, province, selected medical conditions, family history of cancer, use of proxy respondent, smoking status No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional logistic regression to obtain OR and 95% CI; trends examined using multiple logistic regression	Recall bias, exposure misclassification, control selection based on three different sources depending on province of residence, low participation rates among controls, use of proxy respondents	Moderate
Karunanayake *et al.* (2012)	Population-based case-control Males only	Questionnaire answered by subjects; pesticide use collected via detailed telephone interview on all participants with 10+ hours of pesticide use during lifetime and 15%	Cancer registries or hospital in 6 Canadian provinces with histopathological verification for 49% of samples; difficulty recruiting control	Adjusted for age, province of residence, and significant medical history variables No adjustment for co-	Conditional logistic regression to obtain OR and 95% CI	Recall bias, exposure misclassification, control selection based on three different sources depending on province of residence, low participation rates among	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
		random sample of those who did not; exposure based on lifetime exposure to glyphosate	participants for older age groups	exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)		controls, unable to evaluate Epstein-barr virus exposure	
Koureas *et al*. (2014)	Cross-sectional	Questionnaire answered by pesticide sprayers	Genomic DNA extracted from peripheral blood samples and oxidation by-product 8-hydroxydeoxyguanosine (8-OHdG) was determined by enzyme immunoassay; more specific methods (HPLC, GC-MS) are available for measurement	No adjustments. In univariate, occupational exposure, sex and alcohol consumption were statistically significant while DAP concentrations and smoking were not.	For univariate, chi-square test used to obtain RR and 95% CI; 8-OHdG levels transformed into binary variables (categorized as high and low using the 75th percentile cut-off); unknown number of exposed and unexposed cases (questionable power possible given total number of subjects is only 80)	Recall bias, did not control for risk factors identified as statistically significant for univariate analysis, does not measure the consequence of genetic damage	Low
Koutros *et al.* (2013)	Nested case-control Males only	Questionnaire answered by subjects at study enrollment; examined exposure as cumulative lifetime days and intensity-weighted cumulative exposure days	State cancer registries with histopathological verification; total and aggressive prostate cancers evaluated	Adjustment for age, state, race, smoking, fruit servings, family history of prostate cancer, and leisure time physical activity in the winter. No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Poisson regression to obtain RRs and 95% CIs; also included unlagged vs. lagged analysis	Exposure misclassification	High
Landgren *et al*. (2009)	Nested case-control[a] Males only	Questionnaire answered by subjects at enrollment in AHS cohort and subsequent take-home questionnaire to collect	Venous blood collected from antecubital vein and analyzed for MGUS; same method as used for controls group in	Adjusted for age and education level Association with other pesticides examined	Logistic regression models to obtain OR and 95% CI comparing to population-based	Exposure misclassification, control group not from geographical area (used control group with	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
		information on 50 pesticides; occupational expoures, medical histories, and lifestyle factors updated with 5-year follow-up interview; subjects with prior history of lymphoproliferative malignancy excluded	Minnesota	and not found to be significant so no adjustment performed No adjustment for other potential confounders (e.g., solvents, diesel fumes, UV radiation)	screening study in Olmsted County, Minnesota; questionable power (27 cases; 11 controls)	similar demographics from Minnesota)	
Lee *et al*. (2004a)	This study was not included in the study quality ranking because the data were used in the pooled analysis conducted by De Roos *et al.* (2003).						
Lee *et al*. (2004b)	Population-based case-control White males and females only	Subjects or proxies were interviewed by telephone; those living/working on a farm asked for detailed history of pesticide use and farming information	State cancer registry or review of discharge diagnosis and pathology records at 14 hospitals; only newly diagnosed cases with confirmed adenocarcinoma of stomach or esophagus retained; controls randomly selected from a prior study conducted in geographical area	Adjusted for age and sex; evaluated BMI, smoking, alcohol consumption, educational level, family history of stomach or esophageal cancer, respondent type, dietary intake of particular vitamins and minerals, protein, and carbohydrates (included in model if changed value of OR by more than 10%) No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional logistic regression to obtain OR and 95% CI; questionable power (12 cases for stomach; 12 cases for esophagus)	Recall bias, exposure misclassification, use of proxy respondents, control selection	Moderate
Lee *et al*. (2005)	Population-based case-control	Questionnaire and/or interview with subject or proxy individuals to collect information on use of specific pesticides; telephone follow-up for unclear responses	Referral by hospitals or through state cancer registries with histopathological verification; controls selected from a previous study	Adjusted for age and respondent type; evaluated history of head injury, marital status, education level, alcohol consumption, medical history of diabetes mellitus,	Unconditional logistic regression to obtain OR and 95% CI	Recall bias, exposure misclassification, large number of proxy respondents, control selection (historical control group from another cancer evaluation, differences in	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
				dietary intake of α- and β-carotene, and dietary fiber (included in model if changed value of OR by more than 10%) No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)		exposure time period evaluated, needed to add younger controls, exposure information collected for different time periods for cases vs. controls)	
Lee *et al*. (2007)	Nested case-control	Questionnaire answered by subjects at enrollment in AHS cohort and subsequent take-home questionnaire to collect information on 50 pesticides	State cancer registries without histopathological verification; follow-up ~ 7 years	Adjustment for age, smoking, state, total days of pesticide application No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional multivariate logistic regression to obtain OR and 95% CI	Exposure misclassification, limited data on dietary factors, NSAID drug use and family cancer history	Moderate
McDuffie *et al*., 2001	Population based case-control Males only	Questionnaire answered by subjects; pesticide use collected via detailed telephone interview on all participants with 10+ hours of pesticide use during lifetime and 15% random sample of those who did not; exposure based on lifetime exposure to glyphosate	Cancer registries or hospital in 6 Canadian provinces with histopathological verification for 84% of samples; ascertainment of cases stopped in each province once target numbers were reached	Adjustment for age, province, and significant medical variables (including history of cancer in study participants and family history). No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Conditional logistic regression to obtain OR and 95% CI	Recall bias, exposure misclassification, control selection based on three different sources depending on province of residence, relatively low participation rates	Moderate
Nordstrom *et al*., 1998	This study was not included in the study quality ranking because the data were used in the pooled analysis conducted by Hardell *et al.* (2002).						
Orsi *et al*., 2009	Hospital-based case-control	Data collection in 2 stages: 1) self-	Hospital catchment area with histopathological/	Adjustment for age, center, and	Unconditional logistic regression	Recall bias, exposure misclassification,	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
	Males only (occupationally exposed)	administered questionnaire on socioeconomic characteristics, family medical history, and lifelong residential and occupational histories and more specific information for each job held for at least 6 months, and 2) face-to-face interview with trained staff (blinded) using standardized questionnaire	cytological verification Controls were hospital based with no prior history of lymphoid neoplasms, excluding patients with cancer or a disease directly related to occupation, smoking or alcohol abuse (but history of any of these did not prevent selection as a control)	socioeconomic category. Education and housing not found to impact results. Flu immunization, previous history of mononucleosis, skin type, smoking, and drinking did not change results. Evaluated particular crops and animal husbandry as well. No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	to obtain OR and 95% CI. Questionable power (12 cases/24 controls)	hospital-based controls	
Pahwa *et al*. (2011)	Population-based case-control Males only	Questionnaire answered by subjects; pesticide use collected via detailed telephone interview on all participants with 10+ hours of pesticide use during lifetime and 15% random sample of those who did not; exposure based on lifetime exposure to glyphosate	Cancer registries or hospitals in 6 Canadian provinces with histopathological verification for 30% of samples	Adjustment for age group, province of residence, and statistically significant medical history variables No adjustment for co-exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Conditional logistic regression to obtain OR and 95% CI; trends examined using multiple logistic regression	Recall bias, exposure misclassification, control selection based on three different sources depending on province of residence, low participation rates among controls	Moderate
Pahwa *et al*. (2012)	Population-based case-control Males only	Questionnaire answered by subjects; pesticide use collected via detailed telephone interview on all participants with 10+ hours of pesticide use during lifetime and 15% random sample of those	Cancer registries or hospitals in 6 Canadian provinces with histopathological verification for 36.5% of samples	Adjustment for age group, province of residence, and statistically significant medical history variables No adjustment for co-	Conditional logistic regression to obtain OR and 95% CI; trends examined using multiple logistic regression	Recall bias, exposure misclassification, control selection based on three different sources depending on province of residence, low participation rates among controls	Moderate

Table 3.2. Summary of Study Design Elements Impacting Study Quality Assignment and Overall Ranking.							
Journal Article	**Study Design**	**Exposure Assessment**	**Outcome Assessment**	**Confounder Control**	**Statistical Analyses**	**Risk of (Other) Bias**	**Overall Ranking**
		who did not; exposure based on lifetime exposure to glyphosate		exposure to other pesticides or other potential confounders (e.g., solvents, diesel fumes, UV radiation)			
Yiin *et al.* (2012)	Population-based case-control Pooled analysis of men with women analyzed in Carreon *et al*. (2005)	Questionnaire and/or interview for chemical-specific exposure answered by subjects or proxy individuals	Cases referred by physicians or through state cancer registries with histopathological verification; controls matched within state, but not county of residence	Adjustment for age, education, sex, and , sex, and farm pesticide exposure (yes/no) No adjustment for other potential confounders (e.g., solvents, diesel fumes, UV radiation)	Unconditional logistic regression to obtain ORs and 95% CIs	Acknowledge other sources of bias. Recall bias, exposure misclassification, control selection (low number of deceased controls obtained)	Moderate

[a] Mixed methods used in the Landgren et al (2009) study, with cross-sectional study design used to calculate prevalence rates comparing the AHS to a reference population MN. Pesticide risk estimates (including glyphosate) calculated using nested case-control approach, comparing AHS exposed/unexposed (ever/never) study participants.

3.4 Assessment of Epidemiological Studies for Relevance to Analysis

Using the criteria summarized in Section 3.2, a total of 58 individual literature studies were identified in the literature review and were judged as high, moderate, or low quality. Overall, 3 studies, 21 studies, and 34 studies were assigned high, moderate, or low rankings, respectively. All of the high and moderate quality studies were considered relevant to the current evaluation.

The majority of the studies were case-control studies evaluating a wide-range of cancers in the United States and Canada. There were several case-control studies from Canada that utilized the same study population (Kachuri *et al.*, 2013; Karunanayake *et al.*, 2012; McDuffie *et al.*, 2001; Pahwa *et al.*, 2011; Pahwa *et al.*, 2012). In a similar fashion, numerous studies in the United States were nested case-control studies, where the AHS cohort served as the source population for selecting cases and controls (Andreotti *et al.*, 2009; Engel *et al.*, 2005; Flower *et al.*, 2004; Koutros *et al.*, 2013; Landgren *et al.*, 2009; Lee *et al.*, 2007). In these studies, a subset of the AHS cohort were selected based on their outcome status for a particular cancer and exposure information was used from the AHS enrollment questionnaire and/or during follow-up interviews. Nested case-control studies allow for testing of hypotheses not anticipated when the cohort was initially assembled. In the AHS prospective cohort study (De Roos *et al.*, 2005), exposure and demographic information were also obtained from the questionnaires at enrollment; however, subjects were enrolled prior to developing cancer outcomes of interest. Subjects were then followed from enrollment to a subsequent time point to determine if subjects developed cancer outcomes of interest. As such, all available subjects in the cohort are included in the evaluation of whether there was an association between a risk factor (e.g., glyphosate exposure) and outcome.

The moderate studies included a varying degree of control for confounding and biases across studies. As moderate studies, they encompass a combination of strengths and limitations. In particular, important factors that impacted the quality assessment for these studies included whether there was control for known confounders, identification of control selection issues, study power issues, and length of follow-up. As noted previously, most people in these epidemiological studies used pesticides occupationally and were exposed to multiple pesticides over their working lifetime. Therefore, exposure to other pesticides is a particularly important factor to control for and studies that made this adjustment were given more weight than those that did not. Similarly, control selection issues were noted in a few studies and were given less weighting than those without control selection issues. The issues ranged from concerns using hospital-based controls, using different population sources to ascertain controls within the same study, and appropriateness of using controls ascertained for another research question. Numerous studies had limited power due to small sample size, which results in large confidence intervals and reduces the reliability of the results to demonstrate a true association. Studies demonstrating low or questionable power were therefore given less weighting. Lastly, the length of follow-up time varied across studies.

3.5 Summary of Relevant Epidemiological Studies

A summary of the relevant studies evaluating the association between glyphosate exposure and cancer are discussed below. Results of the studies reporting data on glyphosate exposure and

solid tumors (non-lymphohematopoietic) at various anatomical sites are presented in Table 3.3. Results of the studies reporting data on glyphosate exposure and non-solid tumors (lymphohematopoietic) are presented in Table 3.4. For study details, see Table 3.2 above and Appendix C.

3.5.1 Solid Tumor Cancer Studies

(1) Cancer at Multiple Sites from the AHS Cohort

De Roos *et al*., (2005) evaluated associations between glyphosate exposure and cancer incidence of all cancers combined in the AHS cohort study and did not find an association [ever/never use relative risk ratio (RR) =1.0 with 95% confidence interval (CI) of 0.90–1.2) when adjusting for age, demographic and lifestyle factors, and exposure to other pesticides]. In addition, De Roos *et al*., 2005 evaluated cancer at specific anatomical sites. Along with several nested case-control studies, no statistical evidence of an association with glyphosate was observed at any specific anatomical site (Table 3.3). Specifically, AHS researchers reported no evidence of an association between glyphosate use and cancers of the oral cavity (De Roos *et al*., 2005), colon (De Roos *et al*., 2005; Lee *et al*., 2007), rectum (De Roos *et al*., 2005; Lee *et al*., 2007), lung (De Roos *et al*., 2005), kidney (De Roos *et al*., 2005), bladder (De Roos *et al*., 2005), pancreas (De Roos *et al*., 2005; Andreotti *et al*., 2009), breast (Engel *et al*., 2005), prostate (De Roos et al., 2005; Koutros *et al*., 2013) or melanoma (De Roos *et al*., 2005). The adjusted RR or odds ratio (OR) and 95% CI for these studies are provided in Table 3.3.

(2) Prostate Cancer

In a Canadian population-based study (Band *et al.*, 2011), researchers reported non-statistically significant elevated odds of prostate cancer in relation to glyphosate use (OR=1.36; 95% CI=0.83–2.25). There was no adjustment made for exposure to other pesticides. This study included prostate cancer cases from 1983-1990, prior to the prostate-specific antigen (PSA) era. Consequently, the study included more advanced tumors before diagnosis. The AHS related studies (De Roos et al., 2005; Koutros *et al*., 2013), reflect PSA-era cases (i.e., cases which are typically identified at an earlier stage in the progression of the disease) and also did not identify an association with prostate cancer.

(3) Brain (Glioma) Cancer

Lee *et al*. (2005) investigated the association between brain cancer with farming and agricultural pesticide use. Matching for age, sex, vital status, and region, study authors reported a non-significant elevated odds of glioma (OR=1.5; 95% CI=0.7–3.1) in relation to glyphosate use by male farmers; however, the results were significantly different between those who self-reported pesticide use (OR=0.4; 95% CI=0.1–1.6), and for those for whom a proxy respondent was used (OR=3.1; 95% CI=1.2–8.2), indicating recall bias was a potential factor in this study. Furthermore, there was no adjustment for co-exposure to other pesticides and issues noted with control selection.

A population-based case-control study evaluated the risk of brain cancer, specifically, glioma risk, among men and women participating in the Upper Midwest Health Study (Yiin *et al*.,

2012). Using a quantitative measure of pesticide exposure (in contrast to an ever-use metric), Yiin *et al*. (2012) observed no statistical evidence of an association with glyphosate with effect estimates roughly equal to the null value following adjustment for age, education, sex, and use of other pesticides (home and garden use: OR=0.98; 95% CI=0.67–1.43; non-farm jobs: OR=0.83; 95% CI=0.39–1.73).

(4) Stomach and Esophageal Cancer

In a population-based case-control study in eastern Nebraska, Lee *et al*. (2004b) investigated pesticide use and stomach and esophageal adenocarcinomas. There was no association observed between glyphosate exposure and either stomach cancer (OR=0.8; 95% CI=0.4–1.5) or esophageal cancer (OR=0.7; 95% CI=0.3–1.4) after adjustment for age and sex. No adjustment was made for exposure to other pesticides.

(5) Soft Tissue Sarcoma

A Canadian case-control study (Pahwa *et al*., 2011) examined exposure to pesticides and soft tissue sarcoma and found no relation with the use of glyphosate after adjustment for age, province of residence, and medical history variables (OR=0.90; 95% CI= 0.58–1.40); however, control selection issues were noted, including low response rate and selection from three different sources depending on the province of residence.

(6) Total Childhood Cancer

Flower *et al*. (2004), a nested case-control study in the AHS cohort, examined the relation between parental pesticide use and all pediatric cancers reported to state registries among children of AHS participants and did not observe a significant association with maternal use exposure to glyphosate (OR=0.61; 95% CI= 0.32–1.16) or paternal (prenatal) exposure to glyphosate (OR=0.84; 95% CI= 0.35–2.54). The models adjusted for the child's age at the time of parents' enrollment. There was no adjustment for exposure to other pesticides.

Table 3.3. Summary of Findings: Solid Tumor Cancer Studies					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effect Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
All Cancers Combined					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.0 (0.9-1.2)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.0 (0.9-1.1) 1.0 (0.9-1.1)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 0.9 (0.8-1.0) 0.9 (0.8-1.1)	Age, demographic and lifestyle factors, and other pesticides[b]
Lung					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	0.9 (0.6-1.3)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 0.9 (0.5-1.5) 0.7 (0.4-1.2)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 1.1 (0.7-1.9) 0.6 (0.3-1.0)	Age, demographic and lifestyle factors, and other pesticides[b]
Oral Cavity					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.0 (0.5-1.8)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 0.8 (0.4-1.7) 0.8 (0.4-1.7)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5	 1.0 1.1 (0.5-2.5)	Age, demographic and lifestyle factors, and other pesticides[b]

Table 3.3. Summary of Findings: Solid Tumor Cancer Studies					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effect Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
			79.6-337.1 337.2-18,241	1.0 (0.5-2.3)	
			Kidney		
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.6 (0.7-3.8)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 0.6 (0.3-1.4) 0.7 (0.3-1.6)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 0.3 (0.1-0.7) 0.5 (0.2-1.0)	Age, demographic and lifestyle factors, and other pesticides[b]
			Bladder		
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.5 (0.7-3.2)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.0 (0.5-1.9) 1.2 (0.6-2.2)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 0.5 (0.2-1.3) 0.8 (0.3-1.8)	Age, demographic and lifestyle factors, and other pesticides[b]
			Melanoma		
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.6 (0.8-3.0)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.2 (0.7-2.3) 0.9 (0.5-1.8)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days		Age, demographic and lifestyle factors, and other pesticides[b]

Table 3.3. Summary of Findings: Solid Tumor Cancer Studies					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effect Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
			(by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	1.0 0.6 (0.3-1.1) 0.7 (0.3-1.2)	
Colon					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.4 (0.8-2.2)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.4 (0.9-2.4) 0.9 (0.4-1.7)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 0.8 (0.5-1.5) 1.4 (0.8-2.5)	Age, demographic and lifestyle factors, and other pesticides[b]
Lee *et al.* (2007)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	1.0 (0.7-1.5)	Age, smoking, state, total days of pesticide application
Rectum					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.3 (0.7-2.3)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.3 (0.7-2.5) 1.1 (0.6-2.3)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 1.0 (0.5-2.0) 0.9 (0.5-1.9)	Age, demographic and lifestyle factors, and other pesticides[b]
Lee *et al.* (2007)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	1.6 (0.9-2.9)	Age, smoking, state, total days of pesticide application
Colorectal					
Lee *et al.* (2007)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	1.2 (0.9-1.6)	Age, smoking, state, total days of pesticide application
Pancreas					

Table 3.3. Summary of Findings: Solid Tumor Cancer Studies					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effect Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	0.7 (0.3-2.0)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.6 (0.6-4.1) 1.3 (0.5-3.6)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 2.5 (1.0-6.3) 0.5 (0.1-1.9)	Age, demographic and lifestyle factors, and other pesticides[b]
Andreotti *et al.* (2009)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	1.1 (0.6-1.7)	Age group, cigarette smoking, diabetes, and applicator type
			Intensity-Weighted Exposure Days (by control median): ≤184 ≥185	 1.4 (0.9-3.8) 0.5 (0.2-1.3)	Age group, cigarette smoking, and diabetes
Prostate					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.1 (0.9-1.3)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 0.9 (0.7-1.1) 1.1 (0.9-1.3)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 1.0 (0.8-1.2) 1.1 (0.9-1.3)	Age, demographic and lifestyle factors, and other pesticides[b]
Koutros *et al.* (2013)[c]	Nested Case-Control	USA: Iowa and North Carolina	Intensity-Weighted Cumulative Exposure Days (by quartile): Q1 Q2 Q3 Q4	Total prostate cancer: 0.91 (0.79-1.06) 0.96 (0.83-1.12) 1.01 (0.87-1.17) 0.99 (0.86-1.15)	Age, state, race, smoking, fruit servings, family history of prostate cancer, and leisure time physical activity in the winter

Table 3.3. Summary of Findings: Solid Tumor Cancer Studies					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effect Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
			Intensity-Weighted Cumulative Exposure Days (by quartile): Q1 Q2 Q3 Q4	Aggressive prostate cancer: 0.93 (0.74-1.16) 0.91 (0.73-1.13) 1.01 (0.82-1.25) 0.94 (0.75-1.18)	Age, state, race, smoking, fruit servings, family history of prostate cancer, and leisure time physical activity in the winter
Band *et al.* (2011)	Case-Control	Canada: British Columbia	Ever/never	1.36 (0.83-2.25)	Alcohol consumption, cigarette years, education level, pipe years, and respondent type
Esophagus					
Lee *et al.* (2004b)	Case-Control	USA: Nebraska	Ever/never	0.7 (0.3-1.4)	Age and sex
Stomach					
Lee *et al.* (2004b)	Case-Control	USA: Nebraska	Ever/never	0.8 (0.4-1.5)	Age and sex
Breast					
Engel *et al.* (2005)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	Wives who apply pesticides: 0.9 (0.7-1.1) Wives who never used pesticides: 1.3 (0.8-1.9)	Age, race, and state of residence
Soft Tissue Sarcoma					
Pahwa *et al.* (2011)	Case-Control	Canada	Ever/never	0.90 (0.58-1.40)	Age group, province of residence, and statistically significant medical history variables
Brain (glioma)					
Lee *et al.* (2005)	Case-Control	USA: Nebraska	Ever/never	Overall: 1.5 (0.7-3.1) Self-reported: 0.4 (0.1-1.6) Proxy respondents: 3.1 (1.2-8.2)	Age for overall analysis; age and respondent type for other analyses
Yiin *et al.* (2012)	Case-Control	USA: Iowa, Michigan, Minnesota, and Wisconsin	Ever/never	House/garden use: 0.98 (0.67-1.43) Non-farm jobs: 0.83 (0.39-1.73)	Age, education, sex, and use of other pesticides
Total Childhood					

Table 3.3. Summary of Findings: Solid Tumor Cancer Studies					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effect Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
Flower *et al.* (2004)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	Maternal use: 0.61 (0.32-1.16) Paternal use: 0.84 (0.35-2.34)	Child's age at enrollment

[a] Some studies report multiple quantitative risk measurements. This table reports the most highly adjusted quantitative measurements.
[b] De Roos *et al.* (2005) excluded subjects missing covariate data for demographic and lifestyle factors and exposure to other pesticides; therefore, the number of subjects included in each analysis varies.
[c] Effect estimates for glyphosate reported in the supplemental web material for Koutros *et al.* (2013).

3.5.2 Non-Solid Tumor Cancer Studies

(1) Leukemia

De Roos *et al*. (2005) reported no association between leukemia and glyphosate-exposed (ever/never used) pesticide applicators in the AHS cohort. For applicators with the full data set (54,315), the RR was 1.1 (95% CI=0.6–2.4) with only adjustment for age. In the fully adjusted model, the RR was similar (RR=1.0; 95% CI=0.5–1.9). The number of participants included in the adjusted analysis was lower (n=40,716) due to the exclusion of subjects with missing covariate data. Effect estimates using cumulative lifetime exposure and intensity-weighted cumulative exposure were also found to be non-statistically significant and did not demonstrate a trend with increasing exposure.

In a population-based case-control study in Iowa and Minnesota, Brown *et al*. (1990) did not observe an association with the ever-use of glyphosate (OR=0.9; 95% CI=0.5–1.6). A limitation in the study was the low number of cases exposed to glyphosate (n=15). Adjustments were made for several covariates, including vital status, age, tobacco use, family history of lymphopoietic cancer, high risk occupations, and high risk exposures; however, no adjustment was made for exposure to other pesticides.

Chang and Delzell (2016) conducted a meta-analysis exploring glyphosate exposure and leukemia using 3 studies (De Roos *et al*., 2005; Brown *et al*., 1990; and Kaufman *et al.,* 2009). I^2 values were reported, which represented the percentage of the total variance explained by study heterogeneity and measure inconsistency in results. Larger I^2 values indicate greater inconsistency. A meta-risk ratio of 1.0 (95% CI=0.6-1.5) was obtained with an I^2 value of 0.0%, indicating consistency across the data sets. It should be noted that this analysis included data from Kaufman *et al.* (2009), which is not considered in the current evaluation because it was assigned a low quality ranking because a quantitative measure of an association between glyphosate and a cancer outcome was not reported for that study.

(2) Multiple Myeloma

In a follow-up analysis of the study population from Iowa and Minnesota used in Brown *et al.* (1990), Brown *et al.* (1993) investigated whether pesticide use was related to multiple myeloma. Among men in Iowa, the authors observed a non-statistically significant elevated association with glyphosate use (OR=1.7; 95% CI=0.8–3.6; 11 exposed cases); however, no adjustment was made for exposure to other pesticides. The authors cautioned that while the study may lend support to the role of pesticides in general, the study limitations preclude use of the evidence as a definitive finding for any one compound.

De Roos *et al*. (2005) reported a suggestive association between multiple myeloma and glyphosate-exposed pesticide applicators based on 32 multiple myeloma cases observed in the AHS cohort. For applicators with the full data set, the RR was 1.1 (95% CI=0.5–2.4) with only adjustment for age. In the fully adjusted model excluding subjects with missing covariate data, there was a non-statistically significant elevated risk following adjustment for age, demographic and lifestyle factors, and exposure to other pesticides (RR=2.6; 95% CI=0.7–9.4). The authors

postulated that the increased myeloma risk could be due to bias resulting from a selection of subjects in adjusted analyses that differed from subjects included in unadjusted analyses or may be due to a confounder or effect modifier that is prevalent among the subgroup and has not been accounted for in the analyses. When exposure data were also stratified by tertiles with the lowest tertile of exposure as the referent category, trend analyses were not statistically significant. Non-statistically significant elevated RRs of 1.9 (95% CI: 0.6-6.3) and 2.1 (95% CI: 0.6-7.0) were estimated for the highest tertile of both cumulative and intensity-weighted exposure days, respectively. The study authors did note that small sample size precluded precise estimation (n=19 for adjusted analyses). When using never exposed as the referent category, the trend analysis was again non-statistically significant, but the RRs ranged from 2.3 (95% CI: 0.6-8.9) to 4.4 (95% CI: 1.0-20.2) from the lowest tertile to the highest tertile, respectively. When stratified by quartiles, a statistically significant trend is achieved and the RR increased to 6.6 (95% CI: 1.4-30.6); however, the authors noted that the cases were sparsely distributed for these analyses.

Sorahan (2015)[11] re-analyzed the AHS data reported by De Roos *et al*. (2005) to examine the reason for the disparate findings in relation to the use of a full data set versus the restricted data set. Using Poisson regression, risk ratios were calculated without excluding subjects with missing covariate data. When adjusted for age and sex, the RR for ever-use of glyphosate was 1.12 (95% CI of 0.5–2.49). Additional adjustment for lifestyle factors and use of other pesticides did not have a large impact (RR=1.24; 95% CI=0.52–2.94). The authors concluded that the disparate findings in De Roos *et al.* (2005) could be attributed to the use of a restricted dataset that was unrepresentative.

Landgren *et al*. (2009), within the AHS study population, also investigated the association between pesticide use and prevalence of monoclonal gammopathy of undetermined significance (MGUS). MGUS is considered a pre-clinical marker of multiple myeloma progression. The authors did not observe an association with glyphosate use and MGUS using subjects from the AHS cohort (OR=0.50; 95% CI=0.20–1.0). No adjustment was made for exposure to other pesticides.

In a population-based case-control study (Pahwa *et al*., 2012) among men in six Canadian provinces, a non-statistically significant elevated odds of multiple myeloma was reported in relation to glyphosate use (OR=1.22; 95% CI = 0.77–1.93), based upon 32 glyphosate exposed multiple myeloma cases and 133 controls. There was no adjustment for exposure to other pesticides. Kachuri *et al*. (2013), using the same Canadian study population, further explored multiple myeloma in relation to days per year that glyphosate was used. Adjustment for exposure to other pesticides was also not performed in this study. For ever-use, there was a slight non-statistically significant increased odds ratio (OR=1.19; 95% CI=0.76–1.87). For light users (>0 and ≤2 days/year), there was no association (OR=0.72; 95% CI = 0.39–1.32; 15 exposed cases); whereas, for heavy users (>2 days/ year), there was a non-statistically significant increased odds ratio (OR=2.04; 95% CI=0.98–4.23; 12 exposed cases). Similar results were obtained when proxy respondents were excluded from the analysis. The low number of cases and controls exposed to glyphosate, particularly when exposed subjects were divided into light and heavy users, was a limitation of the study. It would be expected that effect estimates would be reduced if adjustment for co-exposure to other pesticides had been performed.

[11] Funded by Monsanto

In a hospital-based case-control study conducted by Orsi *et al*. (2009) in France, 56 multiple myeloma cases and 313 age- and sex-matched controls were identified. A non-statistically significant elevated risk was observed (OR=2.4; 95% CI=0.8–7.3; 5 exposed cases and 18 exposed controls). The wide CI range can primarily be attributed to the low number of exposed cases indicating the analysis is underpowered. Additionally, the study did not adjust for exposure to multiple pesticides.

Chang and Delzell (2016) conducted a meta-analysis exploring glyphosate exposure and multiple myeloma using data from the 6 studies described above (Brown *et al.*, 1993; De Roos *et al*., 2005; Sorahan, 2015; Pahwa *et al*., 2012; Kachuri *et al*., 2013; Orsi *et al*., 2009). Meta-risk ratios were obtained using data from each of the 4 independent study populations, such that if a study population was already represented in the analysis by one study, then the same population analyzed by another study would not be included (e.g., Sorahan, 2015 and De Roos *et al.*, 2005 could not be used simultaneously in a meta-analysis). The combined meta-risk ratio based on data from prioritized studies (Brown *et al.*, 1993; Kachuri *et al.*, 2013; Orsi *et al.*, 2009; and Sorahan, 2015) was 1.4 (95% CI=1.0-1.9) using random-effects and fixed-effects models and the I^2 value = 0.0% indicating consistency across data sets. There was relatively no impact on the meta-risk ratio and associated 95% CI when secondary analyses were conducted using alternative estimates for a study population (e.g., substituting the data from Sorahan, 2015 for De Roos *et al.*, 2005).

(3) Hodgkin Lymphoma

In a Canadian case-control study, Karunanayake *et al.*, (2012) evaluated Hodgkin lymphoma (HL) and observed no association with glyphosate exposure following adjustment for age, province of residence, and medical history variables (OR=0.99; 95% CI=0.62-1.56; 38 cases). No adjustment was made for exposure to other pesticides.

In a hospital-based case-control study conducted by Orsi *et al*. (2009) in France, authors identified 87 HL cases and 265 age-and sex-matched controls. There was a non-statistically significant elevated odds ratio observed (OR=1.7; 95% CI=0.6–5.0; 6 exposed cases and 15 exposed controls). The wide CI range can primarily be attributed to the low number of exposed cases indicating the analysis is underpowered. Also, as noted earlier, this study did not adjust for exposure to multiple pesticides.

Chang and Delzell (2016) conducted a meta-analysis exploring glyphosate exposure and HL using data from both of these studies. A meta-risk ratio of 1.1 (95% CI=0.7-1.6) was obtained with a I^2 value of 0.0%, indicating consistency across the data sets.

(4) Non-Hodgkin Lymphoma

NHL has about 60 subtypes classified by the WHO, which may have etiological differences (Morton *et al.*, 2014). There are analyses available for particular subtypes of NHL; however, these are particularly limited by the small sample sizes. As a result, this evaluation only presents results for total NHL.

There were six studies available that investigated the association between glyphosate exposure and NHL, which was the most for any type of cancer. As discussed in Section 3.4, these studies encompass a combination of strengths and limitations. These studies are therefore discussed in more detail in this section as compared to discussions of other cancer types in order to highlight the strengths and identify the limitations for each study.

De Roos *et al*. (2005) was the only prospective cohort study available; therefore, subjects were enrolled prior to developing cancer outcomes. Disease status was determined through state cancer registries. Exposure information was obtained from a large number of licensed pesticide applicators and no proxies were used. Exposure was evaluated as ever/never use, cumulative lifetime exposure, and intensity-weighted cumulative exposure. Due to the study design, the potential for many biases were reduced. Additionally, the study adjusted and/or considered numerous factors, including use of other pesticides. Median follow-up time was approximately 7 years and a longer follow-up would increase the ability of the study to detect subjects developing cancer outcomes; however, as discussed in Section 3.3.1, study participants provided exposure information prior to enrollment and this information was incorporated into the cumulative lifetime and intensity-weighted cumulative exposure metrics. As a result, the amount of time exposed was longer than just the follow-up time since enrollment. For applicators with the full data set, the RR for ever/never use was 1.2 (95% CI=0.7–1.9; 92 cases) with only adjustment for age. In the fully adjusted model excluding subjects with missing covariate data, the RR was similar following adjustment for age, demographic and lifestyle factors, and exposure to other pesticides (RR=1.1; 95% CI=0.7-1.9). Effect estimates obtained using cumulative lifetime exposure and intensity-weighted cumulative exposure were below 1 (RR = 0.6-0.9 when comparing to the lowest tertile).

De Roos *et al.* (2003) used pooled data from three case-controls studies evaluating NHL in white males from Nebraska, Kansas, and in Iowa and Minnesota (Cantor *et al.*, 1992; Hoar *et al.*, 1986; Zahm *et al.*, 1990; Appendix B). Exposure information was obtained from exposed individuals or their next of kin (i.e., proxy respondents) if the subjects were dead or incapacitated; however, techniques varied across the three studies. There is potential for selection bias due to exclusion of observations with missing covariate data, but only if the lack of the covariate data was associated with glyphosate exposure. The effect estimates for the association between glyphosate exposure and NHL was significant (OR=2.1; 95% CI=1.1–4.0) in the logistic regression analyses controlling for co-exposure to other pesticides. However, utilizing alternative hierarchical regression techniques to adjust for co-exposure to other pesticide exposures, the odds ratio was still elevated, but the increase was not statistically significant (OR=1.6; 95% CI=0.90–2.8).

Eriksson *et al.* (2008) is a Swedish case-control study that used detailed exposure information from exposed individuals (i.e., no use of proxy respondents), but only minimal demographic information was provided on subjects (age and sex) and a table with subject characteristics (e.g., smoking status, alcohol intake, physical activity, education) was not provided. Cases were identified through physicians and verified histopathologically. Glyphosate exposure, which was reported in 29 cases and 18 controls between 1999 and 2003, produced a statistically significant increased OR in the univariate analysis (OR=2.02; 95% CI=1.10–3.71); however, in the

multivariate analysis adjustments were conducted for co-exposure to different agents including MCPA, "2,4,5-Y and/or 2,4-D", mercurial seed dressing, arsenic, creosote, and tar and the OR reduced to 1.51 (95% CI=0.77–2.94) and was not statistically significant. Additional analyses were conducted to investigate the impact of various exposure times. When exposure was for more than 10 cumulative days (the median number of days among exposed controls), the OR was 2.36 (95% CI=1.04–5.37; 17 exposed cases) and for exposure less than 10 cumulative days, the OR was 1.69 (95% CI=0.7–4.07; 12 exposed cases). By dividing the exposed cases and controls using this exposure metric, wider CIs were observed indicating reduced power from the smaller sample sizes. Additionally, these analyses did not account for co-exposure to other pesticides. Similarly, wider CIs were also observed when exposed cases and controls were divided by a longer exposure metric. ORs of 1.11 (95% CI=0.24-5.08) and 2.26 (95% CI=1.16-4.40) were obtained for 1-10 years and >10 years, respectively. It was not clear whether this analysis controlled for co-exposure to other pesticides based on the statistical methods description and the subjects for each exposure group were not reported. This finding, while limited to a single study, suggests that cohort studies without sufficient follow-up time or other case-control studies which did not stratify by time since first exposure may be less sensitive in detecting risk.

Hardell *et al.* (2002) used pooled data from two case-control studies in Sweden (Hardell and Eriksson, 1999; Nordstrom *et al*., 1998; Appendix B) that examined hairy cell leukemia, a subtype of NHL, and NHL (not including hairy cell leukemia). Exposure information was collected from individuals or proxy respondents based on a working history with specific questions on exposures to different chemicals. Cases were identified from regional cancer registries and verified histopathologically. In the univariate analysis, risk of NHL associated with glyphosate exposure was found to be significantly increased (OR=3.04; 95% CI=1.08–8.52), but when study site, vital status, and co-exposure to other pesticides were considered in the multivariate analysis, the OR noticeably attenuated and was found to be non-statistically significant (OR=1.85; 95% CI=0.55–6.20). The wide range of the CI suggests that the analysis is underpowered (only 8 glyphosate-exposed cases and 8 glyphosate-controls).

McDuffie *et al.* (2001) is a multicenter population-based study among men of six Canadian provinces. This case-control study utilized a well-conducted exposure assessment and cases were ascertained from cancer registries or hospitals in six provinces with histopathological verification for 84% of the samples. There are concerns with control selection. There was low control participation (48%) and different sources were used for selecting controls depending on the province of residence. Effect estimates were obtained using a considerable number of exposed cases and controls (51 cases and 133 controls); however, the study did not assess co-exposure to other pesticides. There was a non-statistically significant increased risk of NHL from glyphosate exposure when adjusting for age and province (OR=1.26; 95% CI=0.87–1.80) and when adjusting for age, province and medical variables (OR=1.20; 95% CI=0.83–1.74). Medical variables found to be statistically significant included history of measles, mumps, previous cancer, skin-prick allergy tests, allergy desensitization shots, and a positive family history of cancer in a first-degree relative. It would be expected that effect estimates would attenuate if control for co-exposure to other pesticides had been performed. Additional analyses were conducted to investigate differences in exposure time. When exposure was for more than 2 days/year, the OR was 2.12 (95% CI=1.20-3.73; 23 exposed cases and 36 exposed controls) compared to unexposed subjects and for exposure more than 0 and $\leq$ 2 days/year, the OR was

1.00 (95% CI=0.63–1.57; 28 exposed cases and 97 exposed controls) compared to unexposed subjects.

Orsi *et al.* (2009) is a French hospital-based case-control study that obtained exposure information from subjects (no proxies used) using a detailed questionnaire with lifelong residential and occupational histories followed by a discussion with a trained interviewer who was blinded to case status. No issues regarding exposure or outcome assessment were identified; however, there is potential for selection bias given the study utilized hospital-based controls. The study evaluated several potential confounders; however, it did not assess co-exposure to other pesticides. There was no association observed between NHL and glyphosate use (OR=1.0; 95% CI=0.5-2.2; 12 exposed cases and 24 exposed controls). The low number of cases and controls exposed to glyphosate and lack of adjustment for exposure to multiple pesticides were limitations of the study.

Schinasi and Leon (2014) conducted a meta-analysis exploring occupational glyphosate exposure and NHL using data from six of the above mentioned studies (McDuffie *et al*., 2001; Hardell *et al*., 2002; De Roos *et al*., 2003; De Roos *et al*., 2005; Eriksson *et al*., 2008; and Orsi *et al*., 2009). Since the authors identified a variety of sources of heterogeneity between publications, they decided a priori to calculate meta-risk ratio estimates and 95% CIs using random effect models, allowing between study heterogeneity to contribute to the variance. I^2 values were reported as a measure of inconsistency in results. For glyphosate, the meta-risk ratio was 1.5 with a 95% CI of 1.1–2.0 and the I^2 value was 32.7% indicating relatively low levels of heterogeneity among these studies. This study combined multiple smaller studies that on their own were very limited in statistical power.

The 2015 IARC evaluation noted that fully adjusted effect estimates in two of the Swedish studies (Hardell *et al*., 2002 and Eriksson *et al*., 2008) were not used in the analysis conducted by Schinasi and Leon (2014). Consequently, the IARC Working Group conducted a reexamination of the results of these studies (IARC 2015). For an association between glyphosate exposure and NHL, the IARC estimated a meta-risk ratio of 1.3 (95% CI=1.03–1.65, I^2 =0%; p=0.589 for heterogeneity).

Chang and Delzell (2016) conducted their own meta-analysis exploring glyphosate exposure and NHL using six independent studies (De Roos *et al.*, 2003; De Roos *et al.*, 2005; Eriksson *et al.*, 2008; Hardell *et al.*, 2002; McDuffie *et al.*, 2001; and Orsi *et al.*, 2009). A meta-risk ratio of 1.3 (95% CI=1.0-1.6) was obtained with an I^2 value of 0.0%. In a secondary analysis, the De Roos *et al.* (2003) OR using hierarchical regression was replaced by the logistic regression OR. This change had no impact on the meta-risk ratio and associated confidence interval (meta-risk ratio=1.3; 95% CI=1.0-1.6). In another secondary analysis, the OR from McDuffie *et al.* (2001) was replaced by the OR from Hohenadel *et al.* (2011), which evaluated the same study population (minus four previously misclassified NHL cases). This analysis also yielded similar results (meta-risk ratio=1.3; 95% CI=1.0-1.7). A final analysis was performed with the replacements for both secondary analyses [i.e., logistic regression OR from De Roos *et al.* (2003) and OR from Hohenadel *et al.* (2011)]. The results were relatively the same as the other meta-analyses (meta-risk ratio=1.4; 95% CI=1.0-1.8). Chang and Delzell (2016) also tested for publication bias using Egger's linear regression approach to evaluating funnel plot asymmetry,

and found no significant asymmetry indicating little evidence of publication bias; however, given the small sample size (n=6), this analysis would lack power and the results are not considered meaningful.

Table 3.4. Summary of Findings: Non-Solid Tumor Cancer Studies.					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effec Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
Leukemia					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.0 (0.5-1.9)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.9 (0.8-4.5) 1.0 (0.4-2.9)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 1.9 (0.8-4.7) 0.7 (0.2-2.1)	Age, demographic and lifestyle factors, and other pesticides[b]
Brown *et al.* (1990)	Case-Control	USA: Iowa and Minnesota	Ever/never	0.9 (0.5-1.6)	Vital status, age, tobacco use, family history of lymphopoietic cancer, high occupations, and high risk exposures
Multiple Myeloma					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	2.6 (0.7-9.4)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 1.1 (0.4-3.5) 1.9 (0.6-6.3)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 1.2 (0.4-3.8) 2.1 (0.6-7.0)	Age, demographic and lifestyle factors, and other pesticides[b]
Brown *et al.* (1993)	Case-Control	USA: Iowa	Ever/never	1.7 (0.8-3.6)	Age and vital status
Kachuri *et al.* (2013)	Case-Control	Canada	Ever/never	1.19 (0.76-1.87)	Age, province of residence, smoking status, selected medical conditions, family history of cancer, and use of a proxy respondent
			Days per year of use: 0 to ≤2 days/year >2 days/year	 0.72 (0.39-1.32) 2.04 (0.98-4.23)	Age, province of residence, smoking status, selected medical conditions, family history of cancer, and use of a proxy respondent
Pahwa *et al.* (2012)	Case-Control	Canada	Ever/never	1.22 (0.77-1.93)	Age group, province of residence, and statistically significant medical history variables

Table 3.4. Summary of Findings: Non-Solid Tumor Cancer Studies.					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effec Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
Orsi *et al.* (2009)	Case-Control	France	Ever/never	2.4 (0.8-7.3)	Age, centre, and socioeconomic category
Monoclonal Gammopathy of Undetermined Significance (MGUS)					
Landgren *et al.* (2009)	Nested Case-Control	USA: Iowa and North Carolina	Ever/never	0.5 (0.2-1.0)	Age and education
Hodgkin Lymphoma (HL)					
Karunanayake *et al.* (2012)	Case-Control	Canada	Ever/never	0.99 (0.62-1.56)	Age group, province of residence, and statistically significant medical history variables
Orsi *et al.* (2009)	Case-Control	France	Ever/never	1.7 (0.6-5.0)	Age, centre, and socioeconomic category
Non-Hodgkin Lymphoma (NHL)					
De Roos *et al.* (2005)	Prospective Cohort	USA: Iowa and North Carolina	Ever/never	1.1 (0.7-1.9)	Age, demographic and lifestyle factors, and other pesticides[b]
			Cumulative Exposure Days (by tertile cut points): 1-20 21-56 57-2,678	 1.0 0.7 (0.4-1.4) 0.9 (0.5-1.6)	Age, demographic and lifestyle factors, and other pesticides[b]
			Intensity-Weighted Cumulative Exposure Days (by tertile cut points): 0.1-79.5 79.6-337.1 337.2-18,241	 1.0 0.6 (0.3-1.1) 0.8 (0.5-1.4)	Age, demographic and lifestyle factors, and other pesticides[b]
De Roos *et al.* (2003)	Case-Control	USA: Iowa, Nebraska, Minnesota, and Kansas	Ever/never	1.6 (0.9-2.8)	Age, study site, and use of other pesticides
Eriksson *et al.* (2008)	Case-Control	Sweden	Ever/never	Multivariate: 1.51 (0.77-2.94)	Age, sex, year of diagnosis or enrollment, and exposure to other pesticides
			Days per year of use: ≤ 10 days >10 days	 1.69 (0.70-4.07) 2.36 (1.04-5.37)	Age, sex, and year of diagnosis or enrollment
			Years of use: 1-10 years >10 years	 1.11 (0.24-5.08) 2.26 (1.16-4.40)	Unknown
Hardell *et al.* (2002)	Case-Control	Sweden	Ever/never	Multivariate: 1.85 (0.55-6.20)	Study, study area, vital status, and exposure to other pesticides
McDuffie *et al.* (2001)	Case-Control	Canada	Ever/never	1.20 (0.83-1.74)	Age, province of residence, and statistically significant medical variables
			Days per year of use: >0 and ≤ 2 days	 1.00 (0.63-1.57)	Age and province of residence

Table 3.4. Summary of Findings: Non-Solid Tumor Cancer Studies.					
Study	**Study Design**	**Study Location**	**Exposure Metric**	**Adjusted Effec Estimate: RR or OR (95% CI)[a]**	**Covariate Adjustments in Analyses**
			>2 days	2.12 (1.20 -3.73)	
Orsi *et al.* (2009)	Case-Control	France	Ever/never	1.0 (0.5-2.2)	Age, centre, and socioeconomic category

[a] Some studies report multiple quantitative risk measurements. This table reports the most highly adjusted quantitative measurements.

[b] De Roos *et al.* (2005) excluded subjects missing covariate data for demographic and lifestyle factors and exposure to other pesticides; therefore, the number of subjects included in each analysis varies.

3.6 Discussion

A total of 24 epidemiological studies from the open literature were identified as appropriate for detailed evaluation. Of these, 23 studies were considered informative with regard to the carcinogenic potential of glyphosate. There was no evidence of an association between glyphosate exposure and solid tumors. There was also no evidence of an association between glyphosate exposure and leukemia, or HL. This conclusion is consistent with those recently conducted by IARC, EFSA, and JMPR who also concluded there is no evidence of an association for these tumors at this time. The data should be considered limited though with only one or two studies available for almost all of the cancer types investigated. Additionally, with the increased use of glyphosate following the introduction of glyphosate-tolerant crops in 1996, there is a need for more recent studies since a large number of studies were conducted prior to 1996. As described in Section 1.1, the use pattern changed following the introduction of transgenic crops, which may impact overall effect estimates. The remainder of this discussion focuses on multiple myeloma and NHL. Study elements for the available studies and their potential to impact effect estimates are examined; however, the discussion is applicable in most cases to all of the epidemiological studies used in this evaluation.

Multiple Myeloma

Five studies were available evaluating the association between glyphosate exposure and risk of multiple myeloma (Brown et al., 1993; De Roos et al., 2005; Kachuri et al., 2013; Orsi et al., 2009; Pahwa et al., 2012). The effect estimates for ever/never use ranged from 1.19 to 2.6 although none were found to be statistically significant. Only one study (De Roos et al., 2005) controlled for co-exposures to other pesticides; therefore, potential confounding was not addressed in the other studies. There was an indication of a possible exposure-response relationship; however, this was the only study that evaluated the exposure-response relationship for multiple myeloma. Furthermore, reanalysis of the full dataset by Sorahan (2015) raised concerns about whether the restricted dataset used for these analyses was representative of the whole cohort. There was a single study of MGUS, a precursor to multiple myeloma, which showed decreased risk with exposure to glyphosate; however, the study did not control for exposure to other pesticides. Overall, the available epidemiologic evidence for an association between glyphosate and risk of multiple myeloma is inadequate to assess the carcinogenic potential at this time due to the potential for confounding in three of the four studies, the limited observation of a possible exposure-response relationship in a single study, and concerns whether restricted datasets were representative of the whole cohort.

NHL

Six studies were available evaluating the association between glyphosate exposure and risk of NHL. Effect estimates for ever/never use ranged from 1.0-1.85 in adjusted analyses with none reaching statistical significance (Figure 3.2). Two of these studies did not adjust for co-exposures to other pesticides (McDuffie et al., 2001; Orsi et al., 2009). Many of the evaluated studies had limited power due to small sample sizes, which resulted in large confidence intervals and reduced the reliability of the results to demonstrate a true association. Meta-analyses were performed by IARC (2015) and Chang and Delzell (2016) using these results for the ever/never

use metric. Both analyses reported similar meta-risk ratios ranging from 1.3-1.5, depending on the effect estimates and studies included in the analyses. All meta-analysis estimates reported were non-statistically significant except the meta-risk ratio reported by IARC (2015), which was borderline significant with the lower limit of the 95% CI at 1.03. It should also be noted that publication bias may play a role in this evaluation given there is a tendency to only publish positive results and potential concerns regarding glyphosate have only been raised in recent years.

With respect to meta-analyses, caution should be taken when interpreting results. Meta-analyses are a systematic way to combine data from several studies to estimate a summary effect. Analyses were performed with 6 studies, which many would consider small for performing meta-analyses. Rarely will meta-analyses synthesize data from studies with identical study designs and methods. In the meta-analyses performed by IARC (2015) and Chang and Delzell (2016), inclusion was primarily based on whether a study addressed the broader question regarding the association between glyphosate exposure and risk of NHL. For meaningful results, careful consideration of whether studies are similar and should be combined in the analysis. Furthermore, the bias and confounding issues inherent for each individual study are carried over into the meta-analyses. Across the NHL studies, study characteristics varied, such as overall study design (i.e., cohort and case-control), source population, proxy respondent use, covariate adjustments, and confounding control. Even if these differences are not detected statistically, the meta-analysis estimate should be considered in the context of the data that are used to generate it.

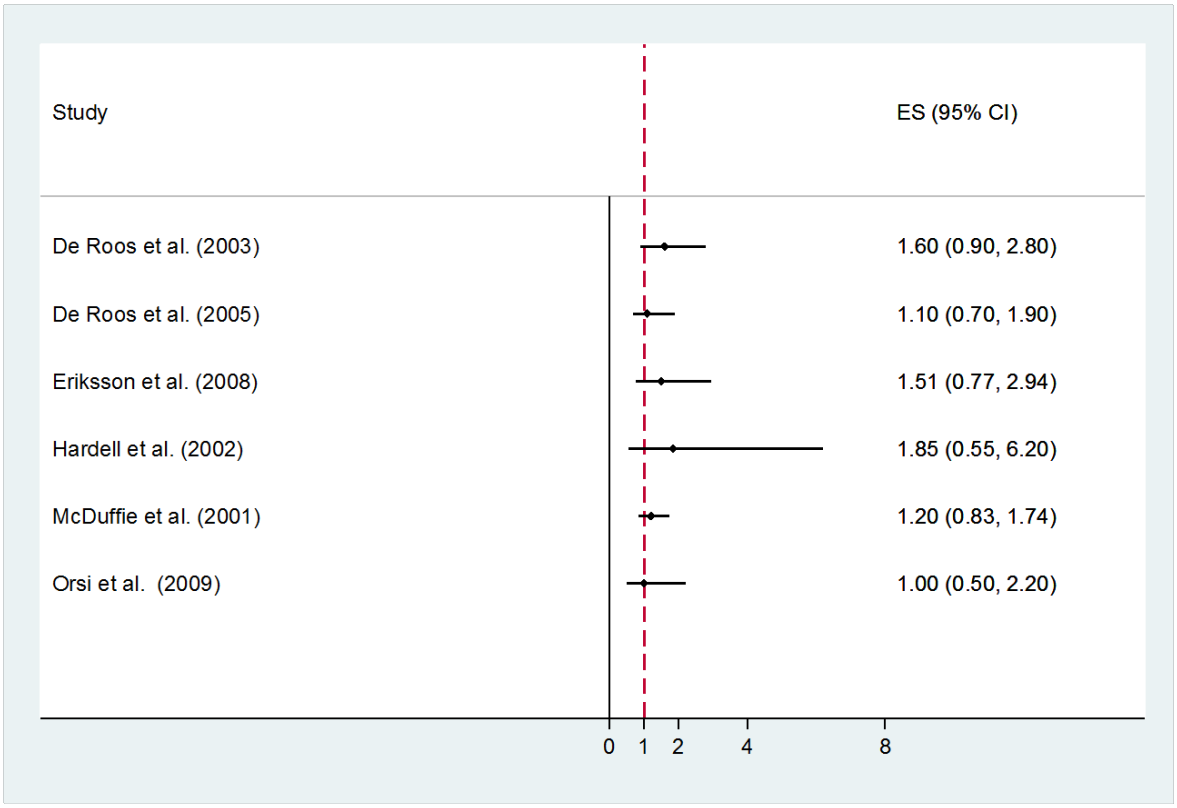

Figure 3.2. Forest plot of effect estimates (denoted as ES for effect sizes) and associated 95% confidence intervals (CI) for Non-Hodgkin lymphoma (NHL).

Using cumulative lifetime and intensity-weighted cumulative exposure metrics, all effect estimates were less than 1 (OR = 0.6-0.9 when comparing to the lowest tertile) in the AHS cohort study (De Roos *et al.*, 2005). Two case-control studies (Eriksson et al., 2008; McDuffie et al., 2001) evaluated the association of glyphosate exposure and NHL stratifying exposure by days per year of use. These studies obtained effect estimates greater than 1, which conflicted with the results in the prospective cohort study; however, these estimates from the case-control studies do not appear to be adjusted for co-exposures to other pesticides. As mentioned previously (and will be discussed further below), there was clearly strong potential for confounding from exposure to other pesticides. In each instance where a study controlled for co-exposure to other pesticides, the adjusted effect estimate decreased in magnitude, including other analyses performed in one of these case-control studies. Consequently, lack of adjustment for co-exposure to other pesticides in these analyses could partially explain the conflicting results between the cohort and case-control studies.

The possible effect of confounding factors, which are related to both the exposure of interest and the risk of disease, may make it difficult to interpret the results. Control for confounding varied considerably across studies (Table 3.2). Studies primarily adjusted for standard variables, such as age, gender, and residency location. Co-exposure to other pesticides was considered for several of the NHL studies for ever/never use (De Roos *et al.*, 2003; De Roos *et al.*, 2005; Eriksson *et al.*, 2008; Hardell *et al.*, 2002); however, analyses of exposure-response and latency effects did not appear to control for these co-exposures.

There is clearly a strong potential for confounding by co-exposures to other pesticides since many are highly correlated and have been reported to be risk factors for NHL. In the studies that did report a quantitative measure adjusted for the use of other pesticides, the risk was always found to be closer to the null than the risk calculated prior to this adjustment. For examples, Eriksson et al. (2008) reported unadjusted and adjusted effect estimates of 2.02 (95% CI: 1.10-3.71) and 1.51 (95% CI:0.77-2.94), respectively. Comparing the magnitude of those effect sizes on the natural log scale, the unadjusted effect was β=0.70 (95% CI: 0.10, 1.31) while the adjusted effect was β=0.41 (95% CI: -0.26, 1.08), suggesting a difference compatible with a degree of confounding by those herbicide co-exposures which appeared to have inflated the unadjusted effect upwards by 70% on the natural log scale (or by 46% on the OR scale). This demonstrates the profound effect this adjustment has on effect estimates and the concern for residual confounding by other pesticides that cause NHL themselves. As discussed in Section 3.2.4, other potential confounders have also been identified. With an association between glyphosate exposure and the outcome of interest, occupational exposure to diesel exhaust fumes, solvents, and UV radiation are highly likely confounders in the NHL studies; however, none of these studies accounted for these potential confounders.

Recall bias and missing data are also limitations in most of the studies. In epidemiologic studies, the quality of the exposure assessment is a major concern since the validity of the evaluations depends in large part on the ability to correctly quantify and classify an individual's exposure. Variation in the quality of exposure assessment, study design and methods, as well as available information concerning potential confounding variables could also explain discrepancies in study findings. During their lifetime, farmers are typically exposed to multiple pesticides and often

several may be used together posing a challenge for identifying specific risk factors. Moreover, there is no direct information on pesticide exposure or absorbed dose because analyses are based on self-reported pesticide use. The studies included in this epidemiology assessment relied primarily on questionnaires and interviews to describe participants' past and/or current exposure to glyphosate. Since the questionnaires are commonly used to account for exposure and capture self-reporting, the results can be subject to misclassification and recall bias.

Furthermore, the use of proxy respondents has the potential to increase recall bias and thus may increase exposure misclassification, especially for proxy respondents not directly involved in farming operations that may be more prone to inaccurate responses than directly interviewed subjects. In some of the NHL studies, the study participants were interviewed directly to assess exposure (De Roos *et al.*, 2005; Eriksson *et al.*, 2008; McDuffie *et al.*, 2001; Orsi *et al.*, 2009), making proxy respondent use a non-issue for these studies. In other studies, however, study participants or proxy respondents were interviewed to assess exposure (Hardell *et al.*, 2002, De Roos *et al.*, 2003). De Roos *et al.* (2003) did not find type of respondent to be statistically significant, but Hardell et al. (2002) did not conduct analyses to evaluate the impact of proxy use In non-NHL studies, proxy analyses were conducted in a small subset (Kachuri *et al.*, 2013; Lee *et al.*, 2004b; Lee *et al.*, 2005; Yiin *et al.*, 2012) and differences in effect estimates were often observed. In a few studies, respondent type was used as an adjustment variable when calculating effect estimates (Band *et al.*, 2011; Kachuri *et al.*, 2013; Lee *et al.*, 2005). As with all study design elements of case-control studies, one concern is whether or not the use of proxy respondents had a differential impact on the cases and controls included in the study because any differential impact may result in differential exposure misclassification. When use of proxy respondents was comparable for cases and controls in the full study population, it could be assumed that there is less concern for potential recall bias from the use of proxy respondents. In Hardell *et al.*, (2002), the percentage of cases and controls with proxy respondents was not fully reported for cases and controls though and this adds a potential source of uncertainty for the study. Moreover, when proxy respondents were used in a study, the percentages were usually reported only for the full study population and were not reported for the specific cases and controls exposed to glyphosate. This lack of information makes it difficult to assess the degree to which recall bias may have occurred due to the use of proxy respondents.

The highest risk measures were reported in studies with subjects developing NHL during a period of relatively low use of glyphosate. For example, Hardell *et al.* (2002) and De Roos *et al.* (2003) acquired cases from 1987-1990 and 1979-1986, respectively. These studies reported the largest adjusted ORs for glyphosate exposure and NHL (1.6 and 1.85); however, these studies investigated subjects prior to the introduction of genetically engineered glyphosate-tolerant crops. As discussed in Section 1.4, glyphosate use dramatically increased following the introduction of genetically engineered glyphosate-tolerant crops in 1996. Prevalence alone would not be expected to result in a corresponding increase in outcomes associated with glyphosate; however, the use pattern changed following the introduction of transgenic crops, such that in addition to new users, individuals already using glyphosate would have a corresponding increase in glyphosate exposure. As a result, if a true association exists between glyphosate exposure and NHL, then a corresponding increase in effect estimates would also be expected during this time. The currently available studies do not display this trend. In more recent years, including the AHS prospective cohort study (De Roos *et al.*, 2005), reported

adjusted risk measures were lower (1.0-1.51). Furthermore, if a true association exists, it would also be expected that the higher effect estimates would be reported in countries where individuals are more exposed to glyphosate, such as the United States and Canada, as compared to countries that exhibit less use[12]. Once again, the expected trend was not observed, such that effect estimates for studies conducted in Sweden (Eriksson *et al.*, 2008; Hardell et al., 2002), where glyphosate-tolerant crops are sparsely grown, were similar or higher than those reported in the United States (De Roos *et al.*, 2003; De Roos *et al.*, 2005) and Canada (McDuffie *et al.*, 2001). These counterintuitive results highlight the need for additional studies to determine the true association between glyphosate exposure and NHL, as well as further elucidate the exposure-response relationship.

Some have argued that the follow-up period (median = 7 years) in De Roos et al. (2005) is not sufficiently long to account for the latency of NHL (Portier et al., 2016); however, the latency period for NHL following environmental exposures is relatively unknown and estimates have ranged from 1-25 years (Fontana et al., 1998; Kato et al., 2005; Weisenburger, 1992). Eriksson et al., 2008) evaluated the impact of time since first exposure. This study found an increased effect estimate for subjects with more than 10 years of glyphosate exposure prior to diagnosis of NHL. This finding suggests a potential for a longer latency for NHL than the follow-up period in De Roos et al. (2005); however, this analysis did not appear to account for co-exposures to other pesticides and the number of subjects in the analysis were not reported. It should be noted that the follow-up time in De Roos et al. (2005) does not represent the amount of time subjects have been exposed. In this study, prior pesticide exposure was provided at time of enrollment and used to evaluate subjects that contribute person-time from enrollment until the point of diagnosis, death, movement from the catchment area, or loss to follow-up. As such, estimated exposure for each subject did not continue to accrue during follow-up. Additionally, subjects were not checked against state registries for inclusion in the cohort. Rather, cancer analyses were restricted to those who are cancer-free at the time of enrollment to remove any issues related to treatment that might impact subsequent cancer risk. At the time of enrollment, the average and median times of exposure 7.5 years and 8 years, respectively, with a standard deviation of 5.3[13]. These values were calculated using the midpoint of exposure categories provided in the questionnaire; therefore, these values represent a range of subject exposure time. Given the majority of the subjects were at least 40 years old at the time of analysis and the recognition that these workers generally start in their profession at a much earlier age and stay in that profession over their lifetime, time of exposure for many of these subjects would be greater than the average and median times. All of this information indicates that subjects within the cohort have ample amount of time for the outcome of interest to develop and be detected during the study. Furthermore, NHL has about 60 subtypes classified by the WHO, which may have etiological differences (Morton *et al.*, 2014). In this evaluation, the analysis of effect estimates was restricted to total NHL due to the small sample sizes in the few instances where NHL subtypes were analyzed. There are concerns with grouping the subtypes together despite etiological differences and the latency period for each NHL subtype may vary due to these etiological differences. Given the latency analysis was limited to Eriksson et al. (2008) and lack of NHL latency understanding in general, further analyses are needed to determine the true

[12] Components in glyphosate formulations in the United States and abroad are similar according to personal communication with Monsanto.
[13] Information provided by email from NIEHS.

latency time of NHL and NHL subtypes. The next update to the AHS cohort study with a longer follow-up would also aid in alleviating any concerns regarding the ability of De Roos et al. (2005) to detect subjects developing NHL.

There are conflicting views on how to interpret the overall results for NHL. Some believe that the data are indicative of a potential association between glyphosate exposure and risk of NHL. This is primarily based on reported effect estimates across studies and the associated meta-analyses greater than 1 despite lack of statistical significance. Additionally, the analysis conducted by Eriksson et al. (2008) observed a slightly statistically significant increase for those with more than 10 years of exposure prior to diagnosis. There were also two case-control studies that investigated the association of glyphosate exposure and NHL by stratifying exposure by days per year of use that reported effect estimates greater than 1 for groups with the highest exposure.

Conversely, others have viewed the effect estimates as relatively small in magnitude and observed associations could be explained by chance and/or bias. All of the effect estimates for ever/never use were non-statistically significant. Sample sizes were small or questionable in some of the studies. Half of the studies reported effect estimates approximately equal to 1, while the other half of the studies reported effect estimates clustered from 1.5-1.85, with the largest effect estimate having the widest confidence interval indicating the estimate was less reliable. As such, the higher effect estimates were contradicted by the results from studies at least equal quality. Meta-analyses were based on studies with varying study characteristics. Given the limitations and concerns discussed above for the studies included in this evaluation, chance and/or bias cannot be excluded as an explanation for the relatively small increase observed in the meta-risk ratios. Meanwhile, analyses performed by De Roos et al. (2005) reported effect estimates less than 1 for cumulative lifetime exposure and intensity-weighted cumulative exposure and these extensive analyses did not detect any exposure-response relationship, which conflicts with the two case-control studies that indicate potential for an exposure-response relationship comparing two groups stratified by days per year of use. Although increased effect estimates were observed in one case-control study (Eriksson et al., 2008) for subjects exposed more than 10 years prior to diagnosis and in two case-control studies (McDuffie et al., 2001; Eriksson et al., 2008) that stratified exposure by days per year of use, none of these analyses appeared to adjust for exposures to other pesticides, which has been found to be particularly important for these analyses and would attenuate these estimates towards the null. Furthermore, none of the studies in this evaluation of glyphosate exposure and risk of NHL accounted for other potential confounders, such as diesel exhaust fumes, solvents, and UV radiation. These adjustments would also be expected to reduce effect estimates towards the null.

Based on the weight-of-evidence, the agency cannot exclude chance and/or bias as an explanation for observed associations in the database. Due to study limitations and contradictory results across studies of at least equal quality, a conclusion regarding the association between glyphosate exposure and risk of NHL cannot be determined based on the available data. The agency will continue to monitor the literature for studies and any updates to the AHS will be considered when available.

4.0 Data Evaluation of Animal Carcinogenicity Studies

4.1 Introduction

Cancer bioassays in animals have historically been the primary studies available to evaluate cancer hazard in humans, since until recently epidemiological evidence was limited. The results of these bioassays, as well as results from screening assays for genotoxicity, are considered in a weight-of-evidence approach to determine the potential of a chemical to induce cancer in humans. Carcinogenicity studies in two rodent species are required for the registration of food use pesticides or when the use of a pesticide is likely to result in repeated human exposure over a considerable portion of the human lifespan (40 CFR Part 158.500). Rodent carcinogenicity studies identified from the data collection phase of the systematic review were evaluated for study quality and acceptable studies were evaluated in the context of the 2005 EPA Guidelines for Carcinogen Risk Assessment as described in Sections 4.2 and 4.3 below, respectively.

4.2 Consideration of Study Quality for Animal Carcinogenicity Studies

The agency has published test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200) and combined chronic/carcinogenicity studies (OCSPP 870.4300) in rodents which have been harmonized with OECD guidelines (Test Nos. 451 and 453). Test substances are typically administered in animal carcinogenicity studies by the oral route for food use pesticides. The studies are generally conducted in mice and rats with exposure durations of 18-24 months for mice and 24 months for rats, which represent exposures of the majority of the expected lifespan in these animals. Guideline carcinogenicity studies are designed to test three or more doses in both sexes (with at least 50 animals/sex/dose) with adequate dose spacing to characterize tumor dose-response relationships. Key considerations when evaluating carcinogenicity studies for cancer hazard assessment include identification of target organs of carcinogenicity, increased incidence of tumors or proportion of malignant neoplasms, and reduction in the time to appearance of tumors relative to the concurrent control group (OECD TG 451).

There are a number of criteria the agency uses when evaluating the technical adequacy of animal carcinogenicity studies. A primary criterion is the determination of the adequacy of dosing. The 2005 EPA Guidelines for Carcinogen Risk Assessment recommends that the highest dose level selected should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors; or without inducing inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms); however, the high dose need not exceed 1,000 mg/kg/day (i.e., limit dose) (OCSPP 870.4200; OCSPP 870.4300). Additional criteria to judge the technical adequacy and acceptability of animal carcinogenicity studies are provided in the test guidelines as well as other published sources (NTP, 1984; OSTP, 1985; Chhabra et al., 1990). As stated in the 2005 EPA Guidelines for Carcinogen Risk Assessment, studies that are judged to be wholly inadequate in protocol, conduct or results, should be discarded from analysis. Studies the agency consider acceptable are further evaluated for potential tumor effects.

Following study quality evaluation, a total of 9 chronic/carcinogenicity studies in the rat and 6 carcinogenicity studies in the mouse were considered acceptable for use in the current evaluation for the active ingredient glyphosate and were subsequently evaluated in the context of the 2005 EPA Guidelines for Carcinogen Risk Assessment as described in Section 4.3. A number of studies were judged to be inadequate in protocol, conduct or reporting and were not considered in the analysis of glyphosate. These studies and the justification for not including them in the analysis are listed below:

1. A two-year chronic oral toxicity study in Albino rats by Reyna (1974)[14]. The study was considered inadequate to assess carcinogenicity due to insufficient reporting on the histopathology findings in the control and treatment groups. Approximately 70 animals were unaccounted for across the study.

2. A two-year drinking water study in Wistar rats with a formulated product (13.6% ammonium salt) by Chruscielska et al., (2000). In addition to deficiencies including inadequate reporting of water consumption and body weight data, this study was conducted with a glyphosate formulated product and not the active ingredient glyphosate, which is the focus of this review. Glyphosate formulations contain various components other than glyphosate and it has been hypothesized these components are more toxic than glyphosate alone. The agency is collaborating with NTP to systematically investigate the mechanism(s) of toxicity for glyphosate and glyphosate formulations. This project is discussed in more detail in Section 7.0 of this document.

3. An initiation-promotion study (George et al., 2010) in male Swiss mice that tested a commercial formulation of glyphosate (41%) on the skin. Study deficiencies included small number (20) of animals, tested only males, and lack of histopathological examination.

4. A carcinogenicity study in Swiss albino mice (Kumar, 2001)[15]. This study was not included due to the presence of a viral infection within the colony, which confounded the interpretation of the study findings. Malignant lymphomas were reported in this study in all dose groups. However, lymphomas are one of the most common types of spontaneous neoplastic lesions in aging mice (Brayton et al., 2012). Murine leukemia viruses (MuLVs) are also a common cause of lymphoma in many different strains of mice (Ward, 2006). For example, Tadesse-Heath et al. (2000) reported 50% lymphoma (mostly B-cell origin) incidence in a colony of Swiss mice infected with MuLVs. Although the lymphoma incidences in Kumar (2001) were within or near normal background variation, it is not clear whether or not the viral infection may have contributed to the lymphoma incidence reported or the lower survival seen at the high dose in this study.

[14] MRID 00062507.

[15] MRID 49987403. In Greim et al. (2015), the same study is cited as Feinchemie Schwebda (2001).

5. A two year feeding study in Sprague-Dawley rats (Excel, 1997) was not included. The agency does not have access to this study to perform an independent assessment of its conduct and; however, Greim et al. (2015) stated that the study "is considered unreliable for carcinogenicity evaluation" and there were "several deviations from the OECD Test Guideline 453".

4.3 Assessment of Animal Carcinogenicity Studies

The agency considers many factors when interpreting the results of carcinogenicity studies. The 2005 EPA Guidelines for Carcinogen Risk Assessment are intended as a guidance only and does not provide a checklist for determining whether tumor findings are related to treatment. These guidelines emphasize the importance of weighing multiple lines of evidence in reaching conclusions regarding human carcinogenic potential of chemicals. Evaluation of observed tumor findings takes into consideration both biological and statistical significance. There are several factors in the 2005 EPA Guidelines for Carcinogen Risk Assessment used in the weight-of-evidence evaluation of individual studies. For this evaluation, the interpretation of the evidence related to tumor findings is described below. The agency is soliciting comment from the SAP regarding several of these factors as they relate to the interpretation of studies as part of Charge Question #3.

Dose Selection

Doses should be selected based on relevant toxicological information. Caution is taken in administering an excessively high dose that would confound the interpretation of the results to humans. As mentioned above, the 2005 EPA Guidelines for Carcinogen Risk Assessment recommends that the highest dose level selected should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors; or without inducing inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms); however, the high dose is not recommended to exceed 1,000 mg/kg/day (OCSPP 870.4200; OCSPP 870.4300). Doses should provide relevant dose-response data for evaluating human hazard for human health risk assessment. In the case of glyphosate, the low (oral) systemic toxicity and limited pharmacokinetic (PK) data for this chemical make it difficult to define a maximum tolerated dose (MTD) for the cancer bioassays. A large number of the carcinogenicity studies conducted with glyphosate approach or exceed the limit dose. The 2005 EPA Guidelines for Carcinogen Risk Assessment state that "weighing of the evidence includes addressing not only the likelihood of human carcinogenic effects of the agent but also the conditions under which such effects may be expressed". As such, the agency puts less weight on observations of tumors that occur near or above the limit dose.

Statistical analyses to evaluate dose response and tumor incidences

The main aim of statistical evaluation is to determine whether exposure to the test agent is associated with an increase in tumor development, rather than due to chance alone. Statistical analyses should be performed on each tumor type separately. The incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered

separately, but may be combined when scientifically defensible (McConnell *et al*., 1986). Trend tests and pairwise comparison tests are the recommended tests for determining whether chance, rather than a treatment-related effect, is a plausible explanation for an apparent increase in tumor incidence. The 2005 Guidelines for Carcinogen Risk Assessment states that

"A trend test such as the Cochran-Armitage test (Snedecor and Cochran, 1967) asks whether the results in all dose groups together increase as dose increases. A pairwise comparison test such as the Fisher exact test (Fisher, 1950) asks whether an incidence in one dose group is increased over that of the control group. By convention, for both tests a statically significant comparison one for which *p* is less than 0.05 that the increased incidence is due to chance. Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result."

In the current evaluation, the Cochran-Armitage Test for Trend (Snedecor and Cochran, 1967; one-sided) was used. For pairwise comparisons, the Fisher Exact Test (Fisher, 1950; one-sided) was used in the current evaluation to determine if incidences observed in treated groups were different from concurrent controls. Furthermore, the 2005 EPA Guidelines for Carcinogen Risk Assessment state that "considerations of multiple comparisons should also be taken into account". Multiple comparison methods control the familywise error rate, such that the probability of Type I error (incorrect rejection of the null hypothesis or "false positive") for the pairwise comparisons in the family does not exceed the alpha level. In the current evaluation, a Sidak correction method was used to adjust for multiple comparisons.

Forthe current evaluation, statistical significance observed in either test is judged in the context of all of the available evidence. Statistically significant responses may or may not be biologically significant and vice versa (Hsu and Stedeford, 2010; EPA, 2005). If a trend was found to be statistically significant, a closer examination of the tumor incidence was taken to determine whether the data demonstrate a monotonic dose-response where an increase in tumor incidence is expected with corresponding increase in dose. Therefore, statistically significant results with fluctuating tumor incidence across doses are not weighed as heavily as those displaying a monotonic dose-response. If a pair-wise comparison was found to be statistically significant, a closer examination of the tumor incidence and other lines of evidence was taken to determine whether the response was biologically significant. Factors considered in determining the biological relevance of a response are discussed below.

Given that statistical evaluations were performed at different times for each study, all statistical analyses were reanalyzed for the purposes of this evaluation to ensure consistent methods were applied (TXR# 0057494).

Historical Control Data

As indicated in the 2005 EPA Guidelines for Carcinogen Risk Assessment (Section 2.2.2.1.3), the standard for determining statistical significance of tumor incidence comes from a comparison of tumors in dosed animals with those in concurrent control animals. Additional insight into the statistical and/or biological significance of a response can come from the consideration of

historical control data (Tarone, 1982; Haseman, 1995; EPA, 2005). Historical control data can add to the analysis, particularly by enabling identification of uncommon tumor types or high spontaneous incidence of a tumor in a given animal strain. Generally speaking, statistically significant increases in tumors should not be discounted simply because incidence rates in the treated groups are within the range of historical controls or because incidence rates in the concurrent controls are somewhat lower than average.

Historical control data are also useful to determine if concurrent control tumor incidences are consistent with previously reported tumor rates (Haseman, 1995). Given the large number of age-related tumor outcomes in long-term rodent bioassays, and thus the large number of potential statistical tests run, caution is taken when interpreting results that have marginal statistical significance or in which incidence rates in concurrent controls are unusually low in comparison with historical controls since there may be an artificial inflation of the differences between concurrent controls and treated groups. Consequently, in the current evaluation, unusually low incidence in concurrent controls was noted when applicable and considered as part of the weight-of-evidence for the tumor findings. Identification of common or uncommon situations prompts further thought about the meaning of the response in the current study in context with other observations in animal studies and with other evidence about the carcinogenic potential of the agent.

Evidence of supporting preneoplastic lesions or related non-neoplastic lesions

Carcinogenicity rodent studies are designed to examine the production of tumors as well as preneoplastic lesions and other indications of chronic toxicity that may provide evidence of treatment-related effects and insights into the way the test agent produces tumors (EPA, 2005). As such, the presence or lack of supporting preneoplastic or other related non-neoplastic changes were noted in the current evaluation of each study and considered in the weight-of-evidence.

Additional Considerations

Other observations can strengthen or lessen the significance of tumor findings in carcinogenicity studies. Such factors include: uncommon tumor types; tumors at multiple sites; tumors in multiple species, strains, or both sexes; progression of lesions from preneoplastic to benign to malignant; reduced latency of neoplastic lesions (i.e., time to tumor); presence of metastases; unusual magnitude of tumor response; and proportion of malignant tumors (EPA, 2005). The agency considers all of the above factors when determining the significance of tumor findings in animal carcinogenicity studies.

4.4 Summary of Animal Carcinogenicity Studies

A total of 9 chronic toxicity/carcinogenicity studies in the rat and 6 carcinogenicity studies in the mouse were considered acceptable and evaluated in the weight-of-evidence analysis for glyphosate. This includes all of the studies that were part of the 2015 CARC evaluation plus an additional 5 studies identified from the systematic review. In the 2015 CARC evaluation, for some of the studies considered, the CARC relied on summary data that was provided in the supplement to the Greim et al. (2015) review article. Due to the ongoing data collection effort and the acquiring of studies not previously submitted, the agency no longer needs to rely on the

Greim et al. (2015) review article for the study data generated in relevant studies, allowing for a more complete and independent analysis. It should be noted that studies have been cited differently in this evaluation as compared to Greim et al. (2015) so these alternative citations have been noted for applicable studies.

The carcinogenicity studies conducted in the rat and mouse that were considered for the analysis are discussed in Sections 4.5 and 4.6, respectively. In these sections, short study summaries are presented which include information on the study design (including test material, strain of animal used, and doses and route of administration) as well as study findings including effects on survival, general toxicity observed, relevant non-neoplastic lesions, and the incidence and characterization of any tumor findings. The characterization of the tumor response(s) is based on the considerations previously discussed in Section 4.3 for interpreting the significance of tumor findings in animal carcinogenicity studies. The rat and mouse carcinogenicity studies are all summarized in Table 4.11 and Table 4.18, respectively.

4.5 Rat Carcinogenicity Studies with Glyphosate

4.5.1 Burnett et al., 1979 (MRID 00105164)

In a two-year chronic/carcinogenicity oral study, glyphosate (as an aqueous monosodium salt solution) was administered to groups of 90 albino rats/sex/dose at doses of 0, 3, 10, or 30 mg/kg/day (M/F) for 24 months through oral intubation (gavage).

A higher mortality rate was noted in the control group in comparison to the treated groups after 12 and 24 months of testing. No histopathological alterations were observed. There were no treatment-related increases in tumor incidences in the study; however, the highest dose tested in this study was 30 mg/kg/day, which was not considered a maximum tolerable dose to assess the carcinogenic potential of glyphosate.

4.5.2 Lankas, 1981 (MRID 00093879)[16]

In a chronic toxicity/carcinogenicity study, groups of Sprague-Dawley rats (50/sex/dose) were fed diets containing glyphosate (98.7%, pure) at dietary doses of 0, 3/3, 10/11, and 31/34 mg/kg/day (M/F).

There were no treatment-related effects on survival at any dose level. As in Burnett (1979), the highest dose tested of approximately 32 mg/kg/day was not considered a maximum tolerable dose to assess the carcinogenic potential of glyphosate. Consequently, a second study (Stout and Ruecker, 1990) was conducted at higher doses, which is summarized in the Section 4.5.3.

Table 4.1. Testicular Interstitial Cell Tumors in Male Sprague-Dawley Rats (Lankas, 1981) Cochran-Armitage Trend Test & Fisher's Exact Test Results

	0 mg/kg/day	3.05 mg/kg/day	10.3 mg/kg/day	31.49 mg/kg/day

[16] In Greim et al. (2015), the same study is cited as Monsanto (1981).

Table 4.1. Testicular Interstitial Cell Tumors in Male Sprague-Dawley Rats (Lankas, 1981) Cochran-Armitage Trend Test & Fisher's Exact Test Results				
Incidence	0/50	3/47	1/49	6/44
(%)	(0)	(6)	(2)	(12)
Raw p-value =	0.009**	0.121	0.500	0.013*
Sidak p-value =	--	0.321	0.875	0.039*

Note: Trend test results denoted at control; * denotes significance at p=0.05; ** denotes significance at p=0.001.

A statistically significant trend was reported for the testicular interstitial tumors; however, closer examination of the tumor incidence indicates that the data do not demonstrate a monotonic dose response with greater incidence observed at the low-dose as compared at the mid-dose. The incidence at the high dose was found to be statistically significant as compared to the concurrent controls. The observed incidence of interstitial cell tumors in concurrent controls (0%) appears to be unusually low for this tumor type as compared to historical controls provided in the study report for this tumor type (mean = 4.5%; range = 3.4%-6.7%) resulting in an artificial difference at the high dose. Furthermore, the observed incidence of interstitial cell tumors in the glyphosate-treated groups were within the normal biological variation for this tumor type in this strain of rat. There was an absence of pre-neoplastic or related non-neoplastic lesions (e.g., interstitial cell hyperplasia). As a result, the statistically significant results do not appear to be biologically significant and are not supported by any histopathological observations. Based on the weight-of-evidence for this study, the agency does not consider the increases in interstitial cell tumors in the testes to be treatment-related.

4.5.3 Stout and Ruecker, 1990 (MRID 41643801)[17]

In a chronic toxicity/carcinogenicity study, groups of Sprague-Dawley rats (60/sex/dose) were fed diets containing glyphosate (96.5%, pure) at dietary doses of 0, 89/113, 362/457 or 940/1183 mg/kg/day M/F) for 24 months. The highest dose tested in this study approaches or exceeds the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300). Tumor findings at these high doses are given less weight.

There was no significant increase in mortality. The most frequently seen tumors were pancreatic cell adenomas, hepatocellular adenomas, and thyroid C-cell adenomas in males. A discussion of each tumor type by organ is presented below:

1. Pancreas: Tumor incidences of pancreatic islet cell tumors in male rats and corresponding historical control values are presented in Tables 4.2 and 4.3, respectively. The incidence of pancreatic islet cell tumors lacked monotonic dose-responses and trend analyses were not statistically significant. Statistical significance was observed with raw (unadjusted) p-values for the incidence of adenomas at the low-dose (89 mg/kg/day) and high-dose (940 mg/kg/day) when comparing to concurrent controls; however, none of the incidences were statistically significant with an adjustment for multiple comparisons (p=0.052 at the low-dose and p=0.120 at the high-dose). The statistical significance of

[17] In Greim et al. (2015), the same study is cited as Monsanto (1990).

the pairwise comparisons with the concurrent control group may have been due to the unusually low incidences in the controls and not to an actual treatment-related response. The mean incidence of pancreatic islet cell adenomas in historical control data provided for laboratory (Monsanto Environmental Health Laboratory; MRID No. 41728701) was 5.3% and ranged from 1.8% to 8.3% indicating the concurrent control incidence for this tumor type was at the lower bound of the range. Carcinomas were only observed in the control group and the combined analyses did not yield any statistically significant pairwise comparisons. There were no supporting preneoplastic or other related non-neoplastic changes observed and no evidence of progression from adenomas to carcinomas. Based on a weight-of-evidence for this study, the agency does not consider these increases in pancreatic islet cell tumors to be treatment-related.

Table 4.2. Pancreatic Islet Cell Tumors in Male Sprague-Dawley Rats (Stout and Ruecker, 1990) Cochran-Armitage Trend Test & Fisher's Exact Test Results.

Tumor Type	0 mg/kg/day	89 mg/kg/day	362 mg/kg/day	940 mg/kg/day
Adenoma Incidence (%) Raw p-value = Sidak p-value =	1/43[a] (2) 0.176 --	8/45 (18) 0.018* 0.052	5/49 (10) 0.135 0.352	7/48[b] (15) 0.042* 0.120
Carcinoma Incidence (%) Raw p-value = Sidak p-value =	1/43[c] (2) -[d] --	0/45 (0) 1.000 1.000	0/49 (0) 1.000 1.000	0/48 (0) 1.000 1.000
Combined Incidence (%) Raw p-value = Sidak p-value =	2/43 (2) 0.242 --	8/45 (18) 0.052 0.149	5/49 (10) 0.275 0.619	7/48 (15) 0.108 0.289

Note: Trend test results denoted at control; * denotes significance at p=0.05.

a. Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed prior to study week 55.

b. First adenoma in the study was observed at week 81 in the 940 mg/kg/day group.

c. First carcinoma in the study was observed at week 105 in the controls.

d. Trend p-value not reported since tumor incidence decreased with increasing dose.

Historical control data on the incidence of pancreatic islet cell adenomas in male Sprague-Dawley rats in 2-year studies (1983–1989) conducted at the testing facility (Monsanto Environmental Health Laboratory; MRID No. 41728701) are presented below in Table 4.3.

Table 4.3. Historical Control Data — Pancreatic Islet Cell Adenomas in Male Sprague- Dawley Rats (MRID No. 41728701).

Study No.	1	2	3	4	5	6	7	Mean
Study Year	07/83	02/85	10/85	6/85	9/88	1/89	3/89	-
Tumor Incidence	2/68	5/59	4/69	1/57	5/60	3/60	3/59	-
Percentage (%)	2.9%	8.5%	5.8%	1.8%	8.3%	5.0%	5.1%	5.3%

2. Liver: Tumor incidences of liver tumors in male rats are presented in Tables 4.4. There was a statistically significant dose trend for liver adenomas only. Closer examination of the incidence indicates a relatively flat response at the low- and mid-dose with only an increase observed at the high-dose (940 mg/kg/day); however, the incidence of liver adenomas at the high-dose was not statistically significant when compared to the concurrent controls. Carcinomas and combined adenomas/carcinomas lacked statistical significance in trend and pairwise comparisons (Table 4.4). Except for a single animal at the mid-dose late in the study (89 weeks), no hyperplasia, preneoplastic foci or other non-neoplastic lesions were observed. Furthermore, there was no evidence of progression from adenomas to carcinomas. Given the lack of both statistical significance and corroborative lesions to support the tumor finding, the agency does not consider these increases in liver tumors to be treatment-related.

Table 4.4. Hepatocellular Tumors in Male Sprague-Dawley Rats (Stout and Ruecker, 1990) Cochran-Armitage Trend Test & Fisher's Exact Test Results

Tumor Type	0 mg/kg/day	89 mg/kg/day	362 mg/kg/day	940 mg/kg/day
Adenoma Incidence (%) Raw p-value = Sidak p-value =	2/44[a] (5) 0.022* --	2/45 (4) 0.700 0.973	3/49 (6) 0.551 0.910	7/48[b] (15) 0.101 0.274
Carcinoma Incidence (%) Raw p-value = Sidak p-value =	3/44 (7) -[d] -	2/45 (4) 0.827 0.995	1/49 (2) 0.954 1.000	2/48[c] (4) 0.845 0.996
Combined Incidence (%) Raw p-value = Sidak p-value =	5/44 (11) 0.078 --	4/45 (9) 0.769 0.988	4/49 (8) 0.808 0.993	9/48 (19) 0.245 0.569

Note: Trend test results denoted at control; * denotes significance at p=0.05.

a. Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed prior to study week 55.
b. First adenoma in the study was observed at week 88 in the 940 mg/kg/day group.
c. First carcinoma in the study was observed at week 85 in the 940 mg/kg/day group.
d. Trend p-value not reported since tumor incidence decreased with increasing dose.

3. Thyroid: Tumor incidences of thyroid tumors in male and female rats are presented in Tables 4.6 and 4.7, respectively. For males, no statistically significant trends were observed for adenomas, carcinomas, or combined adenomas/carcinomas. For females, a statistically significant trend was observed for adenomas and combined adenomas/carcinomas with no statistically significance in pairwise analyses. Therefore, although there may be an indication of a dose-response in females, the increases observed in the glyphosate treated groups were not considered to be different than those observed in the concurrent controls. Non-neoplastic lesions (thyroid C-cell hyperplasia) were

observed; however, there was a lack of a monotonic dose-response for these histopathological findings and no dose-related increase in severity to support tumor findings (Table 4.8). There was also no evidence of progression from adenomas to carcinomas. Based on a weight-of-evidence for this study, the agency does not consider these increases in thyroid tumors to be treatment-related.

Table 4.6. Thyroid C-Cell Tumors in Male Sprague-Dawley Rats (Stout and Ruecker, 1990)
Cochran-Armitage Trend Test & Fisher's Exact Test Results

Tumor Type	0 mg/kg/day	89 mg/kg/day	362 mg/kg/day	940 mg/kg/day
Adenoma				
Incidence	2/54[a, b]	4/55	8/58	7/58
(%)	(4)	(7)	(14)	(12)
Raw p-value =	0.079	0.348	0.060	0.099
Sidak p-value =	--	0.723	0.168	0.269
Carcinoma				
Incidence	0/54	2/55[c]	0/58	1/58
(%)	(0)	(4)	(0)	(4)
Raw p-value =	0.457	0.252	1.000	0.518
Sidak p-value =	--	0.441	1.000	0.768
Combined				
Incidence	2/54	6/55	8/58	8/58
(%)	(4)	(11)	(14)	(14)
Raw p-value =	0.087	0.141	0.060	0.060
Sidak p-value =	--	0.367	0.168	0.168

Note: Trend test results denoted at control.

a. Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed prior to study week 55.

b. First adenoma in the study was observed at week 54 in the controls.

c. First carcinoma in the study was observed at week 93 in the 89 mg/kg/day group.

Table 4.7. Thyroid C-Cell Tumors in Female Sprague Dawley Rats
Cochran-Armitage Trend Test & Fisher's Exact Test Results (Stout and Ruecker, 1990).

Tumor Type	0 mg/kg/day	113 mg/kg/day	457 mg/kg/day	1183 mg/kg/day
Adenoma				
Incidence	2/57[a]	2/60	6/59[b]	6/55
(%)	(4)	(7)	(10)	(11)
Raw p-value =	0.040*	0.710	0.147	0.124
Sidak p-value =	--	0.976	0.380	0.328
Carcinoma				
Incidence	0/57	0/60	1/59[c]	0/55
(%)	(0)	(0)	(2)	(0)
Raw p-value =	0.494	1.000	0.509	1.000
Sidak p-value =	--	1.000	0.509	1.000
Adenoma/Carcinoma				
Incidence	2/57	2/60	7/59	6/55
(%)	(4)	(3)	(12)	(11)
Raw p-value =	0.042*	0.710	0.090	0.124
Sidak p-value =	--	0.976	0.246	0.328

Note: Trend test results denoted at control; * denotes significant at p=0.05.

a. Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed prior to study week 55.

b. First adenoma in the study was observed at week 72 in the controls.
c. First carcinoma in the study was observed at week 93 in the 457 mg/kg/day group.

Table 4.8. Thyroid Non-Neoplastic Lesions (Stout and Ruecker, 1990)				
Males				
Dose	0 mg/kg/day	89 mg/kg/day	362 mg/kg/day	940 mg/kg/day
Total Incidences of thyroid C-cell hyperplasia and severity scores	5/60 (8%) Diffuse (moderate) – 1 Multi-focal (minimal) – 3 Focal (mild) – 1	1/60 (2%) Focal (mild) – 1	6/60 (10%) Focal (minimal) – 4 Multi-focal (minimal) – 1 Multi-Focal (mild) – 1	5/60 (8%) Focal (minimal) – 2 Focal (mild) – 1 Multi-focal (mild) – 1 Multi-focal (moderate) – 1
Females				
	0 mg/kg/day	113 mg/kg/day	457 mg/kg/day	1183 mg/kg/day
Thyroid C-cell hyperplasia and severity scores	10/60 (17%) Diffuse (moderate) – 1 Focal (mild) – 1 Focal (minimal) – 1 Focal (mild) – 1 Focal (moderate) – 1 Multi-focal (minimal) – 3 Multi-focal (moderate) – 1 Diffuse (moderate) – 1	5/60 (8%) Focal (mild) – 3 Focal (minimal) – 1 Multi-focal (minimal) – 1	9/60 (15%) Focal (minimal) – 4 Multi-focal (minimal) – 2 Multi-focal (mild) – 3	5/60 (8%) Focal (mild) – 1 Focal (minimal) – 1 Multi-focal (mild) – 2 Diffuse (moderate) – 1

*Data taken from pages 1071-2114 of the study report.

4.5.4 Atkinson et al., 1993a (MRID 496317023)[18]

In a combined chronic toxicity/carcinogenicity study, glyphosate (98.9% pure) was administered to 50 Sprague-Dawley rats/sex/dose in the diet at doses of 0, 11/12, 112/109, 320/347, and 1147/1134 mg/kg/day for 104 weeks (M/F) for 104 weeks. An additional 35 rats/sex/dose were included for 1-year interim sacrifice.

No adverse effects on survival were seen in either sex across the doses tested. There were no changes in histopathological findings observed. There were no treatment-related increases in tumor incidences in the study.

4.5.5 Brammer, 2001 (MRID 49704601)[19]

In a combined chronic toxicity/carcinogenicity study, glyphosate acid (97.6% pure) was administered to groups of Wistar rats in the diet. Groups of 52 rats/sex received diets containing doses of 0, 121/145, 361/437 or 1214/1498 mg/kg/day for 24 months, in males/females, respectively. The highest dose tested in this study exceeds the highest dose recommended in the

[18] Note: In Greim et al. (2015), the same study is cited as Cheminova (1993a).
[19] Note: In Greim et al. (2015), the same study is cited as Syngenta (2001).

test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300).

A statistically significant higher survival (p=0.02) was observed in males at the highest dose tested at the end of 104 weeks relative to concurrent controls, and a statistically significant trend for improved survival was observed in treated males (p=0.03). The inter-current (early) deaths were 37/52, 36/52, 35/52, and 26/52 for the control, low-, mid-, and high-dose groups, respectively. The terminal deaths were 16/52, 17/52, 18/52, and 26/52 for the control, low-, mid- and high-dose groups, respectively. There were no treatment-related non-neoplastic lesions in any organs of either sex at any dose level tested. As shown in Table 4.9, a statistically significant trend in the incidences of liver adenomas was observed in male rats; however, a monotonic dose-response was not observed upon closer examination of the incidence data. Tumor incidences appear to fluctuate with increases observed at the low- and high-dose and no tumors observed in the control and mid-dose. Statistical significance with raw (unadjusted) p-values was observed for the tumor incidence at the high-dose (1214 mg/kg/day) when compared to concurrent controls; however, it was not statistically significant with an adjustment for multiple comparisons (p= 0.056). Tumor findings at these high doses are given less weight. The improved survival in the high-dose group may help explain a modestly higher incidence of an age-related background tumor like liver adenomas and this corresponds with the lack of associated lesions. Given that the tumor findings did not reflect a monotonic dose response and the high dose tumors were not statistically significant with an adjustment for multiple comparisons, the agency does not consider these increases in liver adenomas to be treatment-related.

Table 4.9. Liver Adenomas in Male Wistar Rats (Brammer, 2001)
Cochran-Armitage Trend Test and Fisher's Exact Test Results.

	0 mg/kg/day	121 mg/kg/day	361 mg/kg/day	1214 mg/kg/day
Adenoma Incidence (%)	0/52[a] (0)	2/52 (4)	0/52 (0)	5/52 (10)
Raw p-value =	0.008**	0.248	1.000	0.028*
Sidak p-value =	--	0.434	1.000	0.056

Note: Trend test results denoted at control; * denotes significance at p=0.05; ** denotes significance at p=0.01
a. Number of tumor-bearing animals/Number of animals examined.

4.5.6 Pavkov and Wyand 1987 (MRIDs 40214007, 41209905, 41209907)

Glyphosate trimesium salt (sulfosate, 56.2% pure) was tested in a 2-year chronic feeding/carcinogenicity study in male and female Sprague-Dawley (Crl:CD[SD]BR) rats. Sixty animals/sex were tested in control group 1 (basal diet, no vehicle), 80/sex were tested in control group 2 (basal diet plus propylene g1ycol at 1% w/w vehicle) and in the low and mid-dose groups, and 90/sex were tested in the high dose group. The following dose levels were tested: 0, 4.2/5.4, 21.2/27 or 41.8/55.7 mg/kg/day in males and females respectively.

Treatment had no effect on survival. There were no changes in histopathological findings observed. There were no treatment-related increases in tumor incidences in the study.

4.5.7 Suresh, 1996 (MRID 49987401)[20]

In a combined chronic toxicity/carcinogenicity study, glyphosate (96.0-96.8% pure) was administered to groups of Wistar rats in the diet. Groups of 50 rats/sex/group received diets containing 0, 6.3/8.6, 59.4/88.5, and 595.2/886 mg/kg/day glyphosate for 24 months in males and females respectively. The highest dose tested in females in this study approaches the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300).

No adverse effects on survival were observed in either sex across the doses tested. There were no changes in histopathological findings observed. There were no treatment-related increases in tumor incidence observed in the study.

4.5.8 Enemoto, 1997 (MRID 50017103-50017105)[21]

In a combined chronic toxicity and carcinogenicity study, groups of 50 Sprague-Dawley rats/sex/group received daily dietary doses of 0, 104/115, 354/393 and 1127/1247 mg/kg bw/day glyphosate for males and females, respectively. In addition, 10 rats/sex/group were included for interim sacrifices at 26, 52, and 78 weeks. The highest dose tested in this study exceeds the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300).

There were no changes in mortality at any of the doses tested. There were no changes in histopathological findings observed. There were no treatment-related increases in tumor incidence observed in the study.

4.5.9 Wood et al., 2009a (MRID 49957404)[22]

In a combined chronic toxicity/carcinogenicity study, glyphosate (95.7% pure) was administered to groups of Wistar rats in the diet. Groups of 51 rats/sex/group received diets containing 0, 95.0, 316.9, and 1229.7 mg/kg/day glyphosate for males and female, respectively. The highest dose tested in this study exceeds the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300).

No adverse effects on survival were seen in either sex across the doses tested. There were no treatment-related preneoplastic or related non-neoplastic lesions in either sex at any dose level.

In female rats, mammary gland tumors were noted. Tumor incidences for mammary gland adenomas, adenocarcinomas, and combined adenomas/adenocarcinomas in female mice are presented in Table 4.10. Statistically significant trends were observed for the adenocarcinoma and combined analyses. Tumor incidence for adenocarcinomas was not statistically significant

[20] Note: In Greim et al. (2015), the same study is cited as Feinchemie Schwebda (1996).
[21] Note: In Greim et al. (2015), the same study is cited as Arysta Life Sciences (1997b).
[22] Note: In Greim et al. (2015), the same study is cited as NuFarm (2009b).

in pairwise comparisons as compared to concurrent controls. Marginal statistical significance was observed with the raw (unadjusted) p-value for combined mammary gland tumors at the high-dose (1229.7 mg/kg/day) when comparing to concurrent controls; however, with an adjustment for multiple comparisons, the increased incidence at the high-dose was not statistically significant (p=0.132). There was also no evidence of progression from adenomas to carcinomas. Based on a weight-of-evidence for this study, the agency does not consider these increases in mammary gland tumors in female rats to be treatment-related.

Table 4.10. Mammary Gland Tumor Incidences in Female Rats (Wood et al., 2009a) Fisher's Exact Test and Cochran-Armitage Trend Test Results

Tumor Type	0 mg/kg/day	95.0 mg/kg/day	316.9 mg/kg/day	1229.7 mg/kg/day
Adenoma				
Incidence	0/51	0/51	0/51	2/51
(%)	(0)	(0)	(0)	(4)
Raw p-value =	0.062	1.000	1.000	0.248
Sidak p-value =	--	1.000	1.000	0.248
Adenocarcinoma				
Incidence	2/51	3/51	1/51	6/51
(%)	(4)	(6)	(2)	(12)
Raw p-value =	0.042*	0.500	0.879	0.135
Sidak p-value =	--	0.875	0.998	0.352
Combined				
Incidence	2/51	3/51	1/51	8/51
(%)	(4)	(6)	(2)	(16)
Raw p-value =	0.007**	0.500	0.879	0.046*
Sidak p-value =	--	0.875	0.998	0.132

Note: Trend test results denoted at control; * denotes significance at p=0.05; ** denotes significant at p=0.01.

4.5.10 Summary of Rat Data

In 5 of the 9 rat studies conducted with glyphosate, no tumors were identified for detailed evaluation. Of the remaining 4 rat studies, a statistically significant trend was observed for tumor incidences in the testes, pancreas, liver, thyroid, or mammary gland; however, the agency determined that these tumor findings are not considered to be related to treatment. Although a statistically significant trend was obtained, closer examination of the incidence data across doses did not demonstrate a monotonic dose response in several instances. Some of the tumor incidences at the highest dose tested (approaching or exceeding 1,000 mg/kg/day for almost all studies) were statistically significant from concurrent controls using raw (unadjusted) p-values; however, none of the pairwise comparisons were found to be statistically significant following adjustment for multiple comparisons, except the testicular tumors seen in a single study. Furthermore, these high-dose tumors were given less weight. There was no evidence of corroborating pre-neoplastic or related non-neoplastic lesions or evidence of tumor progression (progression from pre-neoplastic to malignancy) to support biological significance of tumor findings. In a limited number of cases, the agency considered historical control data to inform the relevance of a tumor increase when incidence rates in the concurrent controls were unusually low.

Table 4.11. Summary of Rat Carcinogenicity Studies			
Study	**Dose Range**	**Pre-Neoplastic or Related Non-Neoplastic Lesions**	**Tumors Incidences, Statistical Significance, and Related Comments**
Burnett et al. (1979) Albino rats	0, 3, 10 or 30 mg/kg/day for 24 months [M/F]	None observed	There were no treatment-related increases in tumor incidences.
Lankas (1981) Sprague-Dawley rats	98.7% Technical in diet 0, 3/3, 10/11, and 31/34 mg/kg/day [M/F]	None observed	Statistically significant trend observed for testicular interstitial cell tumors; however, did not observe monotonic dose-response with higher incidence at low-dose than mid-dose. Incidences were 0/50 in controls, 3/47 at low-dose, 1/49 at mid-dose, and 6/44 at high-dose. Increased incidence at high-dose statistically significant, but unusually low control incidence (based on historical control data in study report) inflated increase at high-dose.
Stout and Ruecker (1990) Sprague-Dawley rats	96.5% Technical in diet 0, 89/113, 362/457 and 940/1183 mg/kg/day [M/F] for 24 months	None observed	Pancreatic tumors lacked statistically significant trend. Tumor incidence for pancreatic adenomas in males were 1/43 in controls, 8/45 at the low-dose, 5/49 at the mid-dose, and 7/48 at the high-dose. Concurrent control incidence for this tumor type was at the lower bound of the historical control range. No statistically significant pairwise comparisons, including the highest dose tested which is approaching/exceeding 1,000 mg/kg/day. Statistically significant trend for liver adenomas in males with only an increase at high-dose. Incidences were 2/44 in controls, 2/45 at the low-dose, 3/49 at the mid-dose, and 7/48 at the high-dose. No statistically significant pairwise comparisons, including the highest dose tested which is approaching/exceeding 1,000 mg/kg/day. No statistically significant trend for thyroid C-cell tumors in males. For females, statistically significant trend for adenomas and combined adenomas/carcinomas. Incidences for adenomas were 2/57 in controls, 2/60 at the low-dose, 6/59 at the mid-dose, and 6/55 at the high-dose. Similar incidences were seen for combined except the mid-dose was 7/59. No statistically significant pairwise comparisons, including the highest dose tested which is approaching/exceeding 1,000 mg/kg/day.
Atkinson et al. (1993a) Sprague-Dawley rats	98.9% Technical in diet 0, 11/12, 112/109, 320/347, and 1147/1134 mg/kg/day for 104 weeks (M/F)	None observed	There were no treatment-related increases in tumor incidences, including the highest dose tested which exceeded 1,000 mg/kg/day.

Table 4.11. Summary of Rat Carcinogenicity Studies			
Study	**Dose Range**	**Pre-Neoplastic or Related Non-Neoplastic Lesions**	**Tumors Incidences, Statistical Significance, and Related Comments**
Brammer. (2001) Wistar rats	97.6% Technical in diet 0, 121/145, 361/437 and 1214/1498 mg/kg/day [M/F]	None observed	Statistically significant trend in liver adenomas in males. Incidences were 0/52 in controls, 2/52 at the low-dose, 0/52 at the mid-dose, and 5/52 at the high-dose. No statistically significant pairwise comparisons when adjusting for multiple comparisons, including the highest dose tested which exceeded 1,000 mg/kg/day.
Pavkov and Wyand (1987) Sprague-Dawley rats	56.2% Technical (Trimesium salt; Sulfosate) 0, 4.2/5.4, 21.2/27 and 41.8/55.7 mg/kg/day [M/F]	None observed	There were no treatment-related increases in tumor incidences.
Suresh (1996) Wistar rats	96.0-96.8% Technical in diet 0, 6.3/8.6, 59.4/88.5, and 595.2/886 mg/kg/day [M/F]	None observed	There were no treatment-related increases in tumor incidences, including the highest dose tested which exceeded 1,000 mg/kg/day.
Enemoto (1997) Sprague-Dawley rats	94.61-97.56% Technical in diet 0, 104/115, 354/393 and 1127/1247 mg/kg/day [M/F]	None observed	There were no treatment-related increases in tumor incidences, including the highest dose tested which exceeded 1,000 mg/kg/day.
Wood et al. (2009a) Wistar rats	95.7% Technical in diet 0, 86/105, 285/349 or 1077/1382 mg/kg/day [M/F]	None observed	Statistically significant trends were observed for the mammary gland adenocarcinoma and combined adenoma/adenocarcinoma analyses. Incidences for adenocarcinomas were 2/51 in controls, 3/51 at the low-dose, 1/51 at the mid-dose, and 6/51 at the high-dose. Similar incidences observed for combined adenoma/adenocarcinomas except incidence at high-dose was 8/51. No statistically significant pairwise comparisons when adjusting for multiple comparisons, including the highest dose tested which exceed 1,000 mg/kg/day.

4.6 Mouse Carcinogenicity Studies with Glyphosate

4.6.1 Reyna and Gordon, 1973 (MRID 00061113)

In an 18-month carcinogenicity study, groups of 50 Swiss white mice/sex/dose were fed glyphosate at dietary levels of approximately 17 mg/kg/day and 50 mg/kg/day. There was no effect on survival at any of the doses tested. There were no changes in histopathological findings observed. There were no treatment-related increases in tumor incidence observed in the study. Although only ten mice/sex/dose were examined for histopathological changes, there were no statistically significant increases in tumors observed in the study; therefore, this deficiency would not impact the overall conclusion regarding tumor findings.

4.6.2 Knezevich and Hogan, 1983 (MRID 00130406)[23]

Groups of 50 male and female CD-1 mice received glyphosate (99.78%, pure) at dietary doses of 0, 161/195, 835/968, 4945/6069 mg/kg/day for males and females, respectively for 24 months. The highest dose tested in this study far exceeds the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300). Furthermore, the mid-dose tested in this study was approaching 1,000 mg/kg/day. Tumor findings at these high doses are given less weight.

No effect on survival was observed. There were no corroborating lesions to support any tumor findings in this study.

A low incidence of renal tubule adenomas, which are considered rare, were noted in males. The incidences of renal tubule adenomas following initial evaluation of the study were reported as follows: 0/49 in the controls; 0/49 at the low-dose; 1/50 at the mid-dose; and 3/50 at the high dose (TXR No. 0004370). In 1985, the registrant directed a re-evaluation of the original renal sections by a consulting pathologist. This re-evaluation identified a small renal tubule adenoma in one control male mouse, which was not diagnosed as such in the original pathology report. In 1986, at the request of the agency, additional renal sections (3 sections/kidney/mouse spaced at 150 micron intervals) were evaluated in all control and all glyphosate-treated male mice in order to determine if additional tumors were present. The additional pathological and statistical evaluations concluded that the renal tumors in male mice were not compound-related.

Subsequently, the agency requested a Pathology Work Group (PWG) evaluate the kidney sections. The PWG examined all sections of the kidney, including the additional renal sections, and were blinded to treatment group. The renal tubular-cell lesions diagnosed by the PWG are presented below in Table 4.12 with results from statistical analyses. The PWG noted that because differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and because both lesions are derived from the same cell type, it is appropriate to combine the incidences from these two tumor types for purposes of evaluation and statistical

[23] Note: In Greim et al. (2015), the same study is cited as Monsanto (1983).

analysis. The PWG unanimously concluded that these lesions are not compound-related based on the following considerations: 1) renal tubular cell tumors are spontaneous lesions for which there is a paucity of historical control data for this mouse stock; 2) there was no statistical significance in a pairwise comparison of treated groups with the concurrent controls and there was no evidence of a statistically significant linear trend; 3) multiple renal tumors were not found in any animal; and 4) compound-related nephrotoxic lesions, including pre-neoplastic changes, were not present in male mice in this study (TXR No. 0005590).

Table 4.12. Kidney Tubular Cell Tumors in Male CD-1 Mice (Knezevich and Hogan, 1983) Cochran-Armitage Trend Test & Fisher's Exact Test Results.

Tumor Type	0 mg/kg/day	161 mg/kg/day	835 mg/kg/day	4945 mg/kg/day
Adenoma				
Incidence	1/49	0/49	0/50	1/50
(%)	(2)	(0)	(0)	(2)
Raw p-value =	0.4422	1.000	1.000	0.758
Sidak p-value =	--	1.000	1.000	0.986
Carcinoma				
Incidence	0/49	0/49	1/50	2/50
(%)	(0)	(0)	(2)	(4)
Raw p-value =	0.063	1.000	0.505	0.253
Sidak p-value =	--	1.000	0.755	0.441
Combined				
Incidence	1/49	0/49	1/50	3/50
(%)	(2)	(0)	(2)	(6)
Raw p-value =	0.065	1.000	0.758	0.316
Sidak p-value =	--	1.000	0.986	0.680

Note: Trend test results denoted at control.

Histopathological examinations noted chronic interstitial nephritis and tubular epithelial changes (basophilia and hypertrophy) in the kidneys of male rats in the study (Table 4.13). The increased incidence of chronic interstitial nephritis in males lacked a dose-response. The incidence in controls of bilateral interstitial nephritis was higher than low-dose group and approximately the same as the mid-dose group. Unilateral chronic interstitial nephritis was only seen in 1 animal in the low- and high-dose groups. Furthermore, chronic interstitial nephritis is not considered to be a precursor lesion for tubular neoplasms. A monotonic dose-response was not observed for the epithelial basophilia and hypertrophy, such that the incidence fluctuated with dose and the lowest incidence was observed at the highest dose tested. There was no increase in supporting preneoplastic or related non-neoplastic renal tubular lesions (e.g., tubular epithelial necrosis/regeneration, hyperplasia) observed in male mice.

Table 4.13. Kidney Histopathological Alterations in Male CD-1 Mice (Knezevich and Hogan, 1983)

Males				
Dose	0 mg/kg/day	161 mg/kg/day	835 mg/kg/day	4945 mg/kg/day
Bilateral Chronic Interstitial Nephritis	5/49 (10%)	1/49 (2%)	7/50 (14%)	11/50 (22%)

Unilateral Chronic Interstitial Nephritis	0/49 (0%)	1/49 (2%)	0/49 (0%)	1/50 (2%)
Proximal Tubule Epithelial Basophilia and Hypertrophy	15/49 (31%)	10/49 (20%)	15/50 (30%)	7/50 (14%)

*Data taken from page 305 and 306, and the study pathology report; incidences were moderate diffuse

Based on the weight-of-evidence for this study, the agency concurs with the PWG conclusion, following a thorough examination of all kidney sections, that the renal tubular neoplasms are not treatment-related with a lack of statistical significance in the trend and pairwise tests. Although there was an increase in chronic interstitial nephritis at the highest dose tested, this finding is not considered relevant to the tubular neoplasms.

4.6.3 Atkinson, 1993b (MRID 49631702)[24]

In a carcinogenicity study, glyphosate (>97% pure) was administered to groups of 50 CD-1 mice/sex/dose in the diet for 104 weeks at doses of 0, 98/102, 297/298, 988/1000 mg/kg/day for males and females, respectively. No interim sacrifices were performed.
There was no effect on survival in the study. There were no preneoplastic lesions or related non-neoplastic lesions observed. As shown in Table 4.14, hemangiosarcomas were found in 4/45 (9%) of high-dose male mice (1000 mg/kg/day) compared to none in the concurrent controls or other treated groups. Hemangiosarcomas are commonly observed in mice (generally more common in males for CD-1 strain) as both spontaneous and treatment-related tumors arising from endothelial cells. As vascular tumors, they can occur at different sites, with liver and spleen tending to be the most common sites in mice. In the high-dose mice with hemangiosarcomas, one had the tumors present in the liver and spleen, one had the tumor present in the liver only, one had the tumors present in the liver, spleen, and prostate, and one had the tumor present in the spleen only. A statistically significant trend was observed (p=0.00296). Closer examination of the incidence indicates a relatively flat response at the low- and mid-dose with only an increase observed at the high-dose; however, the incidence of hemangiosarcomas at the high-dose was not statistically significant when compared to the concurrent controls. Based on a weight-of-evidence for this study, the agency does not consider these increases in hemangiosarcomas in male mice to be treatment-related.

Table 4.14. Hemangiosarcomas in Male CD-1 Mice (Atkinson, 1993b) Cochran-Armitage Trend Test and Fisher's Exact Test Results.

Dose (mg/kg/day)	0	100	300	1000
Hemangiosarcoma				
Incidence	0/47[a]	0/46	0/50	4/45
(%)	(0)	(0)	(0)	(9)
Raw p-value =	0.003**	1.000	1.000	0.053
Sidak p-value =	--	1.000	1.000	0.053

Note: Trend test results denoted at control; * denotes significance at p=0.05; ** denotes significance at p=0.01
a= Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.

[24] Note: In Greim et al. (2015), the same study is cited as Cheminova (1993b).

4.6.4 Wood et al., 2009b (MRID 49957402)[25]

In a feeding study conducted in 2009, CD-1 mice (50/sex/dose) received glyphosate (95.7%) for 80 weeks at dietary dose levels of 0, 71.4/97.9, 234.2/299.5, or 810/1081.2 mg/kg/day for males and females, respectively. The highest dose tested in this study approaches or exceeds the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300).

There was no effect on survival in the study. In male mice at the high dose, there were increases in the incidences of lung adenocarcinomas and malignant lymphomas. A discussion of each tumor type is presented below:

1. Lung: Tumor incidence for lung adenomas, adenocarcinomas, and combined adenomas/adenocarcinomas are presented in Table 4.15. A statistically significant trend was only noted for the adenocarcinomas. Closer examination of the tumor incidence indicates the dose-response was relatively flat at the low- and mid-dose with only an increase observed at the high-dose; however, the incidence of lung adenocarcinomas at the high-dose (810 mg/kg/day) was not statistically significant when compared to the concurrent controls. There were no treatment-related preneoplastic or related non-neoplastic lesions observed. There was also no evidence of progression from adenomas to carcinomas. Based on a weight-of-evidence for this study, the agency does not consider these increases in lung tumors to be treatment-related.

2. Malignant lymphoma: Tumor incidence for malignant lymphoma are also presented in Table 4.16. A statistically significant trend was observed and the incidence at the high-dose (810 mg/kg/day) was statistically significantly elevated as compared to concurrent controls with the raw (unadjusted) p-value; however, with an adjustment for multiple comparisons, the increased incidence at the high-dose was not statistically significant (p= 0.082). Historical control data were also considered to better understand the significance of the reported increased incidence of lymphoma. Historical control data from the same laboratory and same supplier are preferred; however, this data were not available for consideration with the study report. The 2005 EPA Guidelines for Carcinogen Risk Assessment does not prohibit the use of historical control data from other sources; however, it does state it should be used with caution. For this strain of mouse, the mean incidence for untreated animals is approximately 4.5% (range: 1.5%-21.7%) based on historical control data from Charles River (59 studies performed from 1987-2000; Giknis and Clifford, 2005) and Huntingdon Laboratories (20 studies from 1990-2002; Son and Gopinath, 2004). Although the data are not from the performing laboratory, it does indicate that the incidence in concurrent controls in this study was low, which can contribute to the pairwise significance observed at the highest dose tested with the raw (unadjusted) p-value. Based on a weight-of-evidence for this study, the agency does not consider the increase in malignant lymphoma to be treatment-related.

[25] Note: In Greim et al. (2015), the same study is cited as NuFarm (2009a).

Table 4.15. Lung Tumors in Male CD-1 Mice (Wood et al., 2009b) Fisher's Exact Test and Cochran-Armitage Trend Test Results.				
Dose (mg/kg/day)	0	71.4	234.2	810
Lung Adenoma Incidence	9/51	7/51	9/51	4/51
(%)	(18)	(14)	(18)	(8)
Raw p-value =	-[b]	0.793	0.602	0.964
Sidak p-value =	-	0.991	0.937	1.000
Lung Adenocarcinoma	5/51[a]	5/51	7/51	11/51
(%)	(10)	(10)	(14)	(22)
Raw p-value =	0.028*	0.630	0.380	0.086
Sidak p-value =	--	0.949	0.762	0.237
Lung Combined Incidence	14/51	12/51	16/51	15/51
(%)	(27)	(24)	(31)	(29)
Raw p-value =	0.336	0.752	0.414	0.500
Sidak p-value =	--	0.985	0.799	0.875

Note: Trend test results denoted at control; * denotes significance at p=0.05;** denotes significance at p=0.01
a= Number of tumor bearing animals/Number of animals examined.
b = Trend p-value not reported since tumor incidence decreased with increasing dose.

Table 4.16. Malignant Lymphomas in Male CD-1 Mice (Wood et al., 2009b) Fisher's Exact Test and Cochran-Armitage Trend Test Results.				
Dose (mg/kg/day)	0	71.4	234.2	810
Malignant Lymphoma Incidence	0/51	1/51	2/51	5/51
(%)	(0)	(2)	(4)	(10)
Raw p-value =	0.007**	0.500	0.248	0.028*
Sidak p-value =	--	0.875	0.574	0.082

Note: Trend test results denoted at control; * denotes significance at p=0.05;** denotes significance at p=0.01
a= Number of tumor bearing animals/Number of animals examined.

4.6.5 Sugimoto, 1997 (MRID 50017108 - 50017109)[26]

In a carcinogenicity study, glyphosate (purity 97.56 and 94.61%; two lots) was administered to groups of 50 male and 50 female Specific-Pathogen-Free (SPF) ICR (Crj: CD-1) mice/dose in the diet at dose levels of 0, 165/153.2, 838.1/786.8, or 4348/4116 mg/kg/day for males and females, respectively, for 18 months. The highest dose tested in this study far exceeds the highest dose recommended in the test guidelines on how to conduct carcinogenicity studies (OCSPP 870.4200 and OCSPP 870.4300). Furthermore, the mid-dose tested in this study was approaching 1,000 mg/kg/day. Tumor findings at these high doses are given less weight.

There were no treatment-related effects on mortality or survival. There were no changes in histopathological findings observed.

[26]Note: In Greim et al. (2015), the same study is cited as Arysta Life Sciences (1997b)

Hemangiomas in female mice were found to occur at different sites. The tumor incidences are presented in Table 4.17. A statistically significant trend was observed. Tumor incidence at the high-dose, which was approximately 4 times the recommended high-dose in test guidelines (4116 mg/kg/day), was statistically significant with the raw (unadjusted) p-value as compared to concurrent controls; however, with an adjustment for multiple comparisons, the high dose tumors were not statistically significant (p=0.055). Based on a weight-of-evidence for this study, the agency does not consider these increases in hemangiomas in female rats to be treatment-related.

Table 4.17. Hemangioma Incidences (Sugimoto, 1997)
Fisher's Exact Test and Cochran-Armitage Trend Test Results

Tumor Type	0 mg/kg/day	153.2 mg/kg/day	786.8 mg/kg/day	4116 mg/kg/day
Hemangioma				
Incidence	0/50	0/50	2/50	5/50
(%)	(0)	(0)	(4)	(10)
Raw p-value =	0.002**	1.000	0.247	0.028*
Sidak p-value =	--	1.000	0.434	0.055

Note: Trend test results denoted at control; * denotes significance at p=0.05; ** denotes significance at p=0.01.

4.6.6 Pavkov and Turnier, 1987 (MRIDs 40214006, 41209907)

Glyphosate trimesium salt (sulfosate, 56.2% pure) was tested in a 2-year chronic feeding/carcinogenicity study in male and female CD-1 mice. Sixty animals/sex were tested in control group 1 (basal diet, no vehicle), 80/sex were tested in control group 2 (basal diet plus propylene glycol at 1% w/w vehicle) and in the low- and mid-dose groups, and 90/sex were tested in the high-dose group. The following dose levels were tested: 0, 11.7/16, 118/159, and 991/1341 mg/kg/day for males and females, respectively.

No adverse effects on survival were seen in either sex across the doses tested. There were no changes in histopathological findings observed. There were no treatment-related increases in tumor incidence observed in the study.

4.6.7 Summary of Mouse Data

No tumors were identified for detailed evaluation in 2 of the 6 mouse carcinogenicity studies. In the remaining 4 mouse studies, 3 observed a statistically significant trend in tumor incidences in the hemangiosarcomas, lung adenomas, malignant lymphomas or hemangiomas; however, the agency determined that none of the tumors observed in the mouse are treatment related. Although a statistically significant trend was obtained, closer examination of the incidence data across doses did not demonstrate a monotonic dose response in several instances. Some of the tumor incidences at the highest dose tested (approaching or exceeding 1,000 mg/kg/day for almost all studies) were statistically significant from concurrent controls using raw (unadjusted) p-values; however, none of the pairwise comparisons were found to be statistically significant following adjustment for multiple comparisons. Furthermore, these high-dose tumors were given less weight. There was no evidence of corroborating pre-neoplastic or related non-neoplastic lesions or evidence of tumor progression (progression from pre-neoplastic to malignancy) to support

biological significance of tumor findings. In a limited number of cases, the agency considered historical control data to inform the relevance of a tumor increase when incidence rates in the concurrent controls were unusually low.

Table 4.18. Summary of Mouse Carcinogenicity Studies

Study	Dose Range	Pre-Neoplastic or Related Non-Neoplastic Lesions	Tumors Incidences, Statistical Significance, and Related Comments
Reyna and Gordon (1973) Swiss white mice	0, 17 or 50 mg/kg/day for 18 months	None observed	There were no treatment-related increases in tumor incidence.
Knezevich and Hogan (1983) CD-1 mice	99.78% Technical in diet 0, 161/195, 835/968, 4945/6069 mg/kg/day for [M/F] for 24 months.	Chronic interstitial nephritis lacked dose-response and not considered relevant to renal tumors. Tubular epithelial changes in kidney were approximately the same in controls, low- and mid-doses and then decreased at high-dose.	The incidences of renal tubule adenomas were: 1/49 (2%) in the controls; 0/49 at the low-dose; 1/50 at the mid-dose; and 3/50 (6%) at the high dose. No statistical significance in trend or pairwise comparisons, including the mid- and high-doses which approached or exceeded 1,000 mg/kg/day.
Atkinson et al. (1993b). CD-1 mice	97.5 - 100.2% Technical in diet 0, 98/102, 297/298, 988/1000 mg/kg/day for 104 weeks (M/F)	None observed	Statistically significant trend for hemangiosarcomas that were only observed in 4/45 (9%) high-dose male mice. Increased incidence was not statistically significant from the concurrent controls at all doses, including the highest dose tested which is approximately 1,000 mg/kg/day.
Wood et al. (2009b) CD-1 mice	95.7% Technical in diet 0, 71.4/97.9, 234.2/299.5, or 810/1081.2 mg/kg/day [M/F] for 80 weeks	None observed	Statistically significant trend for lung adenocarcinomas with incidences of 5/51 in controls, 5/51 at the low-dose, 7/51 at the mid-dose, and 11/51 at the high-dose. No statistical significance in pairwise comparisons. Statistically significant trend for malignant lymphoma with incidences of 0/51 in controls, 1/51 at the low-dose, 2/51 at the mid-dose, and 5/51 at the high-dose. Incidence in concurrent controls for this tumor type was low. No statistically significant pairwise results with multiple comparison adjustment, including the highest dose tested which was approaching 1,000 mg/kg/day.
Sugimoto (1997) CD-1 mice	94.61 – 97.56% Technical in diet 0, 165/153.2, 838.1/786.8, or 4348/4116 mg/kg/day [M/F] for 18 months	None observed	Statistically significant trend for hemangiomas female mice with incidences of 0/50 in controls, 0/50 at the low-dose, 2/50 at the mid-dose, and 5/50 at the high-dose. No statistically significant pairwise results with multiple comparison adjustment, including the mid- and high-doses which approached or exceeded 1,000 mg/kg/day.

Table 4.18. Summary of Mouse Carcinogenicity Studies

Study	Dose Range	Pre-Neoplastic or Related Non-Neoplastic Lesions	Tumors Incidences, Statistical Significance, and Related Comments
Pavkov and Turnier (1987) CD-1 mice	56.2% Technical (Trimesium salt; Sulfosate) 0, 11.7/16, 118/159, and 991/1341 mg/kg/day [M/F] for 24 months.	None observed	There were no treatment-related increases in tumor incidence, including the highest dose tested which approached/exceeded 1,000 mg/kg/day.

4.7 Absorption, Distribution, Metabolism, Excretion (ADME)

The 2005 EPA Guidelines for Carcinogen Risk Assessment also permit analysis of other key data that may provide valuable insights into the likelihood of human cancer risk from exposure to a chemical, such as information regarding the absorption, distribution, metabolism, and excretion (ADME) of a test chemical. EPA's Harmonized Test Guidelines for pesticides include a series of studies for characterizing a chemical's metabolism and pharmacokinetics. As described in the test guideline (OCSPP 870.7485), testing of the disposition of a test substance is designed to obtain adequate information on its: absorption, distribution, biotransformation (metabolism), and excretion, which can all collectively aid in understanding the chemical's mechanism of toxicity. Basic pharmacokinetic/toxicokinetic parameters determined from these studies can also provide information on the potential for accumulation of the test substance in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance. These data can be used to assess the adequacy and relevance of the extrapolation of animal toxicity data (particularly chronic toxicity and/or carcinogenicity data) to estimate human risk.

Oral exposure is considered the primary route of concern for glyphosate. The maximum absorption from the GI tract for glyphosate was estimated to be ~30% with one study showing up to 40% based upon radiolabel detected in the urine. In general, the amounts of glyphosate detected in tissues were negligible indicating low tissue retention following dosing. Parent glyphosate is the principal form excreted in urine and feces. The primary route of excretion following oral administration of glyphosate is the feces, as verified by the intravenous dosing and bile cannulation experiments. Within the dose ranges tested, elimination was essentially complete by 24 hours indicating that glyphosate does not bioaccumulate.

Multiple studies examined the pharmacokinetics of a single dose of radiolabeled glyphosate ranging from 5.6 – 400 mg/kg. Across these studies, time to reach peak plasma concentrations (T_{max}) appeared to increase with increasing dose; however, the reported range of T_{max} (1-5.5 hours) suggests only a slight shift in absorption kinetics occurs despite large increases in dose. In the one study that tested two doses (NTP, 1992), data graphically show that peak blood levels were only roughly 3-fold with a 10-fold increase between the two doses. Reported area under the curve (AUC) values indicated conflicting results regarding whether linear or non-linear absorption kinetics was occurring at higher doses.

In general, EPA and OECD guideline ADME studies are designed for a different purpose and do not provide the information needed to adequately determine whether linear kinetics is still occurring at high doses of glyphosate. These studies are often limited to one or two doses and do not include time course data. A well-conducted pharmacokinetic study testing multiple doses is needed to conclusively make this determination.

4.8 Discussion

Glyphosate has been extensively tested in rodents to evaluate its carcinogenic potential. A total of 15 rodent carcinogenicity studies were considered to be adequate for this analysis. Nine studies were conducted in the rat and 6 studies were conducted in the mouse. When a potential tumor signal was identified in a study, the agency considered several factors. Consistent with the EPA's 2005 Guidelines for Carcinogen Risk Assessment, the agency evaluated the tumor responses for both statistical and biological significance by considering factors such as historical control data; rarity of tumor types; tumors at multiple sites; tumors in multiple species, strains, or both sexes; progression of lesions from preneoplastic to benign to malignant; reduced latency of neoplastic lesions (i.e., time to tumor); presence of metastases; unusual magnitude of tumor response; proportion of malignant tumors; and dose-related increases. When these factors were considered together, the agency made a determination of whether or not the observed tumor was related to treatment with glyphosate. A weight of the evidence approach was used to determine the carcinogenic potential of glyphosate in rodents.

In 5 of the 9 rat studies conducted with glyphosate, no tumors were identified for detailed evaluation. Of the remaining 4 rat studies, a statistically significant trend was observed for tumor incidences in the testes, pancreas, liver, thyroid, or mammary gland; however, the agency determined that these tumor findings are not considered to be related to treatment, as described in Section 4.5, due to lack of pairwise statistical significance, lack of a monotonic dose response, absence of preneoplastic or non-neoplastic lesions, no evidence of tumor progression, and/or historical control information (in limited instances). Lastly, tumors seen in individual rat studies were not reproduced in other studies, including those conducted in the same animal species and strain at similar or higher doses.

In 2 of the 6 mouse studies, no tumors were identified for detailed evaluation. In the remaining 4 mouse studies, 3 observed a statistically significant trend in tumor incidences in the hemangiosarcomas, lung adenomas, malignant lymphomas or hemangiomas; however, the agency determined that none of the tumors observed in the mouse are treatment related, as described in Section 4.6, due to lack of pairwise statistical significance, lack of a monotonic dose response, absence of preneoplastic or non-neoplastic lesions, no evidence of tumor progression, and/or historical control information (in limited instances). Lastly, tumors seen in individual mouse studies were not reproduced in other studies, including those conducted in the same animal species and strain at similar or higher doses.

In addition to the lines of evidence considered when determining if a tumor was treatment-related within in a study, the agency also looked across all of the relevant studies to determine if the tumor findings were reproducible in other studies conducted in the same species and strain. Increased incidence of testicular, pancreatic, thyroid and mammary gland tumors were seen in only one study and were not reproduced in the other four studies for that strain at similar or higher doses. An increased incidence of hepatocellular adenomas were seen in one study with Sprague-Dawley rats and one study with Wistar rats, but this tumor type was not significantly

increased in the other six studies tested in these rat strains at similar or higher doses. In the mice, an increase in the incidence of renal tumors, hemangiosarcomas, lung adenomas, malignant lymphoma and hemangiomas were reported only in a single study and findings were not seen in the four other studies conducted in CD-1 mice at similar or higher doses.

When looking across the studies at doses where potential tumor signals were identified, doses below 500 mg/kg/day consistently showed no increased incidence of tumors with the single exception of the testicular tumors in SD rats (Lankas, 1981), where an increase in incidence was seen at approximately 31.5 mg/kg/day. However, as discussed in Section 4.5.2, the testicular tumor data do not show a monotonic dose response, the concurrent controls appear to be unusually low for this tumor, there were no pre-neoplastic or related non-neoplastic lesions, and this tumor type was not seen in other studies at doses up to 35-fold higher in the same strain of rat. As a result, the increased incidence in testicular tumors was not considered treatment-related based on the weight-of-evidence for the study. Even if the tumor findings observed above 500 mg/kg/day were considered indicative of treatment-related effects, the 2005 EPA Guidelines for Carcinogen Risk Assessment state that the "weighing of the evidence includes addressing not only the likelihood of human carcinogenic effects of the agent but also the conditions under which such effects may be expressed". As such, the high doses (~1,000 mg/kg/day or greater) where these tumor findings were observed were considered in the context of potential exposure to glyphosate in residential and occupational settings. As previously discussed in Section 1.4, oral exposure is the primary route of concern for glyphosate. In residential/non-occupational settings, children 1-2 years old are considered the most highly exposed subpopulation with an estimate of potential combined exposure of 0.47 mg/kg/day. The estimated maximum potential exposure for occupational workers is 7 mg/kg/day. The estimate of exposure children and occupational workers is at least 2,000-fold and 140-fold lower, respectively, than the doses (~1000 mg/kg/day) where increases in tumor incidences were typically observed in the rodent studies. Based on these exposure estimates, the high dose tumor findings are not considered relevant for human health risk assessment.

Based on the weight-of-evidence, the agency has determined that any tumor findings observed in the rat and mouse carcinogenicity studies for glyphosate are not considered treatment-related. Tumor findings observed at the highest doses tested were also not reproduced in studies in the same animal strain at similar or higher doses. Furthermore, even if the high-dose tumors were considered treatment-related, these findings are not considered relevant for human health risk assessment based on the use pattern and potential exposures for glyphosate.

5.0 Data Evaluation of Genetic Toxicity

5.1 Introduction

Genotoxicity is a broad term for any damage to the genetic material, whether the damage is transient or permanent. Transient damage refers to unintended modifications to the structure of DNA, which may or may not undergo successful repair. Permanent damage refers to heritable changes in the DNA sequence, known as mutations. Types of mutations include: 1) changes in single base pairs, partial, single or multiple genes, or chromosomes, 2) breaks in chromosomes that result in transmissible deletion, duplication or rearrangement of chromosome segments, and 3) mitotic recombination (OECD, 2015). In somatic cells, DNA-reactive chemicals can cause cancer if the mutations occur within regulatory genes that control cell growth, cell division and differentiation, such as proto-oncogenes, tumor suppressor genes and/or DNA damage response genes (OECD, 2015). Additionally, DNA damage may signal the cell to undergo apoptosis (cell death) rather than cell division and, therefore, the damage is not "fixed" as a mutation and is not passed along to daughter cells.

Evaluation of genotoxicity data entails a weight-of-evidence approach that includes consideration of the various types of genetic damage that can occur. Since no single genotoxicity assay evaluates the many types of genetic alterations that can be induced by a chemical, one must employ a battery of genotoxicity tests to adequately cover all the genetic endpoints important for regulatory decisions. EPA, like other regulatory agencies, considers genotoxicity information as part of the weight of evidence when assessing the potential of a chemical to induce cancer in humans. Under FIFRA, OPP requires genotoxicity tests of the technical grade active ingredient for the registration of both food and non-food use pesticides. The current genotoxicity test battery (40 CFR Part 158.500) for pesticide registration consists of:

> 1) Bacterial reverse mutation test (typically conducted in bacteria strains *Salmonella typhimurium* and *Escherichia coli*),
> 2) *in vitro* mammalian (forward) gene mutation *and in vitro* mammalian chromosomal aberration test, and
> 3) *in vivo* test for micronucleus induction (mammalian erythrocyte micronucleus test) *or in vivo* chromosomal aberration test (mammalian bone marrow chromosomal aberration test).

In cases where equivocal or inconsistent results are obtained for the same endpoint in different test systems, additional testing may be required. Test Guidelines on how to conduct the genotoxicity tests have been published by the agency and have been harmonized with the Organization for Economic Cooperation and Development (OECD, 2015; Cimino 2006). These guidelines identify specific test species, genetic endpoints, test conditions, exposure durations as well information on how to report data and interpret the results. The test guidelines provide a level of consistency and predictability for regulatory compliance and regulatory decision making.

5.2 Scope of the Assessment Considerations for Study Quality Evaluation

Previous genotoxicity assessments conducted as part of the CARC reviews for glyphosate in 1991 and 2015, considered only studies conducted with glyphosate technical and included only studies that provided adequate characterization of the test material (*i.e*. purity information provided). In the current analysis, a fit-for-purpose systematic review process was conducted to identify relevant genotoxicity data from regulatory studies and published literature from open sources (published and unpublished) for both glyphosate technical and glyphosate-based formulations. Studies conducted with glyphosate formulations that were identified and considered relevant for genotoxicity evaluation are summarized in table form in Appendix F. As described in Section 7.0 of this document, glyphosate formulations are hypothesized to be more toxic than glyphosate alone. The agency is collaborating with NTP to systematically investigate the mechanism(s) of toxicity for glyphosate and glyphosate formulations. However the focus of this section is the genotoxic potential of glyphosate technical.

As described previously in Section 2.1.3, the list of studies identified in this process were also cross-referenced with genotoxicity review articles for glyphosate from the open literature [Kier and Kirkland (2013), and Williams *et al.* (2000)], as well as recent international evaluations of glyphosate (IARC 2015, EFSA 2015, JMPR 2016). The current analysis also includes studies conducted by other registrants that were not previously available to the agency. Sixteen studies for glyphosate technical that were included in Kier and Kirkland (2013) were not available to the agency; therefore, data and study summaries provided in the review articles were relied upon in the current review and are identified in the data tables with a footnote. The Kier and Kirkland (2013) article serves as the original publication for these studies and provided relevant information on study design and conditions as well as summary data. The data set includes *in vitro* and *in vivo* studies conducted in mammalian systems, with the exception of standard bacterial test strains, which have a long history of detecting chemicals that are mutagenic in humans. Studies conducted in non-mammalian species (e.g. worms, fish, reptiles, plants), were excluded because they were considered to be not relevant for informing genotoxic risk in humans.

When evaluating the quality of the published and unpublished data for inclusion in the analysis, the agency considered the reporting quality (how well a study was reported), the study design and how well the study was conducted. Critical elements in study design and interpretation for genotoxicity tests are described in the various EPA and OECD test guidelines. Elements such as test conditions (e.g. solubility, pH, osmolarity, and cytotoxicity) and study design (e.g. number of test organisms, doses selected, use of positive and negative controls; blinded evaluation) were used to evaluate the quality of published and non-published studies. In cases where inappropriate testing conditions or study design clearly had an impact on the outcome the study, the study was excluded from the analysis. For example, early studies by Majeska (1982) were excluded from the analysis since it was clearly demonstrated that altered pH by the test chemical can result in false positive responses in several of *in vitro* genotoxicity tests (Majeska, 1985d,e,f). In other cases, particularly with the published literature studies, where test conditions and/or study design differed from what is generally considered as acceptable following in the EPA or OECD guidelines, the differences are noted, but the studies were not excluded from analysis unless the condition made the study unreliable. Summaries of relevant genotoxicity studies can be found in TXR# 0057499. Studies that were excluded from the analysis are listed in Appendix G.

The studies evaluating the genetic toxicity of the active ingredient glyphosate are presented in the following sections according to the type of genetic endpoints evaluated: mutations, chromosomal aberrations and other assays evaluating DNA damage. *In vitro* and *in vivo* assays are discussed separately according to the genetic endpoint. For the purpose of this analysis, glyphosate and its salts are considered together when evaluating the genotoxic potential of the active ingredient glyphosate.

5.3 Tests for Gene Mutations for Glyphosate Technical

5.3.1 Bacterial Mutagenicity Assays

Bacteria have traditionally been employed as a primary test organism for the detection of chemical mutagens. The bacterial reverse mutation assay is routinely performed in the test strains of *Salmonella typhimurium* and *Escherichia coli.* These test strains are mutant strains that are deficient for the synthesis of an essential amino acid. The assay detects mutations that revert the test strains back to wild type for amino acid synthesis and the revertants are identified by their ability to grow in culture medium deficient of the specific amino acid(s). This mutagenicity test identifies point mutations, which includes base substitutions and deletions and insertions of up to a few base pairs (OECD 471). The tests are typically conducted in the presence and absence of an exogenous source of metabolic activation (e.g., S9 microsomal fraction of activated liver homogenates) to identify potential mutagenic metabolites.

Glyphosate has been extensively evaluated for its potential to induce mutations in bacteria. Most of the studies considered consist of the full battery of bacterial strains (*i.e.* the recommend strains in EPA and OECD Test Guidelines) and were evaluated at appropriate test concentrations (up to cytotoxic or assay limit concentrations).

EPA identified 27 studies that tested glyphosate technical in bacterial mutagenicity assays by means of the standard plate incorporation method or the pre-incubation modification of the standard assay. Glyphosate was negative in the presence and absence of metabolic activation in all the studies. The results of the bacterial reversion mutation assays evaluating glyphosate technical are presented in Table 5.1

Table 5.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Technical.						
Test/Endpoint	**Test System**	**Concentrations**	**Purity**	**Results**	**Reference**	**Comments**
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA98 and TA100 and WP *uvrA* ± S9	156-5000 µg/plate	95.68%	Negative ± S9	Akanuma (1995) [MRID 50017102]	
Bacterial Reverse Mutation	*S. typhimurium* TA535, TA1537, TA98 and TA100 and *E. coli* WP2P and WP2P *uvrA* ± S9	100-5000 µg/plate in DMSO	95.6% glyphosate acid	Negative ± S9	Callander (1996) [MRID 44320617]	
Bacterial Reverse Mutation	*S. typhimurium* TA 1535, TA1537, TA98 and TA100 and *E. coli* WP2P and WP2P *uvrA* ± S9	100-5000 µg/plate in water	60% potassium glyphosate salt	Negative ± S9	Callander (1999)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA97a, TA98, TA100 and TA102, ± S9	25-2000 µg in aqueous solution	Not provided	Negative ± S9	Chruscielska *et al.* (2000)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 ± S9	10-1000 µg/plate	98.4%	Negative ± S9	Flowers and Kier (1978) [MRID 00078620]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 ± S9	31.6-3160 µg/plate	98.8%	Negative ± S9	Flügge (2009a)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 ± S9	31.6-3160 µg/plate	96.4% technical	Negative ± S9	Flügge (2010b)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA98 and TA100	310-5000 µg/plate (+S9); 160-2500 µg/plate (−S9)	98.6%	Negative ± S9	Jensen (1991a) [MRID 49961502]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 ± S9	1-1000 µg/plate	98.05%	Negative ± S9	Miyaji (2008)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 ± S9	5000 µg/plate	Not reported	Negative ± S9	Moriya et al. (1983)	
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA97, TA98 and TA100 ± S9	33-10,000 µg/plate	99%	Negative ± S9	NTP (1992)	Hamster and rat S9

Table 5.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Technical.						
Test/Endpoint	**Test System**	**Concentrations**	**Purity**	**Results**	**Reference**	**Comments**
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535 and TA97a ± S9	1-5000 µg/plate	61.27 % Glyphosate isopropyl-amine salt	Negative ± S9	Ranzani (2000)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 ± S9	648-5000 µg/plate	98.01%	Negative ± S9	Ribeiro do Val (2007) [MRID 50000903]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. Coli* WP2 *uvrA* ± S9	31.6-5000 µg/plate	96.0% technical	Negative ± S9	Schreib (2010)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98, TA100 and *E. coli* WP2 *hcr* ± S9	10-5000 µg/plate	98.4%	Negative ± S9	Shirasu *et al*. (1978) [MRID 00078619]	Published in Li & Long, 1988
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP *uvrA* ± S9	3-5000 µg/plate (plate-incorporation), 33-5000 µg/plate (pre-incubation test)	95.1%	Negative ± S9	Sokolowski (2007a) [MRID 49957406]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP *uvrA* ± S9	3-5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test)	97.7%	Negative ± S9	Sokolowski (2007b) [MRID 49957407]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP *uvrA* ± S9	3-5000 µg/plate (plate–incorporation) 33-5000 µg/plate (pre-incubation test)	95.0%	Negative ± S9	Sokolowski (2007c) [MRID 49957408]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP *uvrA* ± S9	3-5000 µg/plate	96.66% technical	Negative ± S9	Sokolowski (2009a)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* pKM 101 and WP2 pKM 101 ± S9	3-5000 µg/plate	96.3% glyphosate acid	Negative ± S9	Sokolowski (2009b) [MRID 49961801]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP *uvrA* ± S9	3-5000 µg/plate	97.16 %	Negative ± S9	Sokolowski (2010) [MRID 50000902]	

Table 5.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Technical.						
Test/Endpoint	**Test System**	**Concentrations**	**Purity**	**Results**	**Reference**	**Comments**
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 ± S9	1-1000 µg/plate	96.0%	Negative ± S9	Suresh (1993a)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP *uvrA* ± S9	0-5000 µg/plate	95.3%	Negative ± S9	Thompson (1996) [MRID 49957409]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 ± S9	31.6-5000 µg/plate	98.2%	Negative ± S9	Wallner (2010)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98 and TA100 ± S9	25 µg/plate	Not reported	Negative ± S9	Wilderman and Nazar (1982)	Rat S9 and plant cell-free homogenates were used for metabolic activation
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 ± S9	0.12-10 mg/plate –S9 0.56-15 mg/plate +S9	90% glyphosate trimesium salt	Negative ± S9	Majeska et al. (1982a) [MRID 00126612]	
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA98 and TA100 ± S9	0.005-50 µL/mL	55.6% glyphosate trimesium salt	Negative ± S9	Majeska (1985a) [MRID 00155527]	

[1] Study was cited in Kier and Kirkland (2013). Supplementary information about the study was provided online including test guideline, test material purity, control chemicals and summary data tables.

5.3.2 *In vitro* Tests for Gene Mutations in Mammalian Cells

In vitro gene mutation studies in mammalian cells are conducted in cell lines with reporter genes for forward mutations. The most common reporter genes are the endogenous thymidine kinase (TK) gene, endogenous hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene and the xanthine-guanine phosphoribosyl transferase transgene (XPRT). Mutations that occur within these reporter genes result in mutant cells that are resistant to the cytotoxic effect of the pyrimidine analogue trifluorothymidine (for TK) or the purine analogue 6-thioguanine (for HPRT and XPRT) (OPPTS 870.5330). Suitable cell lines for this assay include L5178Y mouse lymphoma cells, Chinese hamster ovary (CHO) cells, hamster AS52 and V79 lung fibroblasts and human TK6 lymphoblastoid cells. Similar to other *in vitro* assays, chemicals are tested both in the presence and absence of S9 metabolic activation.

A total of four studies were conducted for (forward) mutations in mammalian cells (Table 5.3). Three studies were conducted with a high purity concentration of glyphosate technical (≥95.6%) and the remaining study was performed with glyphosate trimesium salt. In four of the assays, mouse lymphoma L5178Y $TK^{+/-}$ cells were the target organism and one was conducted in CHO cells with the HPRT endpoint. Glyphosate technical and the glyphosate trimesium salt were negative in the mouse lymphoma cell assays (Jensen, 1991b; Clay, 1996; Majesak, 1985b) when tested up to the current guideline limit concentration and glyphosate was negative in CHO/HPRT cells when tested up to cytotoxic concentrations (Li, 1983a).

Table 5.2. *In vitro* Mammalian Gene Mutation Assays: Glyphosate Technical.

Test/Endpoint	Test System	Concentrations/ Conditions	Test Material Purity	Results	Reference	Comments
Gene Mutations in Mammalian Cells	Mouse lymphoma L5178Y $TK^{+/-}$ cells ± S9	296-1000 µg/mL	95.6%	Negative	Clay (1996)[1]	Relative survival was 90% (-S9) and 57% (+S9) at top concentration
Gene Mutations in Mammalian Cells	Mouse lymphoma L5178Y $TK^{+/-}$ cells ± S9	520–4200 µg/mL (+S9); 610–5000 µg/mL (-S9)	98.6%	Negative	Jensen (1991b) [MRID 49961504]	Reported no significant reduction in cloning efficiency at any concentration.
Gene Mutations in Mammalian Cells	Chinese hamster ovary (CHO) cells, HPRT locus ± S9	500–25000 µg/mL (+S9); 500-22500 µg/mL (-S9)	98.7%	Negative	Li (1983a); [MRID 00132681]	Tested S9 from 1-10% Cytotoxic at 22.5 mg/mL (-S9, and with 1,2 and 10% S9) and at 17.5 mg/ml (10% S9)
Gene Mutations in Mammalian Cells	Mouse lymphoma L5178Y $TK^{+/-}$ cells ± S9	1-5 µl/mL	55.6% *Glyphosate trimesium salt*	Negative	Majeska (1985b) [MRID 00155530]	Negative with pH adjusted

[1] Study was cited in Kier and Kirkland (2013). Supplementary information about the study was provided online including test guideline, test material purity, control chemicals and summary data tables.

5.4 *In vitro* Tests for Chromosomal Abnormalities

Cytogenetic assays are tests that can detect chemicals that cause structural chromosomal damage (clastogenicity) or affect the segregation of chromosomes during cell division and alter chromosome number (aneuploidy). Generally, there are two types of *in vitro* cytogenetic assays that identify chemicals inducing chromosomal abnormalities: chromosomal aberration assays and micronucleus assays. Although chromosomal damage observed in these assays are not considered heritable mutations, chemicals that can induce these types of chromosomal damage can also induce transmissible mutations to daughter cells indicating their role in cancer (Yauk *et al.*, 2015; OECD 2015). In addition, assays such as (fluorescence *in situ* hybridization (FISH)) can provide additional mechanistic information on the formation of chromosomal abnormalities. It is important to note that factors such as cytotoxicity, solubility of the test substance, changes in pH or osmolality play a significant role in the outcome of the assay. Like other *in vitro* assays, compounds are generally tested in the presence or absence of S9 metabolic activation to determine if metabolism affects the genotoxic activity of the parent compound and to determine if potential genotoxic metabolites are formed.

5.4.1 *In vitro* Mammalian Chromosomal Aberration Test

Chromosomal aberration assays detect both structural chromosomal and numerical aberrations. Structural chromosomal aberrations are of two types: chromatid and chromosome and include breaks, deletions and rearrangements (OPPTS 870.5375, OECD 2015). Numerical chromosomal aberrations generally results from the loss of an entire chromosome mostly due to damage in the spindle fiber resulting in aneuploidy. The types of cells that are most commonly used in chromosomal aberration assays include established cell lines such as Chinese hamster lung (CHL) and CHO cells or primary cell cultures such as human or other mammalian peripheral blood lymphocytes. In this assay, cells are typically sampled at a time equivalent to the length of approximately 1.5 cell cycles from the start of treatment. Prior to harvesting, cells are treated with Colcemid® or colchicine to arrest cells at the first metaphase stage of the cell cycle following the beginning of exposure to the test article. Once harvested, the cells are stained and metaphase cells are evaluated microscopically for various types of chromosome aberrations. (OECD TG 473). Data should be presented in a way that indicates the percentage of affected cells in the population of cells scored (e.g., % cells with aberrations or # aberrant cells/100 cells). Gaps should not be included in the analysis; they are scored but gaps alone in the absence of any additional chromosomal aberrations (e.g., a fragment or a ring chromosome) are not sufficient to define a cell as aberrant.

Glyphosate technical was evaluated in eight chromosomal aberrations tests to determine its potential to induce clastogenic effects *in vitro*. The findings are presented in Table 5.3. Six of the eight studies were negative. The two positive studies were both from the same laboratory where, Lioi *et al*. reported an increase in chromosomal aberrations at glyphosate concentrations of 8.5μM and above in bovine lymphocytes (Lioi et al., 1998b) and at all concentrations of glyphosate tested (7-170 μM) in human lymphocytes (Lioi et al., 1998a) following a 72-hour exposure period. No chromosomal aberrations were observed as a result of exposure to glyphosate in one study using CHO cells (Majeska, 1985c) and in two studies with CHL cells

(Matsumoto, 1995; and Wright, 1996). Sivikova and Dianovsky (2006) reported no statistically significant increases in chromosomal aberrations in bovine lymphocytes treated with glyphosate (62% pure) at concentrations up 1120 μM following 24-hour exposure. (Sivikova and Dianovsky, 2006). In studies conducted with human lymphocytes treated with glyphosate (≥95%) for 24-96 hours at concentrations, no increase in chromosomal aberrations were seen at concentrations as high as 6000 μM (Fox, 1998; and Manas *et al*., 2009).

5.4.2 *In vitro* Mammalian Micronucleus Test

The *in vitro* micronucleus test can detect the induction of micronuclei in the cytoplasm of cells in the interphase stage of the cell cycle. Micronuclei form from acentric chromosome fragments (i.e., chromosome fragments lacking a centromere) or when whole chromosomes are unable to migrate to the cellular poles during anaphase prior to cell division. (OECD 487). Thus, the micronucleus assay can detect both structural and numerical chromosomal changes. It should be noted, however, that additional work is required to distinguish whether induced micronuclei have arisen from a clastogenic versus an aneugenic mechanism, *e.g*., staining micronuclei to detect the presence of kinetochore proteins. The assay is typically performed with cell lines or primary cell cultures of human or rodent origin. The assay can be conducted with the addition of cytochalasin B which inhibits cytokinesis resulting in the formation of binucleated cells. The presence of binucleated cells, indicates that cells have undergone one round of mitosis, a necessary prerequisite for micronucleus formation.

Six studies evaluated glyphosate technical for its potential to induce micronuclei *in vitro* (Table 5.4). Four of the six studies were positive and the remaining two studies were equivocal. In a study by Koller *et al*. (2012), TR146 cells (derived from a human neck metastasis of buccal epithelial origin) were treated for 20 minutes with up to 20 mg/L (~0.12 mM) glyphosate (95%), the authors reported a statistically significant increase in binucleated cells with micronuclei at 15 (~0.09 mM) and 20 (~0.12 mM) mg/L, and also indicated significant apoptosis and necrosis at 20 mg/L. The short exposure period in this study was unusually short (20 minutes) and was conducted in a tumor cell line that had not been well characterized in regards to its degree of chromosomal instability and DNA damage and repair capacity. In another study, Roustan *et al*. (2014) reported positive findings +S9 only in CHO cells treated with glyphosate (unknown purity) at 10- 100 μg/mL with little evidence of a dose response over that concentration range.

Two other studies evaluated glyphosate technical in human lymphocytes (Mladinic *et al*., 2009a, 2009b). These studies used an exposure protocol that is different from the OECD recommendations for the *in vitro* micronucleus assay. OECD recommends that whole blood or isolated lymphocytes are cultured in the presence of a mitogen (e.g. phytohemagglutinin; PHA) prior to exposure of a test chemical in order to detect micronuclei formed via an aneugenic mechanism. However, in these two studies, blood cells were exposed to glyphosate for 4 hours, washed, and then treated with PHA to stimulate cell division. Both studies reported a statistically significant increase in micronucleated cells at 580 μg/mL (~3.4 mM), but not at lower concentrations, following 4-hour exposures in the presence of S9. The frequency of micronucleated cells (+S9) ranged from 11.3 to 28.7 in one study (Mladinic *et al*., 2009a) and 33.3 to 65.2 in the other study (Mladinic *et al*., 2009b) over the 1000-fold concentration range. No statistically significant increases in micronucleated cells were seen in either study in the absence of S9 activation. When cells were evaluated with vital stains, cells treated with 580

μg/mL showed a significant ($p<0.05$) increase in the percentage of cells undergoing apoptosis and necrosis compared to the negative controls.

Piesova et al. (2004, 2005) conducted two *in vitro* micronucleus studies using glyphosate technical (62%) up to 560 uM in bovine lymphocytes. In the 2004 study, bovine lymphocytes from two donors were treated for 24 or 48 hours without S9 metabolic activation, and for 2 hours (with and without S9 activation) or 48 hours (-S9) in the 2005 study. Both studies yielded similar results following 48-hour exposure to glyphosate. In both cases, the authors reported a weak induction of micronuclei in one donor at 280 μM and at 560 μM in the second donor. The induction was approximately 2-fold ($p < 0.05$), but with no clear dose response. No effects on micronuclei induction were seen at the 2- or 24-hour time points; however, with these early time points it is unlikely that one cell division has occurred during or after treatment. .

Table 5.3. *In vitro* Tests for Chromosome Aberrations in Mammalian Cells- Glyphosate Technical						
Test/Endpoint	**Test System**	**Concentrations/ Conditions**	**Test Material Purity**	**Results**	**Reference**	**Comments**
In vitro Chromosomal Aberration	Chinese hamster ovary (CHO) cells	4-10 µl/mL, ± S9	55.6% *Glyphosate trimesium salt*	Negative	Majeska (1985c) [MRID 00155530]	pH adjusted (7.4-7.6)
In vitro Chromosomal Aberration	Chinese Hamster lung (CHL) cells	±S9: 0, 250, 500, 1000 and 2000 µg/mL; 24 and 48 h treatment - S9; 6 h treatment ±S9 harvest 24 h	95.68%	Negative	Matsumoto (1995) [MRID 50017106]	Decline in pH noted at 500 and 1000 µg/mL.
In vitro Chromosomal Aberration	Chinese hamster lung (CHL) cells	-S9: 24 & 48-hr exposure: 0-1250 µg/mL; +S9: 0-1250 µg/mL	95.3%	Negative	Wright (1996) [MRID 49957410]	Excessive decrease in pH >1250 µg/mL
In vitro Chromosomal Aberration	Bovine lymphocytes	-S9 only: 0, 7, 85 and 170 µM; 72 h exposure	≥98%	Positive (all concs.)	Lioi *et al.* (1998b)	
In vitro Chromosomal Aberration	Bovine lymphocytes	±S9: 0, 28, 56, 140, 280, 560 and 1120 µM; 24 h exposure	62.0%	Negative	Sivikova and Dianovsky (2006)	Decreased MI and PI at ≥ 560 µM
In vitro Chromosomal Aberration	Human lymphocytes	±S9: 100-1250 µg/mL cultures analyzed; 68 & 92 h	95.6%	Negative	Fox (1998) [MRID 49961803]	Excessive decrease in pH >1250 µg/mL
In vitro Chromosomal Aberration	Human lymphocytes	-S9 only: 0, 5.0, 8.5, 17.0 and 51.1 µM; 72 h exposure	≥98%	Positive ≥ 8.5 µM	Lioi *et al*. (1998a)	No significant ↓ in MI observed.
In vitro Chromosomal Aberration	Human lymphocytes	-S9: 0, 200, 1200 and 6000 µM; 48 h exposure	96.0%	Negative	Manas *et al*. (2009)	No toxicity observed up to 6000 µM

[1] Study was cited in Kier and Kirkland (2013). Supplementary information about the study was provided online including test guideline, test material purity, control chemicals and summary data tables.

CA= chromosomal aberrations, MI= mitotic index, PI= proliferation index.

Table 5.4. *In vitro* Tests for Micronuclei Induction in Mammalian Cells- Glyphosate Technical						
Test/Endpoint	**Test System**	**Concentrations/ Conditions**	**Test Material Purity**	**Results**	**Reference**	**Comments**
In vitro Cytokinesis Block Micronucleus Assay (with FISH analysis)	TR146 cells (human-derived buccal carcinoma cell line)	10, 15 and 20 mg/L; 20 minute exposure.	95%	Positive Statistically significant ($p<0.05$) increase in MN at 15 and 20 mg/L.	Koller *et al.* (2012)	Apoptosis and necrosis reported at 20 mg/L Also reported ↑ in NB and NPB
In vitro Cytokinesis Block Micronucleus Test	CHO-K1 cells	5 - 100 µg/mL, ±S9	Not stated	Negative –S9 Positive +S9 at 10-100 µg/mL	Roustan et al., (2014)	No clear dose response
In vitro Cytokinesis Block Micronucleus Test	Bovine lymphocytes (2 donors)	0, 28, 56, 140, 280 and 560 µM 24 & 48 h exposure	62%	24 h: Negative 48 h: Equivocal ↑ MN at 280 µM only (donor A) ↑ MN at 560 µM only (donor B)	Piesova, 2004	No dose-response No significant decrease in CBPI observed.
In vitro Cytokinesis Block Micronucleus Test	Bovine lymphocytes (2 donors)	0, 28, 56, 140, 280 and 560 µM; 2 h (±S9) and 48 h (-S9) exposure	62%	2 h: Negative 48 h: Equivocal ↑ MN at 280 µM only (donor A) and at 560 µM only (donor B)	Piesova, 2005	No dose-response; No significant decrease in CBPI observed. Metabolic activation had no effect on MN formation after 2 h exposure.

Table 5.4. *In vitro* Tests for Micronuclei Induction in Mammalian Cells- Glyphosate Technical						
Test/Endpoint	**Test System**	**Concentrations/ Conditions**	**Test Material Purity**	**Results**	**Reference**	**Comments**
In vitro Cytokinesis Block Micronucleus Assay (with FISH analysis)	Human lymphocytes (treated with cytochalasin B)	4h treatment ±S9; 0.5, 2.91, 3.50, 92.8 and 580 µg/mL; harvested 72 h	98.0%	Negative –S9 Positive +S9, ↑ MN at 580 µg/mL, but not at 0.5-92.8 µg/mL Also observed ↑ in NB at 580 µg/mL (±S9); ↑ NPB at 580 µg/mL (+S9)	Mladinic *et al.* (2009a)	Cells were exposed to glyphosate and washed prior to treatment with PHA. Authors did not report being blind to treatment.
In vitro Cytokinesis Block Micronucleus Assay (with FISH analysis)	Human lymphocytes (treated with cytochalasin B)	4h treatment ±S9; 0.5, 2.91, 3.50, 92.8 and 580 µg/mL	98%	Negative –S9 Positive +S9 ↑ MN at 580 µg/mL, but not at 0.5 -92.8 µg/mL ↑ apoptosis and necrosis at 580 µg/mL (-S9); ↑ apoptosis at ≥ 2.91 µg/mL and necrosis at 580 µg/mL (+S9) ↑ in NB at 580 µg/mL (±S9) and NPB at 580 µg/mL (+S9)	Mladinic *et al.* (2009b)	Cells were exposed to glyphosate and washed prior to treatment with PHA. Authors did not report being blind to treatment. .

CBPI= cytokinesis block proliferation index, FISH= fluorescent in situ hybridization; MN= micronuclei; NB= nuclear buds; NPB= nucleoplasmic bridges.

5.5 *In Vivo* Genetic Toxicology Tests

5.5.1 *In Vivo* Assays for Chromosomal Abnormalities

5.5.1.1 Mammalian Bone Marrow Chromosomal Aberration Assays

The *in vivo* mammalian bone marrow chromosomal assay detects the ability of a chemical to cause structural chromosomal damage in cells in the bone marrow. The assay is typically conducted in rodents (mouse or rat) and detects both chromosome-type and chromatid-type aberrations. Chromatid-type aberrations are expressed when a single chromatid break occurs and/or a reunion between chromatids, and chromosome-type aberrations result from damage expressed in both sister chromatids (OPPTS 870.5385). In this test, animals are exposed (typically via oral route or intraperitoneal injection) and sacrificed at sequential intervals. Prior to sacrifice, animals are treated with a spindle inhibitor such as colchicine or Colcemid® to arrest cells at metaphase. Chromosome preparations from the bone marrow are stained and scored for chromosomal aberrations. (OPPTS 870.5385). Generally, the optimal time to detect chromosomal aberrations in the bone marrow is 24 hours after treatment.

Three *in vivo* mammalian bone marrow chromosomal assays were conducted with glyphosate technical for regulatory purposes and all were negative (Table 5.8). In the first study, Sprague Dawley rats were administered glyphosate (98%) at 0 or 1000 mg/kg and the bone marrow was sampled at 6, 12 or 24 hours after dosing. No significant increase in bone marrow chromosomal aberrations were observed (Li, 1983b). In the second study, Swiss albino mice were treated twice by oral gavage (24 hours apart) with 0 or 5000 mg/kg glyphosate technical (96.8%) resulting in no significant increase in bone marrow chromosomal aberrations (Suresh, 1994). In a third study conducted with glyphosate trimesium salt, no increase in chromosomal aberrations were seen in the bone marrow of rats treated by oral gavage with up to 188 mg/kg (Majeska, 1982c).

5.5.1.2 Rodent Dominant Lethal Test

Dominant lethal mutations cause embryonic or fetal death. The induction of a dominant lethal mutation after exposure to a chemical indicates that the test chemical has affected the germinal tissue (sperm at some point in development, from stem cell to spermatocyte). Dominant lethal effects are considered to result from chromosomal damage (structural or numerical), but may also reflect gene mutations or systemic toxicity (OPPTS 870.5450, OECD 2016). In this test, male rodents are treated with the test material and mated with (untreated) virgin females. The females animals are sacrificed at an appropriate time and the uteri are examined to determine the number of implants, and live and dead embryos. Two dominant lethal studies were identified. One study was conducted in the rat (Suresh, 1992) where male rats were dosed by oral gavage with glyphosate up to 5000 mg/kg. The other study (Rodney, 1980) was conducted in male mice treated with up to 2000 mg/kg glyphosate (98.7%) by oral gavage. No significant increase in dominant lethal mutations were observed in either study (Table 5.5).

5.5.1.3 *In Vivo* Mammalian Erythrocyte Micronucleus Assays

The mammalian micronucleus test is the most commonly conducted *in vivo* test to detect clastogenic or aneugenic chemicals. The test identifies chemicals that induce micronuclei in proerythrocytes (progenitor cells) by assessing micronucleus frequency in immature erythrocytes (polychromatic erythrocytes, PCEs) sampled from the bone marrow or from the peripheral blood (reticulocytes). This test is typically conducted in mice or rats. When bone marrow erythroblasts develop into erythrocytes, the main nucleus is extruded following the final cell division (erythrocytes are the only mammalian cell that does not contain a nucleus). Any micronuclei formed after the final cell division may remain in the cytoplasm following extrusion of the main nucleus. The visualization of micronuclei is facilitated by the lack of a nucleus in these cells (OPPTS 870.5395, OECD 474). Micronuclei can originate from acentric chromosomes, lagging chromosome fragments, or whole chromosomes; thus, micronuclei are biomarkers of both altered chromosome structure or chromosome number. The assay is based on an increase in the frequency of micronucleated erythrocytes in treated animals, in either peripheral blood samples or bone marrow samples (OPPTS 870.5395). Additional mechanistic information on the formation of chromosomal abnormalities can be obtained from the incorporation of centromeric and telomeric fluorescent probes (FISH) assay. . According to EPA test guidelines, a single dose of the test substance may be used in this test if the dose is the maximum tolerated dose (MTD), a dose that produces some indication of bone marrow cytotoxicity (e.g., a reduction in the proportion of immature erythrocytes (PCEs) to total erythrocytes by >50%) or a maximum limit dose of 5000 mg/kg. The routes of administration for this test are typically oral or intraperitoneal injection and generally involve a single administration.

Glyphosate technical has been extensively evaluated for micronuclei induction in *in vivo* studies. Fourteen studies were conducted for regulatory purposes, four were identified from the open literature, and one study was conducted by the U.S. National Toxicology Program (NTP). This included nine studies with administration of glyphosate by the intraperitoneal (i.p.) route and 10 studies by the oral route. The findings are presented in Table 5.10. Of the nine i.p. studies, seven (Costa, 2008; Chruscielska *et al.*, 2000; Durward, 2006; Gava, 2000; Marques, 1999; Rank *et al.*, 1993 and Zaccaria, 1996) were negative. These studies tested doses as high as 2016 mg/kg (single and double administration) with sampling times at 24 and 48 hours post-dose. Two positive findings were reported when glyphosate technical was administered by i.p. Bolognesi *et al.* (1997) reported a significant increase in micronuclei in the bone marrow of male Swiss CD mice 24 hours after i.p. treatment with 300 mg/kg glyphosate technical (99.9%). The dose in this study was administered as ½ dose (150 mg/kg) injections 24 hours apart to 3 male mice. Manas *et al.* (2009) evaluated glyphosate technical (96%) in BALB/c male and female mice (5/sex/dose) administered 50, 100 or 200 mg/kg by two i.p. injections, 24 hours apart. The results showed a significant increase in micronucleated erythrocytes at 200 mg/kg, but not at 50 or 100 mg/kg. It should be noted that doses that resulted in the positive responses in these two studies were above the reported i.p. LD50 value (130 mg/kg) for glyphosate in mice (NTP 1992).

Glyphosate technical was also evaluated in nine micronucleus assays with administration by the oral route in mice and one in the rat. Eight of the nine oral studies in the mouse were negative for micronuclei induction. The single positive response was seen in female mice treated with two 5000 mg/kg (limit dose) doses, 24 hours apart with bone marrow sampling at 24 hours post-dose (Suresh, 1993b). No increase was observed at lower doses (50 and 500 mg/kg) in females or at any dose in males. The eight negative oral studies in mice included single dose administrations of 5000 mg/kg and bone marrow analysis at 24, 48, and/or 72 hours (Jensen, 1991c; Fox and Mackay, 1996) and one or two administrations of glyphosate technical with top doses between 30 and 2000 mg/kg (Honarvar, 2005; Honarvar, 2008; Jones, 1999; and Zoriki-Hosmi, 2007). It should be noted that evaluations at 48 and 72 hours post dose may be too late to detect chemically-induced micronucleated PCEs in the bone marrow as these cells may have already migrated into the peripheral blood. No significant increase in micronucleated erythrocytes were seen in male or female mice following 13-weeks of dietary (feed) administration of glyphosate technical at doses up to 3393 mg/kg/day (NTP, 1992). In the single study that evaluated micronuclei induction in rats, glyphosate technical did not induce significant induce micronuclei in CD1 rats treated by oral gavage at doses up to 2000 mg/kg (Flügge, 2009b). When glyphosate trimesium salt was evaluated, no increase in micronuclei induction was seen in mice treated orally up to 1100 and 800 mg/kg in males and females, respectively (Majeska, 1987).

Table 5.5. *In Vivo* Tests for Chromosomal Aberrations in Mammals- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses	Test Material Purity	Results	Reference	Comments
Bone Marrow Chromosomal Aberration Test	Sprague Dawley rats (males and females)	Intraperitoneal injection; sampled at 6, 12 and 24 h after treatment	0, 1000 mg/kg (6/sex/dose/sampling time)	98%	Negative	Li (1983b) [MRID 00132683]	No toxicity observed. A separate study using ^{14}C-glyphosate showed that glyphosate reaches BM 0.5 h after dosing with ½ life elimination at 7.6 h. Peak BM value was 400 ppm, corresponding to 2000 ppm plasma value.
Bone Marrow Chromosomal Aberration Test	Sprague Dawley rats (males and females) Vehicle: distilled water	Oral gavage, sampling after 6, 12, 24, 48 h and 5 d	0, 21, 63 and 188 mg/kg	58.5% *Glyphosate trimesium salt*	Negative	Majeska (1982c) [MRID 00132176]	
Bone Marrow Chromosomal Aberration Test	Swiss Albino mice (males and females) Vehicle: peanut oil	Oral gavage (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 5000 mg/kg (5/sex/dose)	96.8%	Negative	Suresh (1994) [MRID 49987408]	Significant ($p<0.05$) decrease in bw of females at high dose.
Rodent Dominant Lethal Test	CD-1 mice Each dosed male mated with 2 females/week for 8 weeks	Oral gavage	0, 200, 800, and 2000 mg/kg	98.7%	Negative	Rodwell (1980) [MRID 00046364]	
Rodent Dominant Lethal Test	Wistar rat Each dosed male mated with 1 female/week for 10 weeks	Oral gavage	0, 200, 100 and 5000 mg/kg	96.8%	Negative	Suresh (1992) [MRID 49987404]	

Table 5.6. In Vivo Tests for Micronuclei Induction in Mammals- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses	Test Material Purity	Results	Reference	Comments
Bone Marrow Micronucleus Test	Swiss CD1 mice (males only)	Intraperitoneal injection; 2 injections of half the dosage of 300 mg/kg 24 h apart; sampling at 6 and 24 h	0, 300 mg/kg (3/dose)	99.9%	Positive Stat significant increase in MN at 24 h	Bolognesi *et al.* (1997)	Material and methods indicate 3 animals/dose; however, Table 1 of article indicates 4 animals were evaluated.
Bone Marrow Micronucleus Test	Balb C mice (males and females) Vehicle: Saline	Intraperitoneal Injection (two injections, 24 h apart); sampling after 24 h (last treatment)	0, 50, 100, and 200 mg/kg (5/sex/dose)	96%	Positive ↑MN at 200 mg/kg, but not at 50 or 100 mg/kg	Manas *et al.* (2009)	No significant signs of toxicity observed.
Bone Marrow Micronucleus Test	C3H mice (males only) Vehicle: water	Intraperitoneal Injection (single treatment); sampling after 24, 48 and 72 h	0, 300 mg/kg	Not reported	Negative	Chruscielska *et al.* (2000)	
Bone Marrow Micronucleus Test	Swiss Albino mice (males and females) Vehicle: corn oil	Intraperitoneal Injection (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 15.62, 31.25, and 62.5 mg/kg (5/sex/dose)	980 g/kg Glyphosate technical	Negative#	Costa (2008)[1]	OECD guideline 474 #Was not tested up to limit dose and did not demonstrate that compound was tested up to toxic dose. No mention of BM toxicity or clinical signs.

Table 5.6. In Vivo Tests for Micronuclei Induction in Mammals- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses	Test Material Purity	Results	Reference	Comments
Bone Marrow Micronucleus Test	Crl:CD-1TM(ICR) BR mice (males only[1]) Vehicle: PBS	Intraperitoneal Injection (single treatment); sampling after 24 and 48 h (high dose only)	0, 150, 300 and 600 mg/kg (7/dose)	95.7%	Negative	Durward (2006) [MRID 49957411]	Clinical signs reported at ≥ 150 mg/kg. Significant ↓ in %PCEs reported at 24 h in 600 mg/kg group. ↑in MN PCEs observed at 600 mg/kg (1.9± 0.7 vs. 1.0 ± 1.2 control; $p<0.05$), at 24 h, but not 48 h, within historical control range.
Bone Marrow Micronucleus Test	Swiss Albino mice (males and females) Vehicle: water	Intraperitoneal Injection (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 1008, 2016, and 3024 mg/kg 5/sex/dose	612.7 g/kg (glyphosate technical Nufarm)	Negative	Gava (2000)[1]	LD50 was 4032 mg/kg Mortality observed in 1 animal at high dose (only 4 m/f scored for MPCEs). No effect on PCE/NCE.
Bone Marrow Micronucleus Test	Swiss Albino mice (males and females) Vehicle: water	Intraperitoneal Injection (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 187.5, 375 and 562.5 mg/kg 5/sex/dose	954.9 g/kg (glyphosate technical Nufarm)	Negative	Marques (1999) [MRID 49957412]	LD50 was 750 mg/kg No significant signs of toxicity observed in main study
Bone Marrow Micronucleus Test	NMRI-Bom mice	Intraperitoneal Injection (single treatment); sampling after 24 h (all doses) and 48 h (150 and 200 mg/kg)	0, 150, and 200 mg/kg (5/sex/dose)	glyphosate isopropylamine (purity not specified)	Negative	Rank *et al.* (1993)	

Table 5.6. In Vivo Tests for Micronuclei Induction in Mammals- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses	Test Material Purity	Results	Reference	Comments
Bone Marrow Micronucleus Test	Swiss albino mice (males and females)	Intraperitoneal Injection (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 68, 137, and 206 mg/kg (	360 g/L	Negative	Zaccaria (1996) [MRID 49961501]	Doses selected were reported as corresponding to 25, 50 and 75% LD_{50}
Bone Marrow Micronucleus Test	CD-1 mice (males and females) Vehicle: saline	Oral gavage (single treatment); sampling after 24 and 48 h	0, 5000 mg/kg 5/sex/dose	95.6%	Negative	Fox and Mackay (1996) [MRID 44320619]	No significant signs of toxicity observed
Bone Marrow Micronucleus Test	NMRI mice (males and females) Vehicle: PEG 400	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 mg/kg 5 sex/dose	97.73%	Negative	Honarvar (2005)[1]	OECD guideline 474 No significant signs of toxicity observed
Bone Marrow Micronucleus Test	NMRI mice (males only) Vehicle: 0.5% carboxymethylcellulose	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 mg/kg (5/dose)	99.1%	Negative	Honarvar (2008) [MRID 49961802]	No significant signs of toxicity observed
Bone Marrow Micronucleus Test	NMRI mice (males and females) Vehicle: 0.5% carboxymethylcellulose	Oral gavage (single treatment); sampling after 24, 48 and 72h	0, 5000 mg/kg; 5/sex/dose	98.6%	Negative	Jensen (1991c) [MRID 49961503]	No significant signs of toxicity observed
Bone Marrow Micronucleus Test	CD-1 mice (males only[1]) Vehicle: water	Oral gavage single treatment); sampling after 24 and 48 h	0, 2000 mg/kg 5/dose	59.3% potassium glyphosate salt	Negative	Jones (1999)[1]	OECD guideline 474 No significant signs of toxicity observed
Bone Marrow Micronucleus Test	Swiss albino mice; (males and females)	Oral gavage (2 treatments, 24 h apart); sampling	0, 50, 500, 5000 mg/kg 5/sex/dose	96.8% glyphosate acid	Positive in females at 5000	Suresh (1993b) [MRID 49987407]	No significant signs of toxicity observed

Table 5.6. In Vivo Tests for Micronuclei Induction in Mammals- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses	Test Material Purity	Results	Reference	Comments
	Vehicle: peanut oil	after 24 h (last treatment)			mg/kg only. Negative in males at all doses		
Bone Marrow Micronucleus Test	Swiss mice (males only) Vehicle: corn oil	Oral gavage (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 8, 15 and 30 mg/kg (6/dose)	980.1 g/kg	Negative	Zoriki Hosomi (2007) [MRID 50000901]	OECD guideline 474 No significant signs of toxicity observed
Bone Marrow Micronucleus Test	CD-1 mice (males and females) Vehicle: distilled water	Oral gavage , Sampling 24, 48 and 72 h after treatment	Males: 0, 700, 900 and 1100 mg/kg Females: 0, 400, 600 and 800 mg/kg	55.3% *Glyphosate trimesium salt*	Negative	Majeska (1987) [MRID 40214004]	
Bone Marrow Micronucleus Test	B6CF3 Mice (males and females)	Oral (dietary). MN assay conducted following 13 week feed study.	0, 205/213, 410/421, 811/844, 1678/1690 and 3393/3393 mg/kg (m/f) (10/sex/dose)	99%	Negative	NTP (1992)	
Bone Marrow Micronucleus Test	CD Rats (males and females) Vehicle: 0.8% hydroxypropylmethyl cellulose	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 mg/kg (5/sex/dose)	98.8%	Negative	Flügge (2009b)[1]	OECD guideline 474 No significant signs of toxicity observed

[1] Study was cited in Kier and Kirkland (2013). Supplementary information about the study was provided online including test guideline followed, test material purity, control chemicals and summary data tables.

[2]Only males tested; report indicated that there were no difference between sexes seen in range finding study.

CA= chromosomal aberrations, MPCE= micronucleated polychromatic erythrocytes, NCE= normochromatic erythrocytes, PCE=polychromatic erythrocytes.

5.6 Additional Genotoxicity Assays Evaluating Primary DNA Damage

There are a number of genotoxicity assays that evaluate primary DNA damage, but do not measure the consequence of the genetic damage (*i.e.*, mutation or chromosomal damage). As discussed in the Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines (OECD 2015), the endpoints measured in primary DNA damage tests such as DNA adducts, comet assay, or unscheduled DNA synthesis may lead to cell death or may initiate DNA repair, rather than a mutation. These types of assays can, however, provide mechanistic information when interpreting positive findings in other genotoxicity tests or when determining whether a chemical is acting through a mutagenic mode of action. Additionally, indirect mechanisms of DNA damage such as oxidative DNA damage can be detected by these test systems. Oxidative damage results from oxidative stress, which occurs when there is a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defense systems. Normal cellular metabolism is a source of endogenous reactive oxygen species that accounts for background levels of oxidative damage in normal cells. Some types of oxidative damage are repairable while others lead to serious consequences in the cell. (Cooke et al, 2003). The various assays evaluating primary DNA damage in glyphosate technical are presented in Table 5.7 Details of the findings are discussed below.

Glyphosate technical is not electrophilic and is not considered to be DNA-reactive. In a study to evaluate the potential for glyphosate to directly interact with DNA, Peluso et al. (1998) reported that glyphosate technical did not form DNA adducts in mice when tested up to 270 mg/kg via i.p. Bolognesi et al. (1997) reported an increase in the oxidative damage biomarker 8-hydroxydeoxyguanosine (8-OHdG) in the liver 24 h after i.p. injection of 300 mg/kg in mice. No increase in 8-OHdG was seen in the kidney with glyphosate technical. The dose in this study was high (300 mg/kg) for an i.p. injection and within the i.p. LD_{50} range (134- 545 mg/kg) that has been reported elsewhere (WHO, 1994).

The comet assay, also known as single cell gel electrophoresis (SCGE), is a sensitive and rapid method to detect DNA strand breaks in individual cells. In this assay, individual cells are embedded in agarose. The cells are then lysed (which digests the cellular and nuclear membranes) and the DNA is allowed to unwind under alkaline or neutral conditions. During electrophoresis, chromatin (which is in a supercoiled state) that has undergone steric relaxation due to DNA damage migrates away from the nucleoid (nucleus) toward the anode, yielding images that resemble a comet. The intensity of the comet tail relative to the comet head reflects the amount of DNA breakage (Tice et al., 2000; Collins et al., 2008). The comet assay can detect single and double strand breaks resulting from direct interactions with DNA, alkali labile sites, or transient DNA breaks resulting during DNA excision repair. These types of strand breaks may be, (a) repaired with no persistent effect, (b) be lethal to the cell or (c) be fixed as a mutation (OECD TG 489). DNA strand breaks in the comet assay can be measured by endpoints such as percent tail DNA (also referred to as % tail intensity), tail length, and tail moment. However, % tail DNA is the recommended metric for evaluating and interpreting results using this assay (OECD TG 489).

The five studies that evaluated glyphosate technical using the comet assay are summarized in Table 5.12. Two of the studies were conducted using tumor cell lines. Koller et al. (2012) reported positive comet effects (increased tail intensity) in a human buccal carcinoma cell line (TR146) following a 20-minute treatment with ≥ 20 mg/L (~0.118 mM) glyphosate. Although no evidence of cytotoxicity was reported in this study, the authors did report an increase in apoptosis and necrosis at the same concentrations (≥ 20 mg/L) when the same cell line was tested for *in vitro* micronuclei induction (discussed previously). In a study using Hep-2 cells (presumably a HeLa cell derivative), Manas et al. (2009) reported a statistically significant increase in mean tail length, and tail intensity at all concentrations (3.0-7.5 mM) tested. In a comet study conducted on human lymphocytes, Alvarez-Moya et al. (2014) reported significant increases in tail length only (but not % tail DNA) following treatment with glyphosate concentrations of 0.7-700 µM. Mladinic et al. (2009a) evaluated DNA damage in non-dividing human lymphocytes (±S9) following treatment from 0.5 to 580 µg/mL using the standard alkaline comet method and a modified comet method that detects DNA damage due to oxidative damage (human 8-hydroxyguanidine DNA-glycosylase, hOGG1 comet method). In this study, the authors reported statistically significant increases in tail intensity at 3.5 µg/mL and higher in the absence of S9, with significance only at 580 µg/mL (~3.4 mM) in the presence of S9 using the alkaline method. This concentration also resulted in increased apoptosis and necrosis as well as an increase in plasma total antioxidant capacity (TAC) and changes in plasma lipid peroxidation (thiobarbituric reactive substances, TBARs); however, only a dose-related increase in tail length (not % tail DNA) was observed at 580 µg/mL (+S9) using the hOGG1 method. When the Manas et al. (2013) evaluated blood and liver cells following a 14 day drinking water study in mice treated with 40 and 400 mg/kg/day glyphosate, significant increases in tail intensity, tail length and tail moment were reported were observed at both doses in both tissues (except for DNA tail intensity in liver at 40 mg/kg); however, there were no substantial effects on oxidative stress measurements suggesting that DNA damage reported may not be due to oxidative damage.

The Unscheduled DNA Synthesis (UDS) test with mammalian liver cells *in vitro* identifies substances that induce DNA repair after excision and removal of a segment of damaged DNA. The test is typically conducted in liver cells, which have relatively few cells in the S-phase of the cell cycle. The assay measures the incorporation of radiolabeled nucleotide [^{3}H]-thymidine into DNA during the repair process in non-S phase cells. (OPPTS 870.5555). Substances that produce either a statistically significant dose-related increase or statistically significant and reproducible increase in ^{3}H-TdR incorporation in at least one test point are considered to be positive in this test. A UDS study that evaluated glyphosate technical in rat primary hepatocytes was negative (Williams, 1978). Glyphosate technical was also negative in a DNA repair test conducted in bacteria (Rec-A test) (Shirasu, 1978).

In an alkaline elution assay, which detects single strand DNA breaks, Bolognesi et al. (1997) reported an increase in single strand breaks (i.e. increased DNA elution rate) in the liver and kidney 4 hours after a single i.p. injection of 300 mg/kg. The elution rate returned to control

levels at 24 hours. Glyphosate technical was also negative in a DNA repair test conducted in *Bacillus subtilis* H17 (rec^+) and M45 (rec^-) bacterial Rec-A test (Shirasu, 1978).

Finally, the sister chromatid exchange (SCE) test is an assay that can measure the consequence of primary DNA damage. The mechanism(s) of action for chemical induction of SCE is unclear. The SCE assay detects the exchange of DNA between two sister chromatid arms within a single chromosome. The assay can be performed *in vitro* or *in vivo*. Following exposure, cells/animals are treated with bromodeoxyuridine (BrdU) to allow for the differentiation of the two sister chromatids (harlequin staining) and prior to harvest are treated with a spindle inhibitor to accumulate cells in metaphase. The chromosome preparations are then stained and analyzed for SCEs (OPPTS 870.5900, 870.5915). The SCE studies that evaluated glyphosate technical are also presented in Table 12. Positive SCE findings were reported in all four studies; two evaluating bovine lymphocytes (Lioi, 1988b, Sivikova and Dianovksy, 2006) and two studies evaluating human lymphocytes (Lioi, 1988a; Bolognesi et al., 1997). In all four studies the induction did not demonstrate a clear dose response.

Additionally, although it is recognized that mechanisms other than genotoxicity may be involved in cell transformation, glyphosate trimesium salt was evaluated in the Balb/3T cell transformation assay (an *in vitro* tumor formation assay) and was negative up to 5.0 mg/ml (Majeska, 1982b).

Table 5.7 Assays for Detecting Primary DNA Damage- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses/ Concentrations	Test Material Purity	Results	Reference	Comments
DNA Adducts ^{32}P-postlabeling	Swiss CD1 mice (males and females) Liver and kidney evaluated	Intraperitoneal injection; 24 h exposure	0, 130 and 270 mg/kg	Not reported	Negative	Peluso *et al.* (1998)	
DNA oxidative damage: 8-OHdG formation	Swiss CD-1 mice (males) liver and kidney evaluated	Intraperitoneal injection (single dose); sampling 4 and 24 h after injection	0, 300 mg/kg (3/dose)	99.9%	Kidney: negative Liver: positive (24 h)	Bolognesi *et al.* (1997)	
Single-cell gel electrophoresis (SCGE) assays- Comet assay	TR146 cells (human-derived buccal epithelial cell line).	NA (*in vitro*)	-S9: 10-2000 mg/L; 20 minute exposure.	95%	Positive Increased DNA migration at >20 mg/L	Koller *et al.* (2012)	Also measured multiple cellular integrity parameters to assess cytotoxicity. No clear evidence of cytotoxicity seen except for increase in enzyme activity (indicative of membrane damage) in LDHe (extracellular lactate dehydrogenase) assay at >80 mg/L. No mention of monitoring pH
Single-cell gel electrophoresis (SCGE) assays- Comet assay	Hep-2 cells	NA (*in vitro*)	0, 3, 4.5, 6, 7.5, 9, 12 and 15 mM	96%	Positive Stat. significant increase in mean tail length, and tail intensity at all concs.	Manas *et al.* (2009)	The authors did not report a source for the Hep-2 cells. The agency presumes that this is a HeLa derived cervical carcinoma cell line.

Table 5.7 Assays for Detecting Primary DNA Damage- Glyphosate Technical.

Test/Endpoint	Test System	Route of Administration	Doses/ Concentrations	Test Material Purity	Results	Reference	Comments
Single-cell gel electrophoresis (SCGE) assays- Comet assay	Human lymphocytes	NA (*in vitro*)	0, 0.7, 7, 70, 700 µM	96%	Positive at all doses (increase in tail length only)	Alvarez-Moya *et al*., (2014)	Issues were identified with this study resulting in a low quality ranking. These include: 1) blood was washed with PBS and then held at 4º C for an indeterminate amount of time before exposure to glyphosate. (2) Cells were treated for 20 hours at room temperature. (3) The same amount of damage was reported across 2 orders of magnitude concentration.
Single-cell gel electrophoresis (SCGE) assays- Comet assay	Human lymphocytes; ±S9 Alkaline and hOOG1 Comet assays performed	NA (*in vitro*)	0, 0.5, 2.91, 3.5, 92.8 and 580 µg/mL	98%	Positive ±S9	Mladinic *et al.* (2009a)	<u>The alkaline comet assay</u> -S9: ↑ in mean tail length at 580 µg/mL and ↑ in tail intensity at ≥ 3.5 µg/mL). +S9: ↑ DNA tail length at ≥3.5 µg/mL. Tail intensity ↑ only at 580 µg/mL <u>hOOG1 comet assay</u>: -S9 no effect on tail length, ↑tail intensity only at 3.50 µg/mL +S9: ↑ tail length at 580 µg/mL, no effect on tail intensity compared to controls at any conc.

Table 5.7 Assays for Detecting Primary DNA Damage- Glyphosate Technical.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses/ Concentrations**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Single-cell gel electrophoresis (SCGE) assays- Comet assay with oxidative stress measures	Balb/C mice; evaluated blood and liver	Drinking water (14 days)	0, 40, and 400 mg/kg	96%	Positive Blood and liver at both doses	Manas et al. (2013)	Only minor effects seen on oxidative stress measurements (TBARs, SOD, CAT)
Sister Chromatid Exchange (SCE)	Bovine lymphocytes (3 donors)	NA (*in vitro*)	-S9: 0, 17, 85 and 170 µM; 72 h exposure	≥98%	Positive Significant (p>0.05) increase in SC/cell at all concentrations	Lioi (1998b)	1.8-, 2.1-, 1.6-fold increases, respectively
Sister Chromatid Exchange (SCE)	Human lymphocytes	NA (*in vitro*)	-S9: 0, 5, 8.5, 17 and 51 µM; 72 h exposure	≥98%	Positive Significant (p>0.05) increase in SCE/cell at ≥ 8.5 µM	Lioi (1998a)	1.9-, 2.8-, and 2.6-fold increase at 8.5, 17 and 51 µM, respectively
Sister Chromatid Exchange (SCE)	Human lymphocytes	NA (*in vitro*)	-S9: 0, 0.33, 1,3 and 6 mg/mL; 72 h exposure	99.9%	Positive	Bolognesi *et al.* (1997)	Very limited information was provided on the methods used in this paper. Authors report a dose –dependent increase in SCE frequency; however, no statistical analysis for dose response was reported. Data presented graphically with no error bars.
Sister Chromatid Exchange (SCE)	Human lymphocytes	NA (*in vitro*)	28, 56, 140, 280, 560 and 1120 µM; 24 h exposure ±S9	62%	Positive	Sivikova and Dianovsky (2006)	The increases in SCEs observed did not show a clear concentration related increase across a 40-fold increase in the concentrations tested

Table 5.7 Assays for Detecting Primary DNA Damage- Glyphosate Technical.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses/ Concentrations**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Alkaline elution assay- DNA single strand breaks	Swiss CD-1 mice (males) liver and kidney evaluated	Intraperitoneal injection (single dose); sampling 8 and 24 h after injection	0, 300 mg/kg (3/dose)	99.9%	Positive (Increased elution rate) at 4 hours in liver and kidney At 24 h, elution rate returned to control levels	Bolognesi *et al.* (1997)	Return to control values may indicate DNA repair or reflect rapid elimination of compound
DNA Repair Test (Rec-A test)	*B. subtilis* H17 (rec+) and M45 (rec-)	NA (*in vitro*)	20-2000 µg/disk	98.4%	Negative	Shirasu (1978) [MRID 00078619]	
Unscheduled DNA synthesis (DNA repair)	F-344 rat primary hepatocytes	NA (*in vitro*)	0, 0.0125, 0.0625, 0.125, 0.6.5, 1.25, 12.5, 125 µg/mL	98%	Negative	Li and Long (1988)	
Cell Transformation Assay	BALB/3T cells	NA (*in vitro*)	0.313-5.0 mg/mL	90% *Glyphosate trimesium salt*	Negative	Majeska (1982b) [MRID 00126616]	

h- hour; CAT= catalase, G6PD= glucose 6-phosphate dehydrogenase, NA= not applicable, hOOG1 =, TBARs= thiobarbituric acid reactive substances, SOD= superoxide dismutase

5.7 Summary and Discussion

The genotoxic potential of glyphosate has been extensively investigated using a variety of test systems and genetic endpoints. This assessment focuses only on test systems that the agency considered relevant for assessing genotoxic risks in humans. The totality of the genetic toxicology information was evaluated using a weight of evidence approach to determine the genotoxic potential of glyphosate. This involves the integration of *in vitro* and *in vivo* results as well as an overall evaluation of the quality, consistency, reproducibility, magnitude of response, dose-response relationship and relevance of the findings. In the weight of evidence analysis, studies evaluating endpoints that measured gene mutations and chromosomal aberrations (i.e. permanent DNA damage) were given more weight than endpoints reflecting DNA events that may be transient or reversible such as primary DNA damage (*e.g.*, comet assays). *In vivo* studies in mammals were given the greatest weight and more weight was given to doses and routes of administration that were considered relevant for evaluating genotoxic risk based on human exposure to glyphosate. Also, since the molecular mechanisms underlying the observation of SCEs are unclear and thus, the consequences of increased frequencies of SCEs are unclear, the data from this test were given low weight in the overall analysis. A summary of the various lines of evidence of considered in the weight of evidence evaluation for the genotoxic potential of the active ingredient glyphosate is presented below.

Evidence of primary DNA damage

Glyphosate technical is not considered to be electrophilic and did not induce DNA adducts in the liver or kidney at an i.p. dose of 270 mg/kg. However, evidence of DNA strand breaks were reported in a number mammalian cell studies using the comet assay. Additionally, transient increases in alkali labile sites in the liver and kidney of mice and an induction of 8-OHdG in DNA were seen in the livers of mice following i.p. injections with 300 mg/kg glyphosate. These effects were seen at high doses for the i.p. route in mice (LD_{50} for mouse =130 mg/kg; NTP, 1992). However, due to technical limitations identified in a number of these studies (e.g. use of cancer cell lines that have not been well-characterized, atypical exposure protocols and no indication of blind to treatment), caution should be exercised in interpreting the results.

In vitro mutations

Glyphosate technical was negative in all 39 studies for mutagenicity in bacteria. In the four studies that tested for gene mutations in mammalian cells *in vitro,* no increase in mutations were observed.

In vitro chromosomal alterations

Mixed results were observed in studies evaluating *in vitro* chromosomal alterations with glyphosate treatment. Three SCE studies reported positive findings (Lioi, 1998a, b; Bolognesi *et al*., 1997) bovine and human lymphocytes. As stated previously, low weight is given to SCE results in the overall analysis given the uncertainty regarding the consequence of increases in the frequencies of SCEs. The SCE responses were weak and not concentration dependent. Eight of the 10 studies measuring *in vitro* chromosomal aberrations were negative. The two positive

findings were reported by Lioi et al., one study was conducted with bovine lymphocytes and the other with human lymphocytes. The authors reported positive findings in these studies at concentrations much lower than four other studies that reported negative results using the same cell types. Additionally, in both studies, Lioi et al. used an atypical exposure protocol of 72 hours which is very long for analyzing one round of mitosis. Furthermore, in both studies, nearly the same level effect for aberration frequency and percent of cells with aberrations were observed for the same concentrations of glyphosate and the two other chemicals tested in those experiments.

Four of the six studies evaluating micronuclei induction *in vitro* were positive and two showed equivocal results. Three of the positive responses required S9 activation, two conducted with human lymphocytes and one conducted with CHO cells. The remaining positive micronucleus study was conducted using a TR146 cells which is a tumor cell line derived from human buccal mucosa. The authors state that this cell line had not been previously used for genotoxicity testing. It is difficult to interpret any genotoxicity findings conducted in a tumor cell line that has not been well-characterized regarding its DNA damage response and repair capacity, and its degree of chromosomal instability.

Glyphosate was negative in all three L5178Y mouse lymphoma cell studies which may detect chromosomal damage in addition to mutations.

Mammalian *in vivo* chromosomal alterations

All three *in vivo* mammalian studies evaluating chromosomal aberrations with glyphosate technical were negative. Two studies were conducted in rats (i.p. and oral) and one was conducted in mice (oral). In addition glyphosate was also negative in a rodent dominant lethal test. Glyphosate was negative in 15 of the 19 bone marrow micronucleus studies evaluated. In two of the positive studies, glyphosate technical was administered by i.p. injection. In these studies, the authors reported positive findings at doses of 200-300 mg/kg. Based on the available ADME data for glyphosate, assuming 30% oral absorption, an oral dose of ~700-1000 mg/kg would be needed to achieve a dose of 200-300 mg/kg in the blood. Seven other i.p. studies in mice reported no increase in micronuclei induction at doses up to 3000 mg/kg. The remaining positive finding was reported in an oral gavage study in mice where an approximately 2-fold increase in micronuclei were reported in females only at a dose of 5000 mg/kg, which is considerably higher than the current guideline recommended limit dose of 2000 mg/kg. The effect was not seen in the 7 other oral gavage studies in mice when glyphosate was tested at similar doses. In addition, glyphosate was negative for micronuclei induction following a 13 week dietary study with a dose up to approximately 3000 mg/kg/day. A negative finding was also reported in the only study that evaluated *in vivo* micronuclei induction in the rat using doses up to 2000 mg/kg.

In a meta-analytic review of micronuclei frequency across mammalian and non-mammalian species (primarily fish, amphibians, reptiles and plants), Ghisi et al. (2016), not surprisingly, reported that different responses were observed when comparing mammalian results to phylogenetically distant non-mammalian species for micronuclei induction. Their analyses included most, but not all, of the mammalian studies that the agency evaluated and determined to

be negative for micronuclei induction. The authors reported a statistically significant increase in micronuclei by the i.p. route across the studies in the data set they considered; however, when glyphosate was administered by the oral route (which is the most physiologically relevant route for human exposure to glyphosate), no significant difference was observed.

Conclusion for Glyphosate

The overall weight of evidence indicates that there is no convincing evidence that glyphosate induces mutations *in vivo* via the oral route. When administered by i.p. injection, the micronucleus studies were predominantly negative. In the two cases where an increase in micronuclei were reported via this route, the effects occurred above the reported i.p. LD50 for mice and were not observed in other i.p. injection studies at similar or higher doses. While there is limited evidence genotoxic for effects in some *in vitro* experiments, i*n vivo* effects were given more weight than *in vitro* effects particularly when the same genetic endpoint was measured, which is consistent with current OECD guidance. The only positive findings reported *in vivo* were seen at relatively high doses that are not relevant for human health risk assessment.

6.0 Data Integration & Weight-of-Evidence Analysis Across Multiple Lines of Evidence

6.1 Background

In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of scientific evidence (U.S. EPA, 2010). OPP's draft framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety MOA/human relevance framework, which highlights the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek et al, 2014).

One of the key components of the agency's draft framework is the use of modified Bradford Hill Criteria (Hill, 1965) like those described in the 2005 Guidelines for Carcinogen Risk Assessment. These criteria are used to evaluate the experimental support considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight-of-evidence analysis.

6.2 Dose-Response and Temporal Concordance

Given the lack of consistent positive findings particularly at doses < 1000 mg/kg/day across the lines of evidence, lack of mechanistic understanding, and lack of biological activity in mammalian systems to the parent compound glyphosate, there are few data to assess key events in the biological pathway and any associated temporal or dose concordance. Temporal concordance can be assessed using the experimental animal studies and epidemiological studies that evaluated exposure prior to outcomes. Similarly, dose concordance can be assessed using findings of apical outcomes in experimental animal studies, as well as epidemiological studies that utilize exposure metrics that are stratified by the number of exposure days.

A prospective cohort study is designed to collect exposure information prior to the development of cancer. As such, exposure is known to occur before the outcome. In De Roos *et al.* (2005), a prospective cohort study, no association was observed between glyphosate exposure and numerous cancer subtypes in the AHS cohort. Although the median follow-up time following recruitment into the cohort was approximately 7 years, it does not represent the amount of time subjects were exposed. Study participants provided pesticide exposure information prior to enrollment in the study and this information was used to evaluate has cumulative lifetime days of exposure and intensity-weighted cumulative days of exposure. An updated analysis of the AHS cohort is anticipated with a longer follow-up period, which includes the time period after the introduction of glyphosate-tolerant crops and the subsequent substantial increase in glyphosate use. The updated AHS cohort analysis will further elucidate the impact of increased glyphosate use due to glyphosate-tolerant crops. In De Roos *et al.* (2005), effect estimates did not increase across categories of increasing exposure for almost all cancer types, including NHL, in the prospective cohort study.

Two case-control studies evaluating the risk of NHL (Eriksson *et al.*, 2008 and McDuffie *et al.*, 2001) observed increased effect estimates in the highest exposure categories analyzed. Eriksson *et al.* (2008) found a greater effect estimate for subjects with >10 days (based on the median days of exposure among controls) and >10 years of exposure (for latency analysis) when compared to subjects with ≤10 days and 1-10 years of exposure, respectively; however, this analysis did not appear to adjust for co-exposures to other pesticides. By dividing the total number of exposed cases and controls using these exposure metrics, wider confidence intervals were observed indicating reduced power from smaller sample sizes. This may indicate that a longer follow-up time is needed to detect the risk for NHL; however, given the latency analysis of NHL was limited to Eriksson et al. (2008) and lack of NHL latency understanding in general, further studies are needed to determine the true latency of NHL. McDuffie *et al.* (2001), stratifying based on the average number of days per year of exposure, observed similar effect estimates in the lower exposure category (>0 and ≤2 days/year) while a greater effect estimate was observed in the highest exposure category (>2 days/year). The results from these two case-control studies conflict with the results observed in the cohort study (De Roos *et al.*, 2005), where no dose-response was seen across three exposure categories (stratified by tertiles; however, the case-control studies did not adjust for co-exposure to other pesticides. It is also difficult to make conclusions regarding dose-response with only two exposure categories used for the analyses by Eriksson et al. (2008) and McDuffie et al. (2001). It should also be noted that these analyses combine all NHL subtypes, which may have etiological differences (Morton *et al.*, 2014). Although some studies did provide effect estimates for subtypes, as stated in Section 3.5.2, these were not considered in the current evaluation due to the limited sample sizes. At this time, there are no data available to evaluate dose-response for NHL subtypes.

Furthermore, as discussed in Section 3.6, a dose-response relationship was not observed following the dramatic increase in glyphosate use due to the introduction of genetically engineered glyphosate-tolerant crops in 1996. Due to the change in use pattern, if a true association exists between glyphosate exposure and NHL, this large increase in use would be expected to result in a corresponding increase in risk of NHL associated with glyphosate exposure; therefore, higher effect estimates would be expected in more recent years. This trend was not observed though. For example, some of the highest adjusted risk measures for NHL

were reported for study years prior to 1996. Furthermore, it would also be expected that higher effect estimates would be reported in countries with higher use of glyphosate and/or that use glyphosate-tolerant crops, such as the United States and Canada, as compared to countries that exhibit less use. Once again, this trend was not observed with NHL studies, such that effect estimates for studies conducted in Sweden (Eriksson et al., 2008; Hardell *et al.*, 2002) were similar or higher than those reported in the United States (De Roos *et al.*, 2003; De Roos *et al.*, 2005) and Canada (McDuffie *et al.*, 2001).

With respect to animal carcinogenicity studies, key events in a MOA/AOP are evaluated to confirm that they precede tumor appearance. This temporal concordance evaluation cannot be conducted for glyphosate since a MOA/AOP has not been established. In general, the tumor incidences lacked a monotonic dose-response. It should be noted, however, that no preneoplastic or related non-neoplastic lesions were reported in any of the animal carcinogenicity studies to support any observed tumors. Furthermore, genotoxicity assays did not support a direct mutagenic MOA. While there is limited evidence of genotoxic in some *in vitro* endpoints, multiple *in vivo* do not support a genotoxic risk at relevant human exposure levels.

6.3 Strength, Consistency, and Specificity

A large database is available for evaluating the carcinogenicity potential of glyphosate. Across animal carcinogenicity and genotoxicity studies, results were consistent. For epidemiological studies, only one or two studies were available for almost all cancers investigated. The largest number of studies was available investigating NHL; however, there were conflicting results across studies.

In epidemiological studies, there was no evidence of an association between glyphosate exposure and solid tumors, leukemia, and HL. This conclusion is consistent with those recently conducted by IARC, EFSA, and JMPR. The available data for multiple myeloma are not considered adequate to assess carcinogenic potential at this time.

At this time, a conclusion regarding the association between glyphosate exposure and risk of NHL cannot be supported based on the available data due to conflicting results. Chance and/or bias cannot be excluded as an explanation for observed associations. The magnitude of adjusted risk estimates for never/ever use were relatively small ranging from 1.0 (no association) to 1.85 in adjusted analyses, with the widest confidence interval observed for the highest effect estimate indicating the estimate is less reliable. All of the estimates were not statistically significant with half of the effect estimates approximately equal to 1, while the other half of the effect estimates ranged from 1.5-1.85. As a result, studies of at least equal quality provided conflicting results. There were various limitations identified in Section 3.6 for these studies that could impact calculated effect estimates and explain the weak responses observed in these studies. Meta-risk ratios using these studies were also of small magnitude ranging from 1.3-1.5. As discussed in Section 3.6, meta-analyses should be interpreted with caution and are susceptible to the same limitations identified for individual studies.

Although none of the effect estimates were below 1 using the never/ever exposure metric, risk estimates were all below 1 (0.6-0.9) when using cumulative lifetime and intensity-weighted

cumulative exposure metrics in the prospective cohort study (De Roos et al., 2005). As discussed in Section 6.2, two case-control studies that investigated an exposure-response relationship conflicted with the extensive analyses conducted by De Roos et al. (2005). This may be due to differences in confounding control, differences associated with study design, limitations from small sample sizes, and/or, as some may suggest, a potentially short follow-up time in the cohort. It should also be noted that publication bias may play a role in this evaluation given there is a tendency to only publish positive results and potential concerns regarding glyphosate have only been raised in recent years.

A total of 15 (9 rat and 6 mouse) animal carcinogenicity studies with glyphosate, glyphosate acid, or glyphosate salts were analyzed for the current evaluation. Although increases in tumor incidences were observed in some studies, none were considered treatment-related based on weight-of-evidence evaluations. In 7 of these studies, no tumors were identified for detailed evaluation. In the remaining studies, tumor incidences were not increased at doses <500 mg/kg/day, except for the testicular tumors observed in one study. The high dose tumors, as well as the testicular tumors, were not reproduced in other studies, including those testing the same animal strain with similar or higher dosing. Additionally, the tumors typically lacked a monotonic dose response, pairwise significance, and/or corroborating preneoplastic lesions.

Over 80 genotoxicity studies with the active ingredient glyphosate were analyzed for the current evaluation. The overall weight-of-evidence indicates that there is no convincing evidence that glyphosate is genotoxic *in vivo* via the oral route. When administered via i.p. injection the studies were predominantly negative. In the two cases where an increase in micronuclei were reported via this route, the effects were not observed in other i.p. injection studies at similar or higher doses. Technical glyphosate was negative in all gene mutation studies. There was limited evidence of positive findings in studies evaluating primary DNA damage; however, as discussed in Section 5.6, the endpoints measured in these assays are less specific in regards to detecting permanent DNA changes (mutations) and can be attributed to other factors, such as cytotoxicity or cell culture conditions. Although some positive findings were reported for chromosomal alterations *in vitro*, these findings were limited to a few studies and are not supported by the *in vivo* studies that are the most relevant for human risk assessment.

Overall, there is remarkable consistency in the database for glyphosate across multiple lines of evidence. For NHL, observed associations in epidemiological studies were non-statistically significant and were of relatively small magnitude. Chance and/or bias cannot be excluded as an explanation for the observed associations. For all other cancer types, there were no associations found; however, only one or two studies were available for evaluation of most cancer types. Across species, strain, and laboratory, tumor incidence was not increased at doses <500 mg/kg/day, except the testicular tumors which were only seen in one study. Observed tumors were not reproduced in other studies, including those conducted using the same strain at similar or higher doses. The genotoxicity studies demonstrate that glyphosate is not directly mutagenic or genotoxic *in vivo*.

6.4 Biological Plausibility and Coherence

The Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) include the following guidance regarding the criteria of biological plausibility and coherence:

> "*evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies*. [p.39]."

The genotoxicity studies demonstrate that glyphosate is not directly mutagenic or genotoxic *in vivo*. The available data regarding non-cancer endpoints also do not provide any support for a carcinogenic process for glyphosate, and have shown glyphosate has relatively low toxicity. Laboratory animals generally display non-specific effects (e.g., clinical signs, reduced body weight) following glyphosate exposure at relatively high-doses, and no preneoplastic or related non-neoplastic lesions were observed to corroborate any of the observed tumors in the carcinogenicity studies. As discussed in Section 4.2, metabolism studies demonstrate low oral absorption and rapid excretion of glyphosate. The data are not sufficient to determine whether linear kinetics is occurring at high doses where tumors were observed. In the carcinogenicity test guideline (OCSPP 870.4200) and the 2005 Guidelines for Carcinogen Risk Assessment, inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms) should be avoided. A study evaluating the toxicokinetic profile of glyphosate using multiple doses is needed to further investigate the pharmacokinetic properties between low- and high-dose levels.

Although many of the studies included in this document focus on the potential for glyphosate to cause a cancer outcome, the agency is also aware of a limited number of studies in the open literature that have shown glyphosate and its metabolite, AMPA, can inhibit proliferation and promote apoptosis in cancer cells indicating the compounds have potential to be developed into therapeutic drugs for cancer treatment (Li *et al*, 2013; Parajuli *et al.*, 2015; Parajuli *et al.*, 2016). It is unknown if this is due to lack of additional studies that have investigated these compounds for cancer treatment or if this may be due to publication bias. The bias towards only publishing positive and/or novel results can hamper the ability to evaluate whether there are plausible biological mechanisms for observed outcomes and/or sufficient information to support a cause-and-effect interpretation of an association. Overall, this further supports the need for mechanistic data to elucidate the true mammalian MOA/AOP for glyphosate. There is a distinct lack of mechanistic understanding for the toxicity of glyphosate in mammals and the plant MOA is not relevant for mammalian systems.

As noted previously, tumor incidence in animal carcinogenicity studies was typically only increased at the highest doses tested (≥1000 mg/kg/day). It is very unlikely for people to be exposed to such large doses of glyphosate via the oral route. Glyphosate is registered for pre- and post-emergence application to a variety of fruit, vegetable, and field crops, as well as desiccant applications to several commodities. The highest dietary exposure value for any population subgroup in an unrefined chronic dietary analysis would be 0.23 mg/kg/day for children (1-2 years old). Since glyphosate also has residential uses, including application to turf,

there is also the potential for children at this age to be exposed via incidental oral exposures (e.g., hand to mouth, object to mouth and soil ingestion) from playing on treated lawns. The highest exposure for the incidental oral and dermal exposures would be 0.16 mg/kg/day (from hand-to-mouth behaviors for children) and 0.08 mg/kg/day, respectively. Combining exposures from the dietary and residential exposures for children would, therefore, result in an aggregate exposure of 0.47 mg/kg/day. These calculations use a number of assumptions that have been extensively peer-reviewed[27] and yet the potential oral exposure of glyphosate for the most highly exposed residential population subgroup is more than 2,000 times lower than the highest doses tested in the animal carcinogenicity studies (see Appendix E for more details regarding these calculations). The maximum potential exposure calculated for occupational handlers would be 7 mg/kg/day, which is still significantly lower than the highest doses tested in the animal carcinogenicity studies. As a result, even though tumors were observed in animal carcinogenicity studies, the possibility of being exposed to these excessive dietary doses over time is considered implausible based on the currently registered use pattern and not relevant to human health risk assessment.

6.5 Uncertainty

When evaluating a database, it is also important to assess the uncertainties associated with the available data. When uncertainty is high there is less confidence in the exposure and effect estimates and, therefore, informs the reliability of results. Understanding the sources of uncertainty within a database can help characterize observed results and aid in developing new research with reduced uncertainty.

In some instances, the agency did not have access to all of the data underlying the studies analyzed for the current evaluation. This includes all of the epidemiological studies, 17 genotoxicity studies, and 1 animal carcinogenicity study. For these studies, the agency had to rely upon information the study authors reported. Without the raw data, statistical analyses could not be replicated or recalculated. On the other hand, studies that include full reports with detailed methodology, analytically measured doses, and individual animal data may provide a higher level of confidence. It also allows the agency to perform its own evaluation of the data using current practices and policies.

Several uncertainties have already been identified throughout this document. There are numerous metabolism studies available for glyphosate; however, the data are not sufficient to determine whether linear kinetics is occurring at high doses where tumors were observed in animal carcinogenicity studies. In the carcinogenicity test guideline (OCSPP 870.4200) and the 2005 Guidelines for Carcinogen Risk Assessment, inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms) should be avoided. A study evaluating the toxicokinetic profile of glyphosate using multiple doses is needed to further investigate the pharmacokinetic properties between low- and high-dose levels.

[27] Using the 2012 Standard Operating Procedures for Residential Exposure Assessment. Available: http://www2.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedures-residential-pesticide

With respect to the epidemiological data, the database is limited for each investigated cancer with only one or two studies available. Although six studies were used in the evaluation of NHL, the results were constrained by the limitations of the individual studies, such as small sample size/limited power, missing data, and control selection issues. The quality of the exposure assessment is a major concern since the validity of the overall study results depend in large part on the ability of the study to correctly quantify and classify a subject's exposure. There was no direct information on pesticide exposure or absorbed dose because the exposures were self-reported. All of the studies conducted exposure assessments through questionnaires and interviews that are susceptible to recall bias, which can result in exposure misclassification. The study with the highest ranking (De Roos *et al.*, 2005) did not find an association between glyphosate exposure and NHL; however, it has been noted that the median follow-up time for this study was ~7 years. A longer follow-up from the AHS cohort would be beneficial to better understand whether there is an association between glyphosate exposure and NHL. An update from the AHS cohort would also provide a more recent evaluation of glyphosate exposure and cancer outcomes. Many of the studies were conducted prior to the introduction of glyphosate-tolerant crops in 1996, which resulted in a dramatic increase of glyphosate use in subsequent use. More recent studies will help further elucidate the association between glyphosate exposure and cancer outcomes during this period of time.

Another consideration is that farmers and other applicators apply formulations, not the active ingredient alone. It is possible that different formulations were used across and/or within the different epidemiological studies. Formulations are end-use products that are sold as a mixture of registered pesticidal active ingredients, such as glyphosate, and additional substances that increase the effectiveness of a pesticidal product, which are often referred to as "inert ingredients." For example, inert ingredients may act as a solvent to allow a pesticide active ingredient to penetrate a plant's outer surface, may facilitate and accentuate the dispersion of the product, or may extend the pesticide product's shelf-life[28]. Inert ingredients and the proportion of these inert ingredients vary across formulations. It has been hypothesized that glyphosate formulations may be more toxic than glyphosate alone. Glyphosate has been studied in a multitude of studies and there are studies that have been conducted on numerous formulations that contain glyphosate; however, there are relatively few research projects that have attempted to systematically compare glyphosate and the formulations in the same experimental design. Furthermore, there are even less instances of studies comparing toxicity across formulations. This is one aspect of the uncertainty in the database that the agency has been working to address by developing a strategic research plan in collaboration with NTP (see Section 7.0).

It is recognized that these uncertainties exist for the current database; however, the available data are adequate for evaluating the carcinogenic potential of glyphosate and determine the cancer classification using the 2005 Guidelines for Carcinogen Risk Assessment. As discussed in Section 6.3, there are a large number of studies available and the database is remarkably consistent across these studies.

[28] https://www.epa.gov/pesticide-registration/inert-ingredients-overview-and-guidance

6.6 Evaluation of Cancer Classification per the 2005 EPA Guidelines for Carcinogen Risk Assessment

6.6.1 Introduction

In the 2005 Guidelines for Carcinogen Risk Assessment, five classification descriptors are provided:

- Carcinogenic to Humans
- Likely to be Carcinogenic to Humans
- Suggestive Evidence of Carcinogenic Potential
- Inadequate Information to Assess Carcinogenic Potential
- Not Likely to be Carcinogenic to Humans

Descriptors are assigned using all available data from the multiple lines of evidence. The following text has been excerpted/summarized from the guidelines regarding these descriptors:

> Choosing a descriptor is a matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence. The weight-of-evidence, including the selected descriptor, is presented as a narrative laying out the complexity of information that is essential to understanding the hazard and its dependence on the quality, quantity, and type(s) of data available. The descriptors and narratives are intended to permit sufficient flexibility to accommodate new scientific understanding and new testing methods. The descriptors represent points along a continuum of evidence; consequently, there are gradations and borderline cases that are clarified by the full weight-of-evidence narrative. Rather than focusing simply on the descriptor, the entire range of information included in the weight-of-evidence narrative should be considered.

The weight-of-evidence presented in Sections 6.2-6.5 and based on the available epidemiological, animal carcinogenicity, and genotoxicity data for glyphosate was considered for each classification descriptor. For each descriptor, the guidelines provide examples and/or conditions for when the descriptor may be appropriate and the weight-of-evidence for glyphosate is assessed to determine which descriptor is supported by the available data. As stated in the 2005 EPA Guidelines for Carcinogen Risk Assessment, "the entire range of information included in the weight-of-evidence should be considered". Based on all of the available data, the weight-of-evidence clearly do not support the descriptors "carcinogenic to humans" and "likely to be carcinogenic to humans" at this time. According to the 2005 Cancer Guidelines, "carcinogenic to humans" is appropriate "when there is convincing epidemiologic evidence of a causal association between human exposure and cancer." Similarly, "likely to be carcinogenic to humans" descriptor is appropriate "when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor."

In epidemiological studies, there was no evidence of an association between glyphosate exposure and solid tumors, leukemia, or HL. The available data for multiple myeloma are not considered adequate to assess carcinogenic potential and a conclusion regarding the association between

glyphosate exposure and risk of NHL cannot be determined based on the available data due to conflicting results and various limitations identified in studies investigating NHL. In 7 of the 15 animal carcinogenicity studies, no tumors were identified for detailed evaluation. In the remaining 8 studies, tumor incidences were not increased at doses <500 mg/kg/day, except for testicular tumors. The tumors observed at doses at or exceeding 1,000 mg/kg/day are not considered relevant to human health risk assessment. Tumor findings were not reproduced in studies in the same animal strain at similar or higher doses. Furthermore, the tumors often lacked a monotonic dose response, pairwise significance, and/or corroborating preneoplastic lesions. The mammalian MOA/AOP is unknown for glyphosate and precursor events are unknown; however, the genotoxicity data were highly reproducible and consistent with a clear demonstration that glyphosate does not have a mutagenic MOA.

The descriptor "inadequate information to assess carcinogenic potential" is used when available data are judged inadequate for applying one of the other descriptors. Given the extensive size of the glyphosate database, which includes a multitude of well-designed and well-conducted studies, this classification descriptor is not supported. The epidemiological data at this time are limited and study results appear to be inconsistent for some cancer types. However, it is important to note that epidemiological studies are not available for most pesticides. Similarly, for most pesticides, generally, only two animal bioassays are available. EPA routinely evaluates human cancer potential using the small, more typical datasets. As such, for glyphosate, given the significant amount of information across multiple lines of evidence, the agency believes the database is sufficient to designate a cancer classification descriptor for glyphosate and that "inadequate information to assess carcinogenic potential" is not appropriate.

The remaining two cancer classification descriptors (*"Suggestive Evidence of Carcinogenic Potential"* and *"Not Likely to Be Carcinogenic to Humans")* from the 2005 EPA Guidelines for Carcinogen Risk Assessment are described in detail below. Subsequently, these descriptors are discussed in the context of whether the available evidence do or do not support them.

"Suggestive Evidence of Carcinogenic Potential"

This descriptor is appropriate when a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. It covers a spectrum of evidence associated with varying levels of concern for carcinogenicity. Depending on the extent of the database, additional studies may or may not provide further insights.

Some examples of when this descriptor may be appropriate include the following:

- If a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight-of-evidence for the descriptor of "likely to be carcinogenic to humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system;
- If there is evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally flawed), but where the carcinogenic potential is strengthened by other lines of evidence;

- If there is a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed (when there is a high background rate of a specific tumor in animals of a particular sex and strain, then there may be biological factors operating independently of the agent being assessed that could be responsible for the development of the tumors). In this case, the reasons for determining that the tumors are not due to the agent are explained; or
- If there is a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend.

"Not Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the available data are considered robust for deciding that there is no basis for human hazard concern. In some instances, there can be positive results in experimental animals when there is strong, consistent evidence that each MOA in experimental animals does not operate in humans. In other cases, there can be convincing evidence in both humans and animals that the agent is not carcinogenic.

This descriptor would be appropriate if any of the following was observed:

- Animal evidence demonstrates lack of carcinogenic effects in both sexes in well-designed and well-conducted studies in at least two appropriate animal species in the absence of other animal or human data suggesting a potential for cancer effects, or
- Convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans, or
- Convincing evidence that carcinogenic effects are not likely by a particular exposure route, or
- Convincing evidence that carcinogenic effects are not likely below a defined dose range.

6.6.2 Discussion of Evidence to Support Cancer Classification Descriptors

As stated above, the available data and weight-of-evidence clearly do not support the descriptors "carcinogenic to humans", "likely to be carcinogenic to humans", or "inadequate information to assess carcinogenic potential". The following discusses the remaining cancer classification descriptors and how the evidence does or does not support the descriptors.

It could be argued that the "suggestive evidence of carcinogenic potential" descriptor would be appropriate. The evidence to support this includes:

- Non-statistically significant effect estimates greater than the null were reported for NHL across studies and meta-analyses based on ever/never use ranged from 1.3-1.5.
- There was limited evidence of a possible exposure-response relationship between glyphosate exposure and NHL.

- In several animal carcinogenicity studies, a statistically significant trend was observed. In some instances, tumor incidences at the highest dose tested were statistically significant as compared to concurrent controls using raw (unadjusted) p-values.
- Positive responses were observed in a limited number of genotoxicity assays evaluating chromosomal and primary DNA damage.

However, according to the 2005 EPA Guidelines for Carcinogen Risk Assessment, in order for the above evidence to support the "suggestive evidence of carcinogenic potential" descriptor, "the study generally would not be contradicted by other studies of equal quality in the same population group or experimental system". Furthermore, the guidelines state that "rather than focusing simply on the descriptor, the entire range of information included in the weight-of-evidence narrative should be considered". For the epidemiological studies evaluating NHL, half of the studies reported effect estimates for ever/never use ranging from 1.5-1.85, with the widest confidence interval observed for the highest effect estimate indicating the effect estimate is less reliable. In the other half of the studies, which were of equal or higher quality, the reported effect estimates were approximately equal to the null. All of the effect estimates were non-statistically significant. There were conflicting results in exposure-response assessments investigating glyphosate exposure and the risk of NHL. Although two-case control studies (McDuffie et al., 2001; Eriksson et al., 2008) reported elevated effect estimates when analyzing for exposure-response relationships across two exposure categories, extensive analyses in a study of equal or higher quality (De Roos et al., 2005) for cumulative lifetime exposure and intensity-weighted cumulative exposure contradicted these results reporting effect estimates less than null (ranging from 0.6-0.9) when analyzing across tertiles. Furthermore, the two-case control studies did not account for co-exposure to other pesticides, which would be expected to cause inflated effect estimates. Various limitations that could impact the calculated effect estimate were identified for these studies and discussed in Section 3.6. The effect estimates greater than the null were not strengthened by other lines of evidence, as described in Sections 6.2-6.5.

In 7 (5 rat and 2 mouse) of the 15 animal carcinogenicity studies conducted with glyphosate, no tumors were identified for detailed evaluation. Of the remaining 8 studies, 7 observed a statistically significant trend for a particular tumor type; however, the agency determined that these tumors findings are not considered to be related to treatment. Although a statistically significant trend was obtained, closer examination of the incidence data across doses did not demonstrate a monotonic dose responses in several instances. Although the incidence at the highest dose tested (approaching or exceeding 1,000 mg/kg/day for almost all studies) for some of these tumors were statistically significant from concurrent controls using raw (unadjusted) p-values, none of the pairwise comparisons were found to be statistically significant following adjustment for multiple comparisons, except the testicular tumors that were seen in a single study. Furthermore, these high-dose tumors were given less weight. There was no evidence of corroborating pre-neoplastic or related non-neoplastic lesions and tumors showed no evidence of tumor progression to support the biological significance of tumor findings. In a limited number of cases, the agency also considered historical control data to inform the relevance of tumor findings when incidence rates in the concurrent controls were unusually low. Lastly, tumors seen in individual studies were not reproduced in studies of equal quality, including studies in the same animal species and strain at similar or higher doses.

Although positive responses were observed in a limited number of genotoxicity assays evaluating chromosomal and primary DNA damage, the overall weight-of-evidence indicates that there is no convincing evidence that glyphosate induces mutations *in vivo* via the oral route. When administered via i.p. injection the studies were predominantly negative. In the two cases where an increase in micronuclei were reported via this route of administration, the results were contradicted by numerous other studies at similar or higher doses using the same assays and route of administration. Technical glyphosate was negative in all gene mutation studies. There was limited evidence of positive findings in studies evaluating primary DNA damage; however, the endpoints measured in these assays are less specific in regards to detecting permanent DNA changes (mutations) and can be attributed to other factors, such as cytotoxicity or cell culture conditions. Although some positive findings were reported for chromosomal alterations *in vitro,* these findings were limited to a few studies and are not supported by the *in vivo* studies that are the most relevant for human risk assessment.

In summary, considering the entire range of information for the weight-of-evidence, the evidence outlined above to potentially support the "suggestive evidence of carcinogenic potential" descriptor are contradicted by other studies of equal or higher quality and, therefore, the data do not support this cancer classification descriptor.

For the "not likely to be carcinogenic to humans" descriptor, one of the considerations is whether there is "convincing evidence that carcinogenic effects are not likely below a defined dose range". In the case of glyphosate, effects are not likely below 500 mg/kg/day based on oral studies. Tumor incidences were not increased in animal carcinogenicity at doses <500 mg/kg/day, except for the testicular tumors observed in a single study that were not considered treatment-related. In genotoxicity studies, assays with oral administration were negative except for one instance where an extremely high dose (5,000 mg/kg/day) was administered.

The 2005 EPA Guidelines for Carcinogen Risk Assessment also state that "weighing of the evidence includes addressing not only the likelihood of human carcinogenic effects of the agent but also the conditions under which such effects may be expressed". Increased tumor incidence was typically observed at doses of 1,000 mg/kg/day or greater. Additionally, the only *in vivo* positive assays seen in the genotoxicity studies were administered via i.p. injection at doses of 200 mg/kg/day and 300 mg/kg/day or orally at 5,000 mg/kg/day. These high doses are not considered relevant to human health risk assessment based on the currently registered use pattern for glyphosate. Maximum potential glyphosate exposure in residential and occupational settings have been estimated at 0.47 mg/kg/day and 7 mg/kg/day, respectively, which are well-below the doses necessary to elicit the effects seen in these animal carcinogenicity and genotoxicity studies. Additionally, non-linear kinetics may also be occurring at the high doses. The carcinogenicity test guidelines (OCSPP 870.4200 and OCSPP 870.4300) and the 2005 Guidelines for Carcinogen Risk Assessment state that inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms) should be avoided. A well-conducted pharmacokinetic study evaluating the toxicokinetic profile of glyphosate is needed to further investigate the toxicokinetic properties between high and low dose levels to ensure that inappropriate toxicokinetics is avoided.

Overall, there is not strong support for the "suggestive evidence of carcinogenic potential" cancer classification descriptor based on the weight-of-evidence, which includes the fact that even small, non-statistically significant changes observed in animal carcinogenicity and epidemiological studies were contradicted by studies of equal or higher quality. The strongest support is for "not likely to be carcinogenic to humans" at the doses relevant to human health risk assessment for glyphosate.

6.7 Proposed Conclusions Regarding the Carcinogenic Potential of Glyphosate

Glyphosate is a non-selective, phosphonomethyl amino acid herbicide registered to control weeds in various agricultural and non-agricultural settings. Labeled uses of glyphosate include over 100 terrestrial food crops as well as other non-agricultural sites, such as greenhouses, aquatic areas, and residential areas. Following the introduction of glyphosate-resistant crops in 1996, glyphosate use increased dramatically; however, glyphosate use has stabilized in recent years due to the increasing number of glyphosate-resistant weed species.

Since its registration in 1974, numerous human and environmental health analyses have been completed for glyphosate, which consider all anticipated exposure pathways. Glyphosate is currently undergoing Registration Review. As part of this process, the hazard and exposure of glyphosate are reevaluated to determine its potential risk to human and environmental health using current practices and policies. The human carcinogenic potential of glyphosate has been evaluated by the agency several times. As part of the current evaluation for Registration Review, the agency has performed a comprehensive analysis of available data from submitted guideline studies and the open literature. This includes epidemiological, animal carcinogenicity, and genotoxicity studies.

An extensive database exists for evaluating the carcinogenic potential of glyphosate, including 23 epidemiological studies, 15 animal carcinogenicity studies, and nearly 90 genotoxicity studies for the active ingredient glyphosate. These studies were evaluated for quality and results were analyzed across studies within each line of evidence. The modified Bradford Hill criteria were then used to evaluate multiple lines of evidence using such concepts as strength, consistency, dose response, temporal concordance and biological plausibility. The available data at this time do no support a carcinogenic process for glyphosate. Overall, animal carcinogenicity and genotoxicity studies were remarkably consistent and did not demonstrate a clear association between glyphosate exposure and outcomes of interest related to carcinogenic potential. In epidemiological studies, there was no evidence of an association between glyphosate exposure and numerous cancer outcomes; however, due to conflicting results and various limitations identified in studies investigating NHL, a conclusion regarding the association between glyphosate exposure and risk of NHL cannot be determined based on the available data. Increases in tumor incidence were not considered treatment-related in any of the animal carcinogenicity studies. In 7 of these studies, no tumors were identified for detailed evaluation. In the remaining studies, tumor incidences were not increased at doses <500 mg/kg/day, except for the testicular tumors observed in a single study. Increased tumor incidences at or exceeding the limit dose (≥1000 mg/kg/day) are not considered relevant to human health. Furthermore, data from epidemiological and animal carcinogenicity studies do not reliably demonstrate expected dose-response relationships.

For cancer descriptors, the available data and weight-of-evidence clearly do not support the descriptors "carcinogenic to humans", "likely to be carcinogenic to humans", or "inadequate information to assess carcinogenic potential". For the "suggestive evidence of carcinogenic potential" descriptor, considerations could be looked at in isolation; however, following a thorough integrative weight-of-evidence evaluation of the available data, the database would not support this cancer descriptor. The strongest support is for "not likely to be carcinogenic to humans" at doses relevant to human health risk assessment.

This analysis integrating multiple lines of evidence highlights the need for mechanistic studies to elucidate the MOA/AOP of glyphosate, as well as additional epidemiology studies and updates from the AHS cohort study to further investigate the carcinogenic potential of glyphosate in humans. This evaluation focused on studies on the active ingredient glyphosate; however, additional research could also be performed to determine whether formulation components, such as surfactants, influence the toxicity of glyphosate formulations. The agency has been working on plans to initiate research given these identified data gaps and these plans are described in Section 7.0.

The agency is soliciting advice from the FIFRA SAP on the evaluation and interpretation of the available data for each line of evidence for the active ingredient glyphosate and the weight-of-evidence analysis, as well as how the available data inform cancer classification descriptors according to the agency's 2005 Guidelines for Carcinogen Risk Assessment.

7.0 Collaborative Research Plan for Glyphosate and Glyphosate Formulations

As previously mentioned, some have believed that glyphosate formulations may be more toxic than glyphosate alone. Glyphosate has been studied in a multitude of studies and there are studies that have been conducted on numerous formulations that contain glyphosate; however, there are relatively few research projects that have attempted to directly compare glyphosate and the formulations in the same experimental design. Furthermore, there are even less instances of studies comparing toxicity across formulations.

The agency has been collaborating with the NTP Division of the National Institute of Environmental Health Sciences to develop a research plan intended to evaluate the role of glyphosate in product formulations and the differences in formulation toxicity. Four objectives were identified that laid out how research by NTP might contribute to these research questions: 1) compare the toxicity of glyphosate vs. formulations, as well as compare formulations vs. formulations, 2) provide publicly available toxicology data on cancer-related endpoints, 3) provide publicly available toxicology data on non-cancer endpoints, and 4) investigate the mechanisms of how glyphosate and formulations cause toxic effects.

As part of the first objective, NTP will investigate the differential biological activity of glyphosate, glyphosate formulations, and the individual components of formulations. . The NTP Laboratory Branch generated preliminary data by exposing human hepatoma cells (HepG2) to five different glyphosate products bought off the shelf. The endpoint in the assay was cell viability, measured by ATP levels. The data, presented in Figure 7.1, demonstrate at-a-glance

that formulations are not equally toxic and that the toxicity is not being driven by the amount of glyphosate in the formulations, at least for the endpoint of cell viability. This observation highlights how informative the data generated from this research can be to the overall research questions.

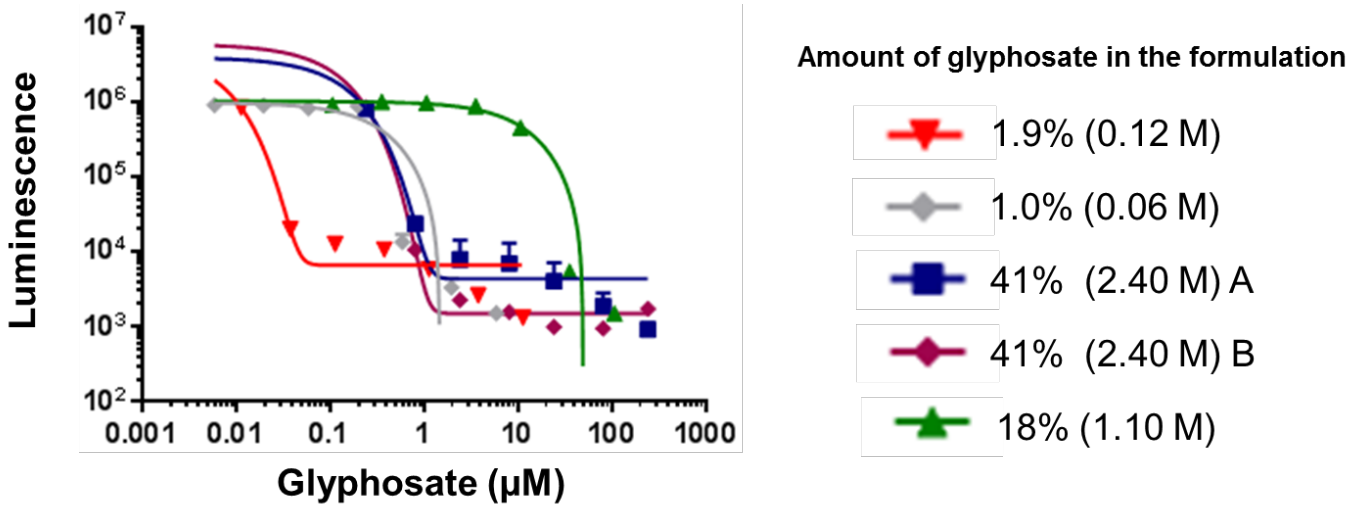

Figure 7.1. Results of HepG2 exposures following 24 hour incubation using different glyphosate formulations.

For the second objective, NTP will contribute to the publicly available knowledge-base describing the biological effects of glyphosate and formulations by conducting guideline studies addressing genotoxicity and studies that evaluate the oxidative stress potential. In order to organize publicly available data on glyphosate and formulations, IARC used 10 key characteristics of carcinogens as a way to help inform their conclusion (Smith *et al.*, 2016). Their review concluded that data were only available for two of these characteristics (genotoxicity and oxidative stress) and little to no information on the remaining characteristics was available. However, when members of a NTP workgroup looked at the available data included in the IARC review, the group did not agree with IARC that the data provided strong or clear evidence for either genotoxicity or induction of oxidative stress given protocol deficiencies that could produce questionable results.

Currently, the publicly available information regarding non-cancer endpoints for glyphosate and glyphosate formulations is limited. To begin to address the third objective, NTP will conduct a screening level analysis of the literature using text mining software, for studies regarding non-cancer endpoints resulting from glyphosate exposure. The resulting scoping report will describe the evidence base for health outcomes investigated in connection to glyphosate, as well as help identify data gaps.

As discussed in Section 6.0, there is a need for mechanistic studies to elucidate the MOA/AOP of glyphosate. Although there are data suggesting glyphosate may be genotoxic or cause oxidative stress, there is little mechanistic information to support these observations. For the last objective, NTP will use *in vitro* screening assays to gain mechanistic information on the effects

of glyphosate and different formulations for a variety of endpoints and allow for direct comparisons among them. The screening approach will also allow for the identification of test substances that would be good candidates for further *in vivo* testing. Since *in vivo* findings in genetic toxicology testing are generally considered as having a greater relevance to humans than *in vitro* findings, it is valuable to confirm the results seen at the cellular level at the whole animal level.

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Appendix A. Journal articles obtained from open literature search

Abstract Only	Cebollero, L. R., et al. (2011). "Glyphosate based herbicides toxicity, a new approach." Toxicology Letters 205, Supplement: S233.
Abstract Only	Monroy, C. M., et al. (2004). "In vitro evaluation of glyphosate-induced DNA damage in fibrosarcoma cells HT1080 and Chinese hamster ovary (CHO) cells." Environ Mol Mutagen 44(3): 216-216.
Abstract Only	Ramos-Morales, P., et al. (2008). "Combined use of multiple biomarkers to evaluate the genotoxic activity of the herbicide Glyphosate." Environ Mol Mutagen 49(7): 577-577.
Abstract Only/Full article already identified	Sorahan, T. (2015). "Multiple Myeloma and Glyphosate Use: A Re-Analysis of US Agricultural Health Study (AHS) Data." Int J Environ Res Public Health 12(2): 1548-1559.
Article not in English	Kwiatkowska, M., et al. (2013). "GLYPHOSATE AND ITS FORMULATIONS - TOXICITY, OCCUPATIONAL AND ENVIRONMENTAL EXPOSURE." Med Pr 64(5): 717-729.
Article not in English	Lawson, R. and E. Estrade-Chapellaz (1999). "Intoxication volontaire par le glufosinate (Basta®)." Annales Françaises d'Anesthésie et de Réanimation 18(9): 1025-1026.
Article not in English	Mañas, F., et al. (2009). "Aberraciones cromosómicas en trabajadores rurales de la Provincia de Córdoba expuestos a plaguicidas." BAG. Journal of basic and applied genetics 20(1): 0-0.
Article not in English	Martinez, A., et al. (2007). "[Cytotoxicity of the herbicide glyphosate in human peripheral blood mononuclear cells]." Biomedica 27(4): 594-604.
Article not in English	Monroy, C. M., et al. (2005). "[Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate]." Biomedica 25(3): 335-345.
Article not in English	Pieniazek, D., et al. (2003). "[Glyphosate--a non-toxic pesticide?]." Med Pr 54(6): 579-583.
Article not in English	Saratovskikh, E. A., et al. (2007). "Genotoxicity of the pestiside in Ames test and the possibility to formate the complexeses with DNA." Ekologicheskaya genetika V(3): 46-54.
Article not in English	В статье представлены результаты генотоксико логических, аллергологических и иммунологических исследований, проведенных в рамках медико-биологической оценки безопасности генно-инженерно-моди-фицированной кукурузы линии MON 88017, устойчивой к глифосату и жуку Diabrotica spp. Анализ данных, полученных при изучении уровня повреждений ДНК и уровня хромосомных аберраций, тяжести активного анафилактического шока и интенсивности гуморального иммунного ответа, состояния гуморального и клеточного звеньев иммунитета, не выявил какого-либо генотоксического, аллергенного, иммуномодулирующего и сенсибилизирующего действия ГМ-кукурузы линии MON 88017 по сравнению с ее традиционным аналогом.
Cancer treatment	Parajuli, K. R., et al. (2015). "Aminomethylphosphonic acid and methoxyacetic acid induce apoptosis in prostate cancer cells." Int J Mol Sci 16(5): 11750-11765.
Cancer treatment	Li, Q., et al. (2013). "Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis." Drug Des Devel Ther 7: 635-643.
Cancer treatment	Parajuli, K. R., et al. (2016). "Aminomethylphosphonic acid inhibits growth and metastasis of human prostate cancer in an orthotopic xenograft mouse model." Oncotarget 7(9): 10616-10626.
Correspondence article	Belle, R., et al. (2012). "LETTER TO THE EDITOR: TOXICITY OF ROUNDUP AND GLYPHOSATE." Journal of Toxicology and Environmental Health-Part B-Critical Reviews 15(4): 233-235.
Correspondence article	Carrasco, A. E. (2011). "Reply to the Letter to the Editor Regarding Our Article (Paganelli et al., 2010)." Chem Res Toxicol 24(5): 610-613.
Correspondence article	de Souza, L. and L. Macedo Oda (2013). "Letter to the editor." Food and Chemical Toxicology 53: 440.
Correspondence article	de Vendomois, J. S., et al. (2010). "Debate on GMOs Health Risks after Statistical Findings in Regulatory Tests." Int J Biol Sci 6(6): 590-598.
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Correspondence article	Grunewald, W. and J. Bury (2013). "Comment on "Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize" by Seralini et al." Food and Chemical Toxicology 53: 447-448.
Correspondence article	Huang, K. and W. Xu (2013). "Reply to letter to the editor." Food and Chemical Toxicology 59: 811-812.
Correspondence article	Korsaeth, A., et al. (2015). "Comments on the recently published study: “Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans”, by T. Bøhn, M.

	Cuhra, T. Traavik, M. Sanden, J. Fagan and R. Primicerio (Food Chemistry 2014, 153: 207–215)." Food Chemistry 172: 921-923.
Correspondence article	Ollivier, L. (2013). "A Comment on "Séralini, G.-E., et al., Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem. Toxicol. (2012)," http://dx.doi.org/10.1016/j.fct.2012.08.005." Food and Chemical Toxicology 53: 458.
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Effects on cellular processes	Benachour, N. and G.-E. Seralini (2009). "Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells." Chem Res Toxicol 22(1): 97-105.
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Not Relevant to current fit for purpose review	Zhang, H., et al. (2008). "Development of one novel multiple-target plasmid for duplex quantitative PCR analysis of roundup ready soybean." Journal of Biotechnology 136, Supplement: S250.
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Not Relevant to current fit for purpose review	Zhang, Q., et al. (2014). "Characterization of cytochalasins from the endophytic Xylaria sp. and their biological functions." J Agric Food Chem 62(45): 10962-10969.
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Not Relevant to current fit for purpose review	Ziegelberger, G., et al. (2006). "International commission on non-ionizing radiation protection." Progress in Biophysics and Molecular Biology 92(1): 1-3.
Not Relevant to current fit for purpose review	Zulet, A., et al. (2015). "Fermentation and alternative oxidase contribute to the action of amino acid biosynthesis-inhibiting herbicides." J Plant Physiol 175: 102-112.

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Relevant- Cancer Epi	Chang, E. T. and E. Delzell (2016). "Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers." J Environ Sci Health B 51(6): 402-434.
Relevant- Cancer Epi	De Roos, A. J., et al. (2005). "Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study." Environ Health Perspect 113(1): 49-54.
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Relevant- Genotoxicity	Alvarez-Moya et al. (2014) "Comparison of the in vivo and in vitro genotoxicity of glyphosate isopropylamine salt in three different organisms". *Genetics and Molecular Biology*, 37, 1, 105-110
Relevant- Genotoxicity	Chan, P. and J. Mahler (1992). "NTP technical report on the toxicity studies of Glyphosate (CAS No. 1071-83-6) Administered In Dosed Feed To F344/N Rats And B6C3F1 Mice." Toxic Rep Ser 16: 1-d3.
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Relevant- Genotoxicity	Da Silva, F. R., et al. (2014). "Genotoxic assessment in tobacco farmers at different crop times." Science of the Total Environment 490: 334-341.
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Relevant- Genotoxicity	El-Shenawy, N. S. (2009). "Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate." Environ Toxicol Pharmacol 28(3): 379-385.
Relevant- Genotoxicity	Fortes, C., et al. (2016). "Occupational Exposure to Pesticides With Occupational Sun Exposure Increases the Risk for Cutaneous Melanoma." J Occup Environ Med 58(4): 370-375.
Relevant- Genotoxicity	Ghisi Nde, C., et al. (2016). "Does exposure to glyphosate lead to an increase in the micronuclei frequency? A systematic and meta-analytic review." Chemosphere 145: 42-54.
Relevant- Genotoxicity	Heydens, W. F., et al. (2008). "Genotoxic potential of glyphosate formulations: mode-of-action investigations." J Agric Food Chem 56(4): 1517-1523.
Relevant- Genotoxicity	Kale, P. G., et al. (1995). "MUTAGENICITY TESTING OF 9 HERBICIDES AND PESTICIDES CURRENTLY USED IN AGRICULTURE." Environ Mol Mutagen 25(2): 148-153.
Relevant- Genotoxicity	Kier, L. D. (2015). "Review of genotoxicity biomonitoring studies of glyphosate-based formulations." Crit Rev Toxicol 45(3): 209-218.
Relevant- Genotoxicity	Koller, V. J., et al. (2012). "Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells." Arch Toxicol 86(5): 805-813.
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Relevant- Genotoxicity	Lioi, M. B., et al. (1998). "Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro." Mutat Res 403(1-2): 13-20.
Relevant- Genotoxicity	Manas, F., et al. (2009). "Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests." Ecotoxicol Environ Saf 72(3): 834-837.
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Relevant- Genotoxicity	Mladinic, M. and D. Zeljezic (2008). "Assessment of oxidative DNA damage by glyphosate applying hOGG1 modified comet and micronucleus assay." Toxicology Letters 180: S170-S171.

Relevant- Genotoxicity	Paz-y-Mino, C., et al. (2011). "Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border." Rev Environ Health 26(1): 45-51.
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Relevant- Genotoxicity	Sivikova, K. and J. Dianovsky (2006). "Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes." Int J Hyg Environ Health 209(1): 15-20.
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Retracted Article	Séralini, G.-E., et al. (2014). "Retraction notice to “Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize” [Food Chem. Toxicol. 50 (2012) 4221–4231]." Food and Chemical Toxicology 63: 244.

Appendix B

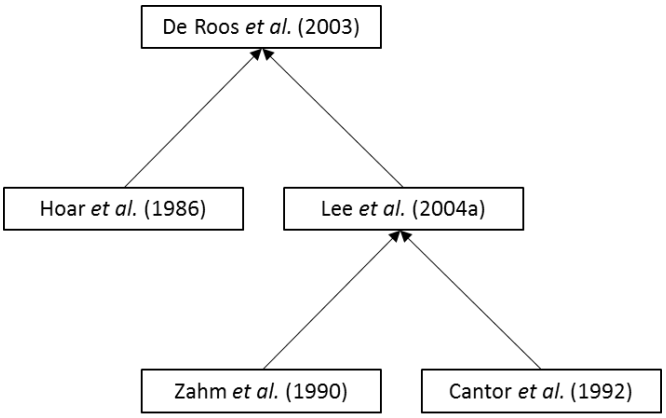

Figure B.1. Visual representation of studies included in De Roos *et al.* (2003).

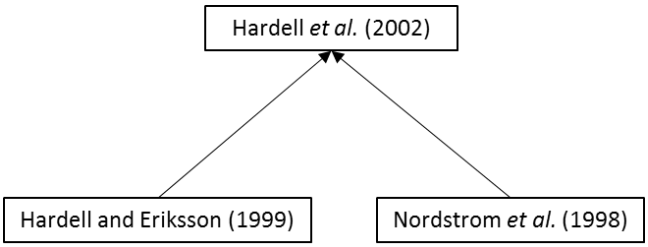

Figure B.2. Visual representation of studies included in Hardell *et al.* (2002).

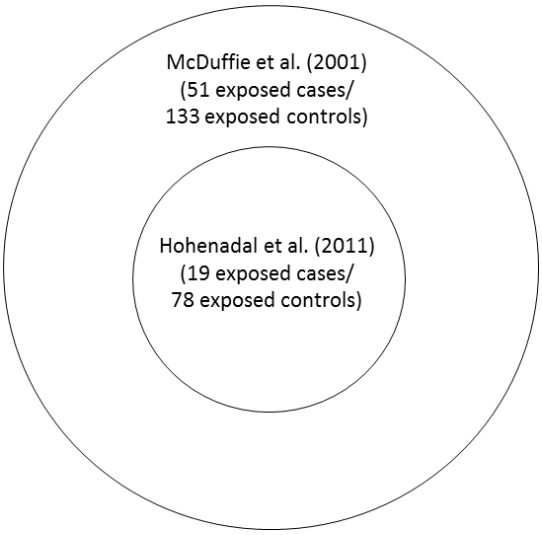

Figure B.3. Visual representation of the association between McDuffie *et al.* (2001) and the follow-up analysis by Hohenadal *et al.* (2011).

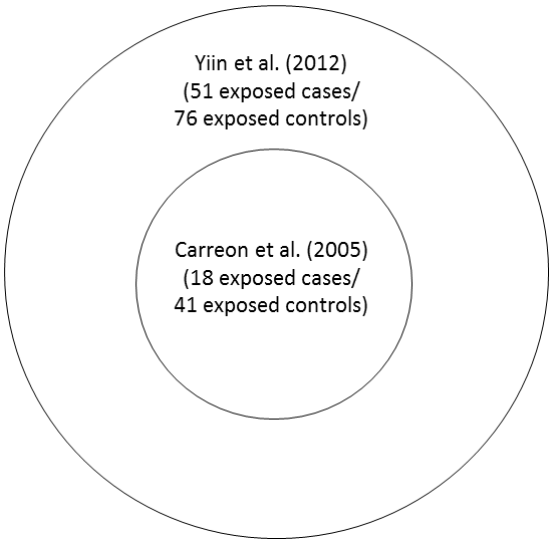

Figure B.4. Visual representation of the association between Carreon *et al.* (2005), which investigated gliomas in women only, and Yiin *et al.* (2012), which investigated both sexes.

Appendix C

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.

Study	Location	Study Years	Case Population	Control Population	Total Number of Subjects	Number of Glyphosate Exposed Cases	Proxy Use
Alavanja *et al.* (2003)	USA: Iowa and North Carolina	Enrollment (1993-1997) through 2001	Males enrolled in AHS; licensed private and commercial applicators	Males enrolled in AHS; licensed private and commercial applicators	566 cases 54,766 controls	not reported	No
Andreotti *et al.* (2009)	USA: Iowa and North Carolina	Enrollment (1993-1997) through 2004	Participants enrolled in AHS; licensed private and commercial applicators and spouses	Participants enrolled in AHS; licensed private and commercial applicators and spouses	93 cases (64 applicators, 29 spouses) 82,503 controls (52,721 applicators, 29,782 spouses)	55 cases 48,461 controls	No
Band *et al.* (2011)	Canada: British Columbia	1983-1990	Male residents in British Columbia identified as cancer patients in British Columbia Cancer Registry (excluding farmers that worked all outside British Columbia)	Male residents in British Columbia identified as cancer patients in British Columbia Cancer Registry (excluding farmers that worked all outside British Columbia) with other cancer sites excluding lung cancer and cancers of unknown primary site	1,153 cases 3,999 controls	25 cases 60 controls	Yes (included in adjustment)
Brown *et al.* (1990)	USA: Iowa and Minnesota	Iowa: 1981-1983; Minnesota: 1980-1982 Initial interview 1981-1984 and supplemental interviews (Iowa only) in 1987	White males (30 years or older) residing in Iowa or Minnesota diagnosed with leukemia	White males without lymphatic or hematopoietic cancer selected by random digit dialing (< age 65), Medicare records (age > 65) and state death certificate files (deceased controls) - frequency matched for 5-year age group, vital status, and state of residence	Initial: 578 cases; 1245 controls Supplemental: 92 cases; 211 controls	15 cases 49 controls	Yes (not evaluated)
Brown *et al.* (1993)	USA: Iowa	Iowa: 1981-1983; Interview 1981-1984	White males (30 years or older) residing in Iowa diagnosed with multiple myeloma	White males without lymphatic or hematopoietic cancer selected by random digit dialing (< age 65), Medicare records (age >	173 cases 650 controls	11 cases 40 controls	Yes (not evaluated)

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.							
Study	**Location**	**Study Years**	**Case Population**	**Control Population**	**Total Number of Subjects**	**Number of Glyphosate Exposed Cases**	**Proxy Use**
				65) and state death certificate files (deceased controls) - frequency matched for 5-year age group, vital status, and state of residence			
Cocco *et al.* (2013)	Czech Republic, France, Germany, Italy, Ireland, and Spain	1998-2004	Adult patients first diagnosed with lymphoma residing in the referral area of the participating centers	Controls from Germany and Italy were randomly selected by sampling from the general population, matched to cases on sex, 5-year age-group, and residence area. The rest of the centers used matched hospital controls, with eligibility criteria limited to diagnoses other than cancer, infectious diseases, and immunodeficient diseases	2,348 cases 2,462 controls	4 cases 2 controls	No
De Roos *et al.* (2003)	USA: Nebraska, Iowa, Minnesota, and Kansas	Nebraska: 1983-1986 Iowa: 1981-1983 Minnesota: 1980-1982 Kansas: 1979-1981	White males diagnosed with NHL in one of the 4 states (21 years or older in Nebraska and Kansas; 30 years or older in Iowa and Minnesota)	Males living in same geographic area obtained by random digit dialing, Medicare records and state mortality files - frequency matched for race, sex, age, and vital status	870 cases 2,569 controls	36 cases 61 controls	Yes (not significant in covariate analysis)
De Roos *et al.* (2005)	USA: Iowa and North Carolina	Enrollment (1993-1997) through 2001	Participants enrolled in AHS; licensed private and commercial applicators and spouses	Participants enrolled in AHS; licensed private and commercial applicators and spouses	54,315 subjects included in this analysis	All cancers – 358 cases Lung – 26 cases Oral cavity – 10 cases Colon – 15 cases Rectum – 14 cases Pancreas – 7 cases Kidney – 9 cases Bladder – 17 cases Prostate – 145 cases Melanoma – 14 cases All lymphohematopoietic cancers – 36 cases NHL – 17 cases Leukemia – 9 cases	No

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.							
Study	**Location**	**Study Years**	**Case Population**	**Control Population**	**Total Number of Subjects**	**Number of Glyphosate Exposed Cases**	**Proxy Use**
						Multiple myeloma – 6 cases (13,280 subjects not exposed to glyphosate used for comparison population)	
Engel *et al.* (2005)	USA: Iowa and North Carolina	Enrollment (1993-1997) through 2000	Wives of applicators enrolled in AHS study with no history of breast cancer	Wives of applicators enrolled in AHS study with no history of breast cancer	309 cases 30,145 controls	82 cases; 10,016 controls	No
Eriksson *et al.* (2008)	Sweden	1999-2002	Patients (18-74 years of age) residing in Sweden and diagnosed with NHL	Swedish residents randomly selected living in same health service regions as cases - frequency matched for age (in 10 years) and sex	910 cases 1,016 controls	29 cases 18 controls	No
Flower *et al.* (2004)	USA: Iowa	1993-1997	Children (born after 1975) of participants enrolled in AHS study who were diagnosed with childhood cancer up to 19 years of age	Children (born after 1975) of participants enrolled in AHS study not diagnosed with childhood cancer up to 19 years of age	50 cases out of 17,357 total study population	Maternal use: 13 cases of 6075 total exposed Paternal use: 6 cases of 3231 total exposed	No
Hardell *et al.* (2002)	Sweden	NHL: 1987-1990 HCL: 1987-1992	NHL: Male residents of one of four northern or three middle counties in Sweden age 25 years and older diagnosed with NHL; identified from regional cancer registries HCL: Living male residents of Sweden age 25 years and older diagnosed with HCl; identified from the Swedish Cancer Registry	NHL: Two male controls for each case matched by age, year of death if deceased, and county HCL: Four male controls for each case matched by age and county	515 cases 1,141 controls	8 cases 8 controls	Yes (not evaluated)
Kachuri *et al.* (2013)	Canada: Alberta, British Columbia, Manitoba, Ontario,	1991–1994	Men aged ≥ 19 years (≥ 30 years in analysis) - pulled from hospital records in Quebec,	Men aged ≥ 19 years (30 years in analysis) - pulled from provincial health insurance records in	342 cases 1,357 controls	32 cases 121 controls	Yes (included in adjustment)

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.							
Study	**Location**	**Study Years**	**Case Population**	**Control Population**	**Total Number of Subjects**	**Number of Glyphosate Exposed Cases**	**Proxy Use**
	Quebec, and Saskatchewan		cancer registries in all other provinces	Alberta, Saskatchewan, Manitoba, and Quebec; computerized telephone listings in Ontario; voter lists in British Columbia			
Karunanayake *et al.* (2012)	Canada: Alberta, British Columbia, Manitoba, Ontario, Quebec, and Saskatchewan	1991–1994	Men aged ≥ 19 years - pulled from hospital records in Quebec, cancer registries in all other provinces	Men aged ≥ 19 years - pulled from provincial health insurance records in Alberta, Saskatchewan, Manitoba, and Quebec; computerized telephone listings in Ontario; voter lists in British Columbia	316 cases 1,506 controls	38 cases 133 controls	No
Koureas *et al.* (2014)	Greece	2010	Inhabitants of the city of Larissa; Eligibility criteria for pesticide sprayers were 1) to personally apply pesticides systematically, and 2) to have recently applied pesticides (no longer than 7 days between last application and sampling).	The rural residents group were occupied in administrative services, public order services, health services, education or trade. Inclusion criteria for this group: absence of any involvement in agricultural activities either as a primary or secondary occupation by participant or any member of household. Also recruited urban residents (mainly blood donors) from the city of Larissa.	80 pesticide sprayers, 85 rural residents, and 121 individuals	Not reported	No
Koutros *et al.* (2013)	USA: Iowa and North Carolina	Enrollment (1993-1997) through 2007	Males enrolled in AHS; licensed private and commercial applicators	Males enrolled in AHS; licensed private and commercial applicators	1,962 incident cases (including 919 aggressive prostate cancers) among 54,412 applicators	1464 cases 42,420 controls	No
Landgren *et al.* (2009)	USA: Iowa and North Carolina	Exposure information: enrollment (1993-1997) and 5-year follow-up interview	Males enrolled in AHS; licensed private and commercial applicators	Males enrolled in AHS; licensed private and commercial applicators	678 participants	27 cases out of 570 total exposed	No

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.							
Study	**Location**	**Study Years**	**Case Population**	**Control Population**	**Total Number of Subjects**	**Number of Glyphosate Exposed Cases**	**Proxy Use**
		Blood samples: 2006-2007 (Iowa) and 2008 (North Carolina)					
Lee *et al.* (2004b)	USA: Nebraska	1988-1993	White residents of 1 of 66 Nebraska counties age 21 years or older with a newly confirmed case of adenocarcinoma of the stomach or Cases identified from the Nebraska Cancer Registry (1988–1990) or from discharge diagnosis and pathology records from 14 Nebraska hospitals (1991–1993)	Frequency matched by age and sex to the combined distribution of glioma, stomach, and esophageal cancer cases from a control group from a previous study (1986–1987) that selected controls from the general population by random digit dialing for those under 65 years, Health Care Financing Administration Medicare files for those over 65 years, mortality records for deceased and matched by race, sex, vital status (or year of death if deceased)	Stomach: 170 cases Esophagus: 137 cases 502 Controls	12 cases 46 controls	Yes (analysis showed differences)
Lee *et al.* (2005)	USA: Nebraska	1988-1993	White residents of 1 of 66 Nebraska counties age 21 years or older with confirmed adult glioma. Cases identified from Nebraska Cancer Registry or from participating hospitals in Lincoln and Omaha, Nebraska	Frequency matched by age, sex, and vital status to the combined distribution of glioma, stomach, and esophageal cancer cases from a control group from a previous study (1986–1987) that selected controls from the general population by random digit dialing for those under 65 years, Medicare files for those over 65 years, mortality records for deceased and matched by race, sex, vital status (or year of death if deceased), and 5-year age groups to the overall case distribution. Additional	251 glioma cases 498 controls	17 cases 32 controls	Yes (analysis showed differences, included in adjustment)

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.							
Study	**Location**	**Study Years**	**Case Population**	**Control Population**	**Total Number of Subjects**	**Number of Glyphosate Exposed Cases**	**Proxy Use**
				younger controls were brought into the study through random digit dialing and from death certificates			
Lee *et al.* (2007)	USA: Iowa and North Carolina	1993-97; follow-up to 2002	Agricultural Health Study participants: private and commercial applicators licensed in Iowa or North Carolina with no history of colorectal cancer at enrollment. Followed through 2002 for incident colorectal cancer	Agricultural Health Study participants: private and commercial applicators licensed in Iowa or North Carolina with no history of colorectal cancer at enrollment. Followed through 2002 for incident colorectal cancer	56,813 licensed pesticide applicators 305 incident colorectal cancer cases (212 colon, 93 rectum) 56,508 controls	Colon - 151 cases; 49 controls Rectum - 74 cases; 18 controls Colorectal - 225 cases; 67 controls	No
McDuffie *et al.* (2001)	Canada: Alberta, British Columbia, Manitoba, Ontario, Quebec, and Saskatchewan	1991-1994	Male residents of six Canadian provinces age 19 years and older diagnosed with STS, HD, NHL, or MM; this study only evaluated those with NHL. Cases were identified from Canadian Cancer Registries; in Quebec, hospital ascertainment was used	Random control subject selection using Health Insurance records, computerized telephone listings, and voters' lists; males 19 years and older from same six Canadian provinces as cases matched by age (within 2 years)	517 cases 1506 controls	Univariate analysis: 51 cases; 133 controls (multivariate analysis also conducted - glyphosate exposed numbers not reported)	No
Orsi *et al.* (2009)	France	2000-2004	Men aged 20–75 years living in the catchment areas of the main hospitals in Brest, Caen, Nantes, Lille, Toulouse, and Bordeaux, with no history of immunosuppression or taking immunosuppressant drugs. Cases ascertained from hospital records.	Patients from the same hospital catchment area as the cases. Patients were hospitalized for orthopedic or rheumatological conditions (89.3%), gastrointestinal or genitourinary tract diseases (4.8%), cardiovascular diseases (1.1%), skin and subcutaneous tissue disease (1.8%), and infections (3.0%), excluding patients admitted for cancer or a disease directly related to	491 cases 456 controls	NHL: 12 cases 24 controls HL: 6 cases 15 controls Lymphoproliferative syndromes: 4 cases 18 controls Multiple myeloma: 5 cases;18 controls Lymphoid neoplasms: 27 cases; 24 controls	No

Table B.1. Design Characteristics of Epidemiological Studies Evaluated for Study Quality.							
Study	**Location**	**Study Years**	**Case Population**	**Control Population**	**Total Number of Subjects**	**Number of Glyphosate Exposed Cases**	**Proxy Use**
				occupation, smoking, or alcohol abuse			
Pahwa *et al.* (2011)	Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, and Saskatchewan)	1991-1994	Men aged ≥ 19 years - pulled from hospital records in Quebec, cancer registries in all other provinces	Men aged ≥ 19 years - pulled from provincial health insurance records in Alberta, Saskatchewan, Manitoba, and Quebec; computerized telephone listings in Ontario; voter lists in British Columbia	342 cases 1,506 age/resident matched controls	32 cases 133 controls	No
Pahwa *et al.* (2012)	Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, and Saskatchewan)	1991-1994	Men aged ≥ 19 years - pulled from hospital records in Quebec, cancer registries in all other provinces	Men aged ≥ 19 years - pulled from provincial health insurance records in Alberta, Saskatchewan, Manitoba, and Quebec; computerized telephone listings in Ontario; voter lists in British Columbia	342 cases 1506 age/resident matched controls	32 cases 133 controls	No
Yiin *et al.* (2012)	USA: Upper Midwest Health Study (Iowa, Michigan, Minnesota and Wisconsin)	1995-1997	Age 18–80 (at ascertainment or diagnosis in 1995 through January 1997) residing in counties where the largest population center had fewer than 250,000 residents. Referral by physicians or through state cancer registries with cases verified by histological evaluation.	Controls age 18–64 randomly selected from state driver's license/nondriver ID records, and those age 65–80 were selected from Health Care Financing Administration's (HCFA) Medicare data within 10-year age group strata, with the proportion/stratum determined by the age distribution of glioma cases in that state from 1992 to 1994. Controls were frequency-matched within a state but not by county of residence. Selected even if they had a self-reported history of cancer other than glioma.	798 glioma cases; 1,175 controls	12 cases 19 controls	Yes (analysis showed no differences)

Appendix D. List of studies assigned a low quality ranking and not evaluated in detail

As described in Section 3.2, if studies did not collect exposure information on glyphosate from all subjects, did not assess an outcome (e.g., biomonitoring studies), and/or did not provide a quantitative measure of an association between glyphosate and a cancer outcome, then these studies were assigned a low quality ranking and were not further evaluated in detail. These studies included the following 32 studies:

Acquavella *et al.* 2006; Andre *et al.*, 2007; Baker *et al.* 2005; Benedetti *et al.*, 2013; Bolognesi *et al.*, 2002; Bolognesi *et al.*, 2004; Bolognesi *et al.* 2009; Bortoli *et al.*, 2009; Costa *et al.*, 2006; Da Silva *et al.* 2014; Dennis *et al.* 2010; Firth *et al.* 2007; Gomez-Arroyo *et al.*, 2013; Gregio D'Arce *et al.*, 2000; El-Zaemey *et al.*, 2013; Fortes *et al.*, 2016; Fritschi *et al.*, 2005; Hernandez *et al.*, 2006; Kaufman *et al.* 2009; Khayat *et al.*, 2013; Lebailly *et al.*, 2003; Mandel *et al.* 2005; Martinez-Valenzuela *et al.*, 2009; Monge *et al.*, 2007; Pastor *et al.*, 2003; Paz-y Mino *et al.*, 2007; Paz-y Mino *et al.* 2011; Ruder *et al.* 2004; Shaham *et al.*, 2001; Silva Kahl *et al.* 2016; Simoniello *et al.*, 2008; Vlastos *et al.*, 2006.

Appendix E

Chronic Dietary Exposure

The agency uses Dietary Exposure Evaluation Model- Food Consumption Intake Database (DEEM-FCID; version 3.16), which incorporates consumption data from United States Department of Agriculture (USDA) National Health and Nutrition Examination Survey, What We Eat in America (NHANES/WWEIA; 2003-2008) to calculate potential chronic dietary exposures. In an unrefined chronic dietary analysis, several conservative assumptions are used to generate high end estimates of potential exposure. These assumptions include tolerance-level residues for all registered commodities, 100% crop treated, and drinking water values from a direct application to water scenario, as well as DEEM default processing factors. For glyphosate, the highest exposure value for any population subgroup in an unrefined chronic dietary analysis would be 0.23 mg/kg/day for children 1-2 years old (Table E.1; DEEM inputs and results attached below).

Table E.1. Chronic dietary exposure estimates

Population Subgroup	Exposure (mg/kg/day)
General U.S. Population	0.091515
All Infants (< 1 year old)	0.142826
Children 1-2 years old	**0.230816**
Children 3-5 years old	0.214117
Children 6-12 years old	0.149269
Youth 13-19 years old	0.089636
Adults 20-49 years old	0.076396
Adults 50-99 years old	0.062987
Females 13-49 years old	0.071057

Post-application Incidental Oral and Dermal Exposure

Glyphosate has residential uses, including application to turf, which would result in the highest potential post-application exposures; therefore, there is potential for children to be exposed via incidental oral and dermal routes from playing on treated lawns. For this assessment, the agency evaluates exposures from hand-to-mouth behavior, object-to-mouth behavior, incidental soil ingestion, and dermal contact using the 2012 Standard Operating Procedures for Residential Pesticide Exposure Assessment[29]. Incidental oral exposures from hand-to-mouth, object-to-mouth, and incidental soil ingestion are considered inter-related and, therefore, not combined. To calculate high end estimates of exposures, the following is assumed according to the 2012 SOP to be health-protective: 1) maximum label rates are applied to the turf, 2) exposures are assumed to occur every day to the residue values on the day of application (i.e., no dissipation), and 3) individuals engage in post-application activities for the maximum amount of time represented by data for children spending time outdoors and not specifically engaged in activities

[29] Available: http://www2.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedures-residential-pesticide

on turf, when in actuality children do not spend all of their outdoor time on turf. The highest exposure from incidental oral scenarios using the maximum application rate for turf applications would be 0.16 mg/kg/day from hand-to-mouth behaviors by children (1 to <2 years old). Dermal post-application to children 1 to <2 years old would be 0.08 mg/kg/day.

Table E.2. Post-application Exposure Estimates for Application of Glyphosate to Turf[1].

Lifestage	Post-application Exposure Scenario		Exposure (mg/kg/day)
Children 1 to <2 year old	Turf – sprays	Hand-to-Mouth	0.16
		Object-to-Mouth	0.005
		Incidental Soil Ingestion	0.0003
		Dermal (high contact activities)	0.08

[1] Based on Roundup® Weed & Grass Super Concentrate, EPA Reg. No. 71995-25.

DEEM-FCID™ Chronic Residue File.

```
Filename: C:\Users\tbloem\Documents\work\glyphosate\registration review\417300C.R08
Chemical: Glyphosate
RfD(Chronic): 1 mg/kg bw/day  NOEL(Chronic): 100 mg/kg bw/day
RfD(Acute): 0 mg/kg bw/day  NOEL(Acute):  0 mg/kg bw/day
Date created/last modified: 06-09-2016/10:37:44       Program ver. 3.16, 03-08-d
Comment: THIS R98 FILE WAS GENERATED USING THE CONVERT TO R98 UTILITY VERSION 1.1.2.
-------------------------------------------------------------------------------
   EPA     Crop                                  Def Res     Adj.Factors   Comment
   Code     Grp  Commodity Name                   (ppm)       #1    #2
---------- ---- -------------------------------  ----------  ------ ------  -------
0101050000 1AB  Beet, garden, roots               0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101050001 1AB  Beet, garden, roots-babyfood      0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101052000 1A   Beet, sugar                      10.000000   1.000  1.000  P 7F04
            Full comment: P 7F04886
0101052001 1A   Beet, sugar-babyfood            10.000000   1.000  1.000  P 7F04
            Full comment: P 7F04886
0101053000 1A   Beet, sugar, molasses           10.000000   1.000  1.000  P 7F04
            Full comment: P 7F04886
0101053001 1A   Beet, sugar, molasses-babyfood  10.000000   1.000  1.000  P 7F04
            Full comment: P 7F04886
0101067000 1AB  Burdock                           0.200000   1.000  1.000
0101078000 1AB  Carrot                            5.000000   1.000  1.000  P 8E36
            Full comment: P 8E3676  7F2016
0101078001 1AB  Carrot-babyfood                   5.000000   1.000  1.000  P 8E36
            Full comment: P 8E3676  7F2016
0101079000 1AB  Carrot, juice                     5.000000   1.000  1.000  P 8E36
            Full comment: P 8E3676  7F2016
0101084000 1AB  Celeriac                          0.200000   1.000  1.000
0101100000 1AB  Chicory, roots                    0.200000   1.000  1.000
0101168000 1AB  Ginseng, dried                    0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101190000 1AB  Horseradish                       0.200000   1.000  1.000  P 8E36
            Full comment: P 8E3676
0101250000 1AB  Parsley, turnip rooted            0.200000   1.000  1.000
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0101251000 1AB  Parsnip                              0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101251001 1AB  Parsnip-babyfood                     0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101314000 1AB  Radish, roots                        0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101316000 1AB  Radish, Oriental, roots              0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0101327000 1AB  Rutabaga                             0.200000   1.000  1.000
0101331000 1AB  Salsify, roots                       0.200000   1.000  1.000
0101388000 1AB  Turnip, roots                        0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103015000 1CD  Arrowroot, flour                     0.200000   1.000  1.000
0103015001 1CD  Arrowroot, flour-babyfood            0.200000   1.000  1.000
0103017000 1CD  Artichoke, Jerusalem                 0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103082000 1CD  Cassava                              0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103082001 1CD  Cassava-babyfood                     0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103139000 1CD  Dasheen, corm                        0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103166000 1CD  Ginger                               0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103166001 1CD  Ginger-babyfood                      0.200000   1.000  1.000
0103167000 1CD  Ginger, dried                        0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103296000 1C   Potato, chips                        0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103297000 1C   Potato, dry (granules/ flakes)       0.200000   6.500  1.000
0103297001 1C   Potato, dry (granules/ flakes)-b     0.200000   6.500  1.000
0103298000 1C   Potato, flour                        0.200000   6.500  1.000  P 7F20
            Full comment: P 7F2016
0103298001 1C   Potato, flour-babyfood               0.200000   6.500  1.000  P 7F20
            Full comment: P 7F2016
0103299000 1C   Potato, tuber, w/peel                0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103299001 1C   Potato, tuber, w/peel-babyfood       0.200000   1.000  1.000
0103300000 1C   Potato, tuber, w/o peel              0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103300001 1C   Potato, tuber, w/o peel-babyfood     0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103366000 1CD  Sweet potato                         3.000000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103366001 1CD  Sweet potato-babyfood                3.000000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103371000 1CD  Tanier, corm                         0.200000   1.000  1.000
0103387000 1CD  Turmeric                             0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103406000 1CD  Yam, true                            0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0103407000 1CD  Yam bean                             0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016
0200051000 2    Beet, garden, tops                   0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0200101000 2    Chicory, tops                        0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016 & 8E2122
0200140000 2    Dasheen, leaves                      0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0200315000 2    Radish, tops                         0.200000   1.000  1.000
0200317000 2    Radish, Oriental, tops               0.200000   1.000  1.000
0200332000 2    Salsify, tops                        0.200000   1.000  1.000
0301165000 3A   Garlic, bulb                         0.200000   1.000  1.000  P 8E36
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            Full comment: P 8E3676
0301165001 3A   Garlic, bulb-babyfood             0.200000   1.000  1.000  P 8E36
            Full comment: P 8E3676
0301237000 3A   Onion, bulb                       0.200000   1.000  1.000  P 8E36
            Full comment: P 8E3676
0301237001 3A   Onion, bulb-babyfood              0.200000   1.000  1.000  P 8E36
            Full comment: P 8E3676
0301238000 3A   Onion, bulb, dried                0.200000   9.000  1.000  P 8E36
            Full comment: P 8E3676
0301238001 3A   Onion, bulb, dried-babyfood       0.200000   9.000  1.000  P 8E36
            Full comment: P 8E3676
0301338000 3A   Shallot, bulb                     0.200000   1.000  1.000
0302103000 3B   Chive, fresh leaves               0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
0302198000 3B   Leek                              0.200000   1.000  1.000  P 8E36
            Full comment: P 8E3676
0302239000 3B   Onion, green                      0.200000   1.000  1.000  P 8E36
            Full comment: P 8E3676
0302338500 3B   Shallot, fresh leaves             0.200000   1.000  1.000
0401005000 4A   Amaranth, leafy                   0.200000   1.000  1.000
0401018000 4A   Arugula                           0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401104000 4A   Chrysanthemum, garland            0.200000   1.000  1.000
0401133000 4A   Cress, garden                     0.200000   1.000  1.000
0401134000 4A   Cress, upland                     0.200000   1.000  1.000
0401138000 4A   Dandelion, leaves                 0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401150000 4A   Endive                            0.200000   1.000  1.000  P 7F20
            Full comment: P 7F2016 & 8E2122
0401204000 4A   Lettuce, head                     0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401205000 4A   Lettuce, leaf                     0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401248000 4A   Parsley, leaves                   0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401313000 4A   Radicchio                         0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401355000 4A   Spinach                           0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0401355001 4A   Spinach-babyfood                  0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0402076000 4B   Cardoon                           0.200000   1.000  1.000
0402085000 4B   Celery                            0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0402085001 4B   Celery-babyfood                   0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0402086000 4B   Celery, juice                     0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0402087000 4B   Celtuce                           0.500000   1.000  1.000
0402152000 4B   Fennel, Florence                  0.500000   1.000  1.000
0402322000 4B   Rhubarb                           0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0402367000 4B   Swiss chard                       0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501061000 5A   Broccoli                          0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501061001 5A   Broccoli-babyfood                 0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501062000 5A   Broccoli, Chinese                 0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501064000 5A   Brussels sprouts                  0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501069000 5A   Cabbage                           0.200000   1.000  1.000  P 8E21
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            Full comment: P 8E2122
0501071000 5A   Cabbage, Chinese, napa            0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501072000 5A   Cabbage, Chinese, mustard         0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501083000 5A   Cauliflower                      0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0501196000 5A   Kohlrabi                         0.500000   1.000  1.000
0502063000 5B   Broccoli raab                    0.200000   1.000  1.000
0502070000 5B   Cabbage, Chinese, bok choy       0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0502117000 5B   Collards                         0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0502194000 5B   Kale                             0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0502229000 5B   Mustard greens                   0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0502318000 5B   Rape greens                      0.200000   1.000  1.000
0502389000 5B   Turnip, greens                   0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
0600347000 6    Soybean, seed                   20.000000   1.000  1.000  P 5F15
            Full comment: P 5F1536
0600349000 6    Soybean, soy milk               20.000000   1.000  1.000  P 5F15
            Full comment: P 5F1536
0600349001 6    Soybean, soy milk-babyfood or in  20.000000   1.000  1.000
0600350000 6    Soybean, oil                    20.000000   1.000  1.000  P 5F15
            Full comment: P 5F1536
0600350001 6    Soybean, oil-babyfood           20.000000   1.000  1.000  P 5F15
            Full comment: P 5F1536
0601043000 6A   Bean, snap, succulent            5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0601043001 6A   Bean, snap, succulent-babyfood   5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0601257000 6A   Pea, edible podded, succulent    5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0601349500 6AB  Soybean, vegetable               5.000000   1.000  1.000
0602031000 6B   Bean, broad, succulent           5.000000   1.000  1.000
0602033000 6B   Bean, cowpea, succulent          5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0602037000 6B   Bean, lima, succulent            5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0602255000 6B   Pea, succulent                   5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0602255001 6B   Pea, succulent-babyfood          5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0602259000 6B   Pea, pigeon, succulent           5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603030000 6C   Bean, black, seed                5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603032000 6C   Bean, broad, seed                5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603034000 6C   Bean, cowpea, seed               5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603035000 6C   Bean,  great northern, seed      5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603036000 6C   Bean, kidney, seed               5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603038000 6C   Bean, lima, seed                 5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603039000 6C   Bean, mung, seed                 5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603040000 6C   Bean, navy, seed                 5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
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0603041000 6C   Bean, pink, seed                    5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603042000 6C   Bean, pinto, seed                   5.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603098000 6C   Chickpea, seed                      8.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603098001 6C   Chickpea, seed-babyfood            8.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603099000 6C   Chickpea, flour                     8.000000   1.000  1.000
0603182000 6C   Guar, seed                          8.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603182001 6C   Guar, seed-babyfood                8.000000   1.000  1.000
0603203000 6C   Lentil, seed                        8.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603256000 6C   Pea, dry                           8.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603256001 6C   Pea, dry-babyfood                  8.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
0603258000 6C   Pea, pigeon, seed                  8.000000   1.000  1.000
0603348000 6C   Soybean, flour                    20.000000   1.000  1.000  P 5F15
            Full comment: P 5F1536
0603348001 6C   Soybean, flour-babyfood           20.000000   1.000  1.000  P 5F15
            Full comment: P 5F1536
0801374000 8A   Tomatillo                          0.100000   1.000  1.000
0801375000 8A   Tomato                             0.100000   1.000  1.000
0801375001 8A   Tomato-babyfood                    0.100000   1.000  1.000
0801376000 8A   Tomato, paste                      0.100000   5.400  1.000
0801376001 8A   Tomato, paste-babyfood             0.100000   5.400  1.000
0801377000 8A   Tomato, puree                      0.100000   3.300  1.000
0801377001 8A   Tomato, puree-babyfood             0.100000   3.300  1.000
0801378000 8A   Tomato, dried                      0.100000  14.300  1.000
0801378001 8A   Tomato, dried-babyfood             0.100000  14.300  1.000
0801379000 8A   Tomato, juice                      0.100000   1.500  1.000
0801380000 8A   Tomato, Tree                       0.100000   1.000  1.000
0802148000 8BC  Eggplant                           0.100000   1.000  1.000
0802234000 8BC  Okra                               0.500000   1.000  1.000  P 9E60
            Full comment: P 9E6003
0802270000 8B   Pepper, bell                       0.100000   1.000  1.000
0802270001 8B   Pepper, bell-babyfood              0.100000   1.000  1.000
0802271000 8B   Pepper, bell, dried                0.100000   1.000  1.000
0802271001 8B   Pepper, bell, dried-babyfood       0.100000   1.000  1.000
0802272000 8BC  Pepper, nonbell                    0.100000   1.000  1.000
0802272001 8BC  Pepper, nonbell-babyfood           0.100000   1.000  1.000
0802273000 8BC  Pepper, nonbell, dried            0.100000   1.000  1.000
0901075000 9A   Cantaloupe                         0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0901187000 9A   Honeydew melon                     0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0901399000 9A   Watermelon                         0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0901400000 9A   Watermelon, juice                  0.500000   1.000  1.000
0902021000 9B   Balsam pear                        0.500000   1.000  1.000
0902088000 9B   Chayote, fruit                     0.500000   1.000  1.000
0902102000 9B   Chinese waxgourd                   0.500000   1.000  1.000
0902135000 9B   Cucumber                           0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0902308000 9B   Pumpkin                            0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0902309000 9B   Pumpkin, seed                      0.500000   1.000  1.000
0902356000 9B   Squash, summer                     0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0902356001 9B   Squash, summer-babyfood            0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
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0902357000 9B   Squash, winter                      0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
0902357001 9B   Squash, winter-babyfood            0.500000   1.000  1.000  P 3E28
            Full comment: P 3E2845
1001106000 10A  Citron                             0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1001107000 10A  Citrus hybrids                     0.500000   1.000  1.000
1001108000 10A  Citrus, oil                        0.500000   1.000  1.000
1001240000 10A  Orange                             0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1001241000 10A  Orange, juice                      0.500000   1.800  1.000  P 4F43
            Full comment: P 4F4338
1001241001 10A  Orange, juice-babyfood             0.500000   1.800  1.000  P 4F43
            Full comment: P 4F4338
1001242000 10A  Orange, peel                       0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1001369000 10A  Tangerine                          0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1001370000 10A  Tangerine, juice                   0.500000   2.300  1.000  P 4F43
            Full comment: P 4F4338
1002197000 10B  Kumquat                            0.500000   1.000  1.000
1002199000 10B  Lemon                              0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1002200000 10B  Lemon, juice                       0.500000   2.000  1.000  P 4F43
            Full comment: P 4F4338
1002200001 10B  Lemon, juice-babyfood              0.500000   2.000  1.000  P 4F43
            Full comment: P 4F4338
1002201000 10B  Lemon, peel                        0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1002206000 10B  Lime                               0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1002207000 10B  Lime, juice                        0.500000   2.000  1.000  P 4F43
            Full comment: P 4F4338
1002207001 10B  Lime, juice-babyfood               0.500000   2.000  1.000  P 4F43
            Full comment: P 4F4338
1003180000 10C  Grapefruit                         0.500000   1.000  1.000  P 4F43
            Full comment: P 4F4338
1003181000 10C  Grapefruit, juice                  0.500000   2.100  1.000  P 4F43
            Full comment: P 4F4338
1003307000 10C  Pummelo                            0.500000   1.000  1.000
1100007000 11   Apple, fruit with peel             0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100008000 11   Apple, peeled fruit                0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100008001 11   Apple, peeled fruit-babyfood       0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100009000 11   Apple, dried                       0.200000   8.000  1.000  P 6F18
            Full comment: P 6F1861
1100009001 11   Apple, dried-babyfood              0.200000   8.000  1.000  P 6F18
            Full comment: P 6F1861
1100010000 11   Apple, juice                       0.200000   1.300  1.000  P 6F18
            Full comment: P 6F1861
1100010001 11   Apple, juice-babyfood              0.200000   1.300  1.000  P 6F18
            Full comment: P 6F1861
1100011000 11   Apple, sauce                       0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100011001 11   Apple, sauce-babyfood              0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100129000 11   Crabapple                          0.200000   1.000  1.000
1100173500 11   Goji berry                         0.100000   1.000  1.000
1100210000 11   Loquat                             0.200000   1.000  1.000
1100266000 11   Pear                               0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
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1100266001 11   Pear-babyfood                      0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100267000 11   Pear, dried                        0.200000   6.250  1.000  P 6F18
            Full comment: P 6F1861
1100268000 11   Pear, juice                        0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100268001 11   Pear, juice-babyfood               0.200000   1.000  1.000  P 6F18
            Full comment: P 6F1861
1100310000 11   Quince                             0.200000   1.000  1.000
1201090000 12A  Cherry                             0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1201090001 12A  Cherry-babyfood                    0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1201091000 12A  Cherry, juice                      0.200000   1.500  1.000  P 2600
            Full comment: P 260044
1201091001 12A  Cherry, juice-babyfood             0.200000   1.500  1.000  P 2600
            Full comment: P 260044
1202012000 12B  Apricot                            0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202012001 12B  Apricot-babyfood                   0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202013000 12B  Apricot, dried                     0.200000   6.000  1.000  P 2600
            Full comment: P 260044
1202014000 12B  Apricot, juice                     0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202014001 12B  Apricot, juice-babyfood            0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202230000 12B  Nectarine                          0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202260000 12B  Peach                              0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202260001 12B  Peach-babyfood                     0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202261000 12B  Peach, dried                       0.200000   7.000  1.000  P 2600
            Full comment: P 260044
1202261001 12B  Peach, dried-babyfood              0.200000   7.000  1.000
1202262000 12B  Peach, juice                       0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1202262001 12B  Peach, juice-babyfood              0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1203285000 12C  Plum                               0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1203285001 12C  Plum-babyfood                      0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1203286000 12C  Plum, prune, fresh                 0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1203286001 12C  Plum, prune, fresh-babyfood        0.200000   1.000  1.000  P 2600
            Full comment: P 260044
1203287000 12C  Plum, prune, dried                 0.200000   5.000  1.000  P 2600
            Full comment: P 260044
1203287001 12C  Plum, prune, dried-babyfood        0.200000   5.000  1.000
1203288000 12C  Plum, prune, juice                 0.200000   1.400  1.000  P 2600
            Full comment: P 260044
1203288001 12C  Plum, prune, juice-babyfood        0.200000   1.400  1.000  P 2600
            Full comment: P 260044
1301055000 13A  Blackberry                         0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1301056000 13A  Blackberry, juice                  0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1301056001 13A  Blackberry, juice-babyfood         0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1301058000 13A  Boysenberry                        0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
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1301208000 13A  Loganberry                          0.200000   1.000  1.000
1301320000 13A  Raspberry                           0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1301320001 13A  Raspberry-babyfood                  0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1301321000 13A  Raspberry, juice                    0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1301321001 13A  Raspberry, juice-babyfood           0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1302057000 13B  Blueberry                           0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1302057001 13B  Blueberry-babyfood                  0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1302136000 13B  Currant                             0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1302137000 13B  Currant, dried                      0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1302149000 13B  Elderberry                          0.200000   1.000  1.000
1302174000 13B  Gooseberry                          0.200000   1.000  1.000
1302191000 13B  Huckleberry                         0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1303227000 13C  Mulberry                            0.200000   1.000  1.000
1304175000 13D  Grape                               0.200000   1.000  1.000  P 5F15
            Full comment: P 5F1560
1304176000 13D  Grape, juice                        0.200000   1.200  1.000  P 5F15
            Full comment: P 5F1560
1304176001 13D  Grape, juice-babyfood               0.200000   1.200  1.000  P 5F15
            Full comment: P 5F1560
1304179000 13D  Grape, wine and sherry              0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1304195000 13D  Kiwifruit, fuzzy                    0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2929
1307130000 13G  Cranberry                           0.200000   1.000  1.000  P 0E24
            Full comment: P 0E2421
1307130001 13G  Cranberry-babyfood                  0.200000   1.000  1.000  P 0E24
            Full comment: P 0E2421
1307131000 13G  Cranberry, dried                    0.200000   1.000  1.000  P 0E24
            Full comment: P 0E2421
1307132000 13G  Cranberry, juice                    0.200000   1.100  1.000  P 0E24
            Full comment: P 0E2421
1307132001 13G  Cranberry, juice-babyfood           0.200000   1.100  1.000  P 0E24
            Full comment: P 0E2421
1307359000 13G  Strawberry                          0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1307359001 13G  Strawberry-babyfood                 0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1307360000 13G  Strawberry, juice                   0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1307360001 13G  Strawberry, juice-babyfood          0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2930
1400003000 14   Almond                              1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400003001 14   Almond-babyfood                     1.000000   1.000  1.000
1400004000 14   Almond, oil                         1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400004001 14   Almond, oil-babyfood                1.000000   1.000  1.000
1400059000 14   Brazil nut                          1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400068000 14   Butternut                           1.000000   1.000  1.000
1400081000 14   Cashew                              1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400092000 14   Chestnut                            1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
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1400155000 14   Hazelnut                            1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400156000 14   Hazelnut, oil                       1.000000   1.000  1.000
1400185000 14   Hickory nut                         1.000000   1.000  1.000
1400213000 14   Macadamia nut                       1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400269000 14   Pecan                               1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1400278000 14   Pine nut                            1.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1400282000 14   Pistachio                           1.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1400391000 14   Walnut                              1.000000   1.000  1.000  P 7F18
            Full comment: P 7F1893
1500025000 15   Barley, pearled barley             30.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
1500025001 15   Barley, pearled barley-babyfood    30.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
1500026000 15   Barley, flour                      30.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
1500026001 15   Barley, flour-babyfood             30.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
1500027000 15   Barley, bran                       30.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
1500065000 15   Buckwheat                          30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500066000 15   Buckwheat, flour                   30.000000   1.000  1.000
1500120000 15   Corn, field, flour                  5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500120001 15   Corn, field, flour-babyfood        5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500121000 15   Corn, field, meal                  5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500121001 15   Corn, field, meal-babyfood         5.000000   1.000  1.000
1500122000 15   Corn, field, bran                  5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500123000 15   Corn, field, starch                5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500123001 15   Corn, field, starch-babyfood       5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500124000 15   Corn, field, syrup                 5.000000   1.500  1.000  P 8F36
            Full comment: P 8F3673
1500124001 15   Corn, field, syrup-babyfood        5.000000   1.500  1.000  P 8F36
            Full comment: P 8F3673
1500125000 15   Corn, field, oil                   5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500125001 15   Corn, field, oil-babyfood          5.000000   1.000  1.000  P 8F36
            Full comment: P 8F3673
1500126000 15   Corn, pop                          0.100000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500127000 15   Corn, sweet                        3.500000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500127001 15   Corn, sweet-babyfood               3.500000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500226000 15   Millet, grain                      30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500231000 15   Oat, bran                          30.000000   1.000  1.000  P 6E46
            Full comment: P 6E4645
1500232000 15   Oat, flour                         30.000000   1.000  1.000  P 6E46
            Full comment: P 6E4645
1500232001 15   Oat, flour-babyfood                30.000000   1.000  1.000  P 6E46
            Full comment: P 6E4645
1500233000 15   Oat, groats/rolled oats            30.000000   1.000  1.000  P 6E46
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            Full comment: P 6E4645
1500233001 15   Oat, groats/rolled oats-babyfood  30.000000   1.000  1.000  P 6E46
            Full comment: P 6E4645
1500323000 15   Rice, white                        0.100000   1.000  1.000
1500323001 15   Rice, white-babyfood               0.100000   1.000  1.000
1500324000 15   Rice, brown                        0.100000   1.000  1.000
1500324001 15   Rice, brown-babyfood               0.100000   1.000  1.000
1500325000 15   Rice, flour                        0.100000   1.000  1.000
1500325001 15   Rice, flour-babyfood               0.100000   1.000  1.000
1500326000 15   Rice, bran                         0.100000   1.000  1.000
1500326001 15   Rice, bran-babyfood                0.100000   1.000  1.000
1500328000 15   Rye, grain                        30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500329000 15   Rye, flour                        30.000000   1.000  1.000
1500344000 15   Sorghum, grain                    30.000000   1.000  1.000
1500345000 15   Sorghum, syrup                    30.000000   1.000  1.000
1500381000 15   Triticale, flour                  30.000000   1.000  1.000
1500381001 15   Triticale, flour-babyfood         30.000000   1.000  1.000
1500401000 15   Wheat, grain                      30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500401001 15   Wheat, grain-babyfood             30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500402000 15   Wheat, flour                      30.000000   1.000  1.000
1500402001 15   Wheat, flour-babyfood             30.000000   1.000  1.000
1500403000 15   Wheat, germ                       30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500404000 15   Wheat, bran                       30.000000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1500405000 15   Wild rice                          0.100000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1800002000 18   Alfalfa, seed                      0.500000   1.000  1.000
1901028000 19A  Basil, fresh leaves                0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901028001 19A  Basil, fresh leaves-babyfood       0.200000   1.000  1.000
1901029000 19A  Basil, dried leaves                0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901029001 19A  Basil, dried leaves-babyfood       0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901102500 19A  Chive, dried leaves                0.200000   1.000  1.000
1901118000 19A  Cilantro, leaves                   0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901118001 19A  Cilantro, leaves-babyfood          0.200000   1.000  1.000
1901144000 19A  Dillweed                           0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901184000 19A  Herbs, other                       0.200000   1.000  1.000
1901184001 19A  Herbs, other-babyfood              0.200000   1.000  1.000
1901202000 19A  Lemongrass                         0.200000   1.000  1.000
1901220000 19A  Marjoram                          0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901220001 19A  Marjoram-babyfood                 0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1901249000 19A  Parsley, dried leaves              0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1901249001 19A  Parsley, dried leaves-babyfood     0.200000   1.000  1.000  P 8E21
            Full comment: P 8E2122
1901334000 19A  Savory                             0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1902105000 19B  Cinnamon                           7.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1902105001 19B  Cinnamon-babyfood                  7.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1902119000 19B  Coriander, seed                    7.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
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1902119001 19B  Coriander, seed-babyfood          7.000000   1.000  1.000
1902143000 19B  Dill, seed                        7.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1902274000 19B  Pepper, black and white          7.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1902274001 19B  Pepper, black and white-babyfood 7.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
1902354000 19B  Spices, other                    7.000000   1.000  1.000
1902354001 19B  Spices, other-babyfood          7.000000   1.000  1.000
2001163000 20A  Flax seed, oil                  40.000000   1.000  1.000  00ND00
            Full comment: 00ND0025 (S18)
2001319000 20A  Rapeseed, oil                   20.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
2001319001 20A  Rapeseed, oil-babyfood          20.000000   1.000  1.000  P 2E41
            Full comment: P 2E4118
2001336000 20A  Sesame, seed                    40.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
2001336001 20A  Sesame, seed-babyfood           40.000000   1.000  1.000
2001337000 20A  Sesame, oil                     40.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
2001337001 20A  Sesame, oil-babyfood            40.000000   1.000  1.000
2002330000 20B  Safflower, oil                  40.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
2002330001 20B  Safflower, oil-babyfood         40.000000   1.000  1.000  P 9E60
            Full comment: P 9E6003
2002364000 20B  Sunflower, seed                 40.000000   1.000  1.000  P 6F34
            Full comment: P 6F3408
2002365000 20B  Sunflower, oil                  40.000000   1.000  1.000  P 6F34
            Full comment: P 6F3408
2002365001 20B  Sunflower, oil-babyfood         40.000000   1.000  1.000  P 6F34
            Full comment: P 6F3408
2003114001 20C  Coconut, oil-babyfood            0.100000   1.000  1.000  P 2F26
            Full comment: P 2F2680
2003128000 20C  Cottonseed, oil                 40.000000   1.000  1.000
2003128001 20C  Cottonseed, oil-babyfood        40.000000   1.000  1.000
3100046000 31   Beef, meat byproducts            5.000000   1.000  1.000
3100046001 31   Beef, meat byproducts-babyfood   5.000000   1.000  1.000
3100048000 31   Beef, kidney                     5.000000   1.000  1.000  P 4F43
            Full comment: P 4F4312
3100049000 31   Beef, liver                      5.000000   1.000  1.000  P OF23
            Full comment: P OF2329
3100049001 31   Beef, liver-babyfood             5.000000   1.000  1.000  P OF23
            Full comment: P OF2329
3200170000 32   Goat, meat byproducts            5.000000   1.000  1.000
3200172000 32   Goat, kidney                     5.000000   1.000  1.000
3200173000 32   Goat, liver                      5.000000   1.000  1.000
3400291000 34   Pork, skin                       5.000000   1.000  1.000
3400292000 34   Pork, meat byproducts            5.000000   1.000  1.000
3400292001 34   Pork, meat byproducts-babyfood   5.000000   1.000  1.000
3400294000 34   Pork, kidney                     5.000000   1.000  1.000
3400295000 34   Pork, liver                      5.000000   1.000  1.000  P 0F23
            Full comment: P 0F2329
3500340000 35   Sheep, meat byproducts           5.000000   1.000  1.000
3500342000 35   Sheep, kidney                    5.000000   1.000  1.000
3500343000 35   Sheep, liver                     5.000000   1.000  1.000
4000093000 40   Chicken, meat                    0.100000   1.000  1.000  P 9F50
            Full comment: P 9F5096
4000093001 40   Chicken, meat-babyfood           0.100000   1.000  1.000  P 9F50
            Full comment: P 9F5096
4000094000 40   Chicken, liver                   1.000000   1.000  1.000  P 9F50
            Full comment: P 9F5096
4000095000 40   Chicken, meat byproducts         1.000000   1.000  1.000  P 9F50
            Full comment: P 9F5096
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4000095001 40   Chicken, meat byproducts-babyfoo   1.000000   1.000  1.000  P 9F50
            Full comment: P 9F5096
4000097000 40   Chicken, skin                      1.000000   1.000  1.000
4000097001 40   Chicken, skin-babyfood             1.000000   1.000  1.000
5000382000 50   Turkey, meat                       0.100000   1.000  1.000  P 0F23
            Full comment: P 0F2329
5000382001 50   Turkey, meat-babyfood              0.100000   1.000  1.000  P 0F23
            Full comment: P 0F2329
5000383000 50   Turkey, liver                      1.000000   1.000  1.000
5000383001 50   Turkey, liver-babyfood             1.000000   1.000  1.000
5000384000 50   Turkey, meat byproducts            1.000000   1.000  1.000  P 0F23
            Full comment: P 0F2329
5000384001 50   Turkey, meat byproducts-babyfood   1.000000   1.000  1.000  P 0F23
            Full comment: P 0F2329
5000386000 50   Turkey, skin                       1.000000   1.000  1.000
5000386001 50   Turkey, skin-babyfood              1.000000   1.000  1.000
6000301000 60   Poultry, other, meat               0.100000   1.000  1.000  P 9E60
            Full comment: P 9E6003
6000302000 60   Poultry, other, liver              1.000000   1.000  1.000
6000303000 60   Poultry, other, meat byproducts    1.000000   1.000  1.000
6000305000 60   Poultry, other, skin               1.000000   1.000  1.000
7000145000 70   Egg, whole                         0.050000   1.000  1.000  P  9F5
            Full comment: P  9F5096
7000145001 70   Egg, whole-babyfood                0.050000   1.000  1.000  P  9F5
            Full comment: P  9F5096
7000146000 70   Egg, white                         0.050000   1.000  1.000  P  9F5
            Full comment: P  9F5096
7000146001 70   Egg, white (solids)-babyfood       0.050000   1.000  1.000
7000147000 70   Egg, yolk                          0.050000   1.000  1.000  P  9F5
            Full comment: P  9F5096
7000147001 70   Egg, yolk-babyfood                 0.050000   1.000  1.000  P  9F5
            Full comment: P  9F5096
8000157000 80   Fish-freshwater finfish            0.250000   1.000  1.000  P 9F21
            Full comment: P 9F2163
8000158000 80   Fish-freshwater finfish, farm ra   0.250000   1.000  1.000  P 9F21
            Full comment: P 9F2163
8000159000 80   Fish-saltwater finfish, tuna       0.250000   1.000  1.000  P 9F21
            Full comment: P 9F2163
8000160000 80   Fish-saltwater finfish, other      0.250000   1.000  1.000  P 9F21
            Full comment: P 9F2163
8000161000 80   Fish-shellfish, crustacean         3.000000   1.000  1.000  P 3F29
            Full comment: P 3F2956
8000162000 80   Fish-shellfish, mollusc            3.000000   1.000  1.000  P 3F29
            Full comment: P 3F2956
8601000000 86A  Water, direct, all sources         0.159000   1.000  1.000
8602000000 86B  Water, indirect, all sources       0.159000   1.000  1.000
9500000500 O    Acai berry                         0.200000   1.000  1.000
9500001000 O    Acerola                            0.200000   1.000  1.000
9500001500 O    Agave                              0.500000   1.000  1.000
9500016000 O    Artichoke, globe                   0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
9500019000 O    Asparagus                          0.500000   1.000  1.000  P 8E36
            Full comment: P 8E3648
9500019500 O    Atemoya                            0.200000   1.000  1.000
9500020000 O    Avocado                            0.200000   1.000  1.000  P 8F20
            Full comment: P 8F2021
9500022000 O    Bamboo, shoots                     0.500000   1.000  1.000  P 9E60
            Full comment: P 9E6003
9500023000 O    Banana                             0.200000   1.000  1.000  P 9F22
            Full comment: P 9F2223
9500023001 O    Banana-babyfood                    0.200000   1.000  1.000  P 9F22
            Full comment: P 9F2223
9500024000 O    Banana, dried                      0.200000   3.900  1.000  P 9F22
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            Full comment: P 9F2223
9500024001 O    Banana, dried-babyfood          0.200000   3.900  1.000  P 9F22
            Full comment: P 9F2223
9500060000 O    Breadfruit                      0.200000   1.000  1.000  P 9E37
            Full comment: P 9E3754
9500073000 O    Cactus                          0.500000   1.000  1.000
9500074000 O    Canistel                        0.200000   1.000  1.000
9500077000 O    Carob                           0.200000   1.000  1.000
9500089000 O    Cherimoya                       0.200000   1.000  1.000
9500109000 O    Cocoa bean, chocolate           0.200000   1.000  1.000  P 0E38
            Full comment: P 0E3857
9500110000 O    Cocoa bean, powder              0.200000   1.000  1.000  P 0E38
            Full comment: P 0E3857
9500111000 O    Coconut, meat                   0.100000   1.000  1.000  P 2F26
            Full comment: P 2F2680
9500111001 O    Coconut, meat-babyfood          0.100000   1.000  1.000  P 2F26
            Full comment: P 2F2680
9500112000 O    Coconut, dried                  0.100000   2.100  1.000  P 2F26
            Full comment: P 2F2680
9500113000 O    Coconut, milk                   0.100000   1.000  1.000  P 2F26
            Full comment: P 2F2680
9500114000 O    Coconut, oil                    0.100000   1.000  1.000  P 2F26
            Full comment: P 2F2680
9500115000 O    Coffee, roasted bean            1.000000   1.000  1.000  P 6E18
            Full comment: P 6E1809
9500116000 O    Coffee, instant                 1.000000   1.000  1.000  P 6E18
            Full comment: P 6E1809
9500141000 O    Date                            0.200000   1.000  1.000  P 9E37
            Full comment: P 9E3754
9500151000 O    Feijoa                          0.200000   1.000  1.000
9500153000 O    Fig                             0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2929
9500154000 O    Fig, dried                      0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2929
9500177000 O    Grape, leaves                   0.200000   1.000  1.000
9500178000 O    Grape, raisin                   0.200000   4.300  1.000  P 5F15
            Full comment: P 5F1560
9500183000 O    Guava                           0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2443
9500183001 O    Guava-babyfood                  0.200000   1.000  1.000
9500188000 O    Hop                             7.000000   1.000  1.000
9500193000 O    Jackfruit                       0.200000   1.000  1.000
9500209000 O    Longan                          0.200000   1.000  1.000
9500211000 O    Lychee                          0.200000   1.000  1.000
9500212000 O    Lychee, dried                   0.200000   1.850  1.000
9500214000 O    Mamey apple                     0.200000   1.000  1.000
9500215000 O    Mango                           0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2490
9500215001 O    Mango-babyfood                  0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2490
9500216000 O    Mango, dried                    0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2490
9500217000 O    Mango, juice                    0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2490
9500217001 O    Mango, juice-babyfood           0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2490
9500235000 O    Olive                           0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2929
9500236000 O    Olive, oil                      0.200000   1.000  1.000  P 3E29
            Full comment: P 3E2929
9500243000 O    Palm heart, leaves              0.500000   1.000  1.000  P 9E60
            Full comment: P 9E6003
9500244000 O    Palm, oil                       0.100000   1.000  1.000  P 6H51
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            Full comment: P 6H5115
9500244001 O    Palm, oil-babyfood                0.100000   1.000  1.000  P 6H51
            Full comment: P 6H5115
9500245000 O    Papaya                            0.200000   1.000  1.000  P 1E24
            Full comment: P 1E2443
9500245001 O    Papaya-babyfood                   0.200000   1.000  1.000
9500246000 O    Papaya, dried                     0.200000   1.800  1.000  P 1E24
            Full comment: P 1E2443
9500247000 O    Papaya, juice                     0.200000   1.500  1.000  P 1E24
            Full comment: P 1E2443
9500252000 O    Passionfruit                      0.200000   1.000  1.000  P 9E37
            Full comment: P 9E3715
9500252001 O    Passionfruit-babyfood             0.200000   1.000  1.000
9500253000 O    Passionfruit, juice               0.200000   1.000  1.000  P 9E37
            Full comment: P 9E3715
9500253001 O    Passionfruit, juice-babyfood      0.200000   1.000  1.000
9500254000 O    Pawpaw                            0.200000   1.000  1.000
9500263000 O    Peanut                            0.100000   1.000  1.000  P 0F23
            Full comment: P 0F2329
9500264000 O    Peanut, butter                    0.100000   1.890  1.000
9500265000 O    Peanut, oil                       0.100000   1.000  1.000  P 0F23
            Full comment: P 0F2329
9500275000 O    Peppermint                      200.000000   1.000  1.000
9500276000 O    Peppermint, oil                 200.000000   1.000  1.000
9500277000 O    Persimmon                         0.200000   1.000  1.000  P 9E37
            Full comment: P 9E3754
9500279000 O    Pineapple                         0.200000   1.000  1.000  P 2F26
            Full comment: P 2F2634
9500279001 O    Pineapple-babyfood                0.200000   1.000  1.000  P 2F26
            Full comment: P 2F2634
9500280000 O    Pineapple, dried                  0.200000   5.000  1.000  P 2F26
            Full comment: P 2F2634
9500281000 O    Pineapple, juice                  0.200000   1.700  1.000  P 2F26
            Full comment: P 2F2634
9500281001 O    Pineapple, juice-babyfood         0.200000   1.700  1.000  P 2F26
            Full comment: P 2F2634
9500283000 O    Plantain                          0.200000   1.000  1.000  P 9F22
            Full comment: P 9F2223
9500284000 O    Plantain, dried                   0.200000   3.900  1.000  P 9F22
            Full comment: P 9F2223
9500289000 O    Pomegranate                       0.200000   1.000  1.000  P 1E39
            Full comment: P 1E3978
9500311000 O    Quinoa, grain                     5.000000   1.000  1.000
9500333000 O    Sapote, Mamey                     0.200000   1.000  1.000
9500346000 O    Soursop                           0.200000   1.000  1.000
9500351000 O    Spanish lime                      0.200000   1.000  1.000
9500352000 O    Spearmint                       200.000000   1.000  1.000
9500353000 O    Spearmint, oil                  200.000000   1.000  1.000
9500358000 O    Starfruit                         0.200000   1.000  1.000  P 6E34
            Full comment: P 6E3424
9500361000 O    Sugar apple                       0.200000   1.000  1.000
9500362000 O    Sugarcane, sugar                  2.000000   1.000  1.000
9500362001 O    Sugarcane, sugar-babyfood         2.000000   1.000  1.000
9500363000 O    Sugarcane, molasses              30.000000   1.000  1.000  P 9H51
            Full comment: P 9H5196
9500363001 O    Sugarcane, molasses-babyfood     30.000000   1.000  1.000  P 9H51
            Full comment: P 9H5196
9500368000 O    Tamarind                          0.200000   1.000  1.000
9500372000 O    Tea, dried                        1.000000   1.000  1.000  P 1H53
            Full comment: P 1H5310 & 8H5568
9500373000 O    Tea, instant                      7.000000   1.000  1.000  P 1H53
            Full comment: P 1H5310 & 8H5568
9500373500 O    Teff, flour                       5.000000   1.000  1.000
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9500398000 O    Watercress                          0.200000   1.000  1.000  P 9E60
            Full comment: P 9E6003
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Attachment 2: DEEM-FCID™ Chronic Exposure Estimates.

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US EPA                                                              Ver. 3.16, 03-08-d
DEEM-FCID Chronic analysis for GLYPHOSATE                    NHANES 2003-2008 2-day
Residue file name: C:\Users\tbloem\Documents\work\glyphosate\registration
review\417300C.R08
                                                 Adjustment factor #2 NOT used.
Analysis Date 06-09-2016/10:40:23     Residue file dated: 06-09-2016/10:37:44
COMMENT 1: THIS R98 FILE WAS GENERATED USING THE CONVERT TO R98 UTILITY VERSION 1.1.2.
================================================================================
                    Total exposure by population subgroup
--------------------------------------------------------------------------------

                                        Total Exposure
                                        --------------
          Population                        mg/kg
           Subgroup                       body wt/day
-------------------------------------   -------------
Total US Population                        0.091530
Hispanic                                   0.094838
Non-Hisp-White                             0.091452
Non-Hisp-Black                             0.086606
Non-Hisp-Other                             0.095659
Nursing Infants                            0.072309
Non-Nursing Infants                        0.174388
Female 13+ PREG                            0.076716
Children 1-6                               0.218895
Children 7-12                              0.139417
Male 13-19                                 0.097324
Female 13-19/NP                            0.082295
Male 20+                                   0.077524
Female 20+/NP                              0.064402
Seniors 55+                                0.061294
All Infants                                0.142873
Female 13-50                               0.070729
Children 1-2                               0.230916
Children 3-5                               0.214174
Children 6-12                              0.149290
Youth 13-19                                0.089645
Adults 20-49                               0.076405
Adults 50-99                               0.062993
Female 13-49                               0.071066

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Note: The reference dose (RfD) and percent of RfD have been removed from this file because these are based on non-cancer endpoints and non-cancer endpoints are not the focus of this SAP.

Appendix F

Genotoxicity Studies with Glyphosate Based Formulations

While the focus of this analysis to determine the genotoxic potential of glyphosate, the agency has identified numerous studies conducted with glyphosate-based formulations that contain various concentrations of the glyphosate as well as other components of the end use products and are presented in Tables F.1-F.5.

Table F.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Formulations.						
Test/Endpoint	**Test System**	**Concentrations**	**Test Material/ Concentration**	**Results**	**Reference**	**Comments**
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100; *E. coli* WP2 *uvrA* pKM101 ± S9	1.6-5000 µg/plate ± S9 (plate incorporation)	ICIA 0224 57.6% in water	Negative ± S9	Callander (1988)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537*; E. coli* WP2P and *uvrA* ± S9	100-5000 µg/plate ±S9 plate incorporation & pre-incubation protocols	TMSC (tri-methyl-sulfonium chloride) 95% purity	Negative ± S9	Callander (1993)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	26, 43, 72, 120, 200 µg/plate	Glyphosate liquid formulation (480 g/L isopropylamine salt)	Negative ± S9	Camolesi (2009)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	26, 43, 72, 120, 200 µg/plate	MON 77280 equivalent of glyphosate acid: 495 g/L	Negative ± S9	Camolesi (2010)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	0.2-2000 µg/plate	MON 76190 53.2% glyphosate	Negative ± S9	Catoyra (2009)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA97a, TA98, TA100 and TA102± S9	2 µg/plate (toxic)	Perzocyd 10 SL formulation	Negative ± S9	Chruscielska *et al.* (2000)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	0.03-3.0 µL/plate	MON 8080 (87.6%)	Negative ± S9	Flowers (1981)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	3.16-1000 µg/plate	TROP M (Glyphosate 480); 35.84% purity based on acid, 48.46% pure based on IPA salt	Negative ± S9	Flügge (2010a)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	0.316-100	Glyphosate 757 g/kg granular formulation (76.1%	Negative ± S9	Flügge (2010d)[1]	

Table F.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Formulations.						
Test/Endpoint	**Test System**	**Concentrations**	**Test Material/ Concentration**	**Results**	**Reference**	**Comments**
			monoammonium glyphosate salt)			
Bacterial Reverse Mutation	*S. typhimurium* TA97a, TA98, TA100, and TA1535 ± S9	1-5000 µg/plate	Roundup WG 784 g/kg ammonium salt equivalent	Negative ± S9	Gava (1998)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537± S9	50-5000 µg/plate	Rodeo® (containing IPA salt and water only); 40% glyphosate (acid equivalent)	Negative ± S9	Kier *et al.*, (1992)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 ± S9	5-500 µg/plate (-S9)/ 15-1500 µg/plate (+S9)	MON 2139 (Roundup®) 31% Glyphosate (acid equivalent)	Negative ± S9	Kier *et al.*, (1992)	Cytotoxic at top concentrations
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 ± S9	5-500 µg/plate (-S9)/ 15-1500 µg/plate (+S9)	MON 14445 (Direct®); 75% Glyphosate (acid equivalent)	Negative ± S9	Kier *et al.*, (1992)	Cytotoxic at the top concentrations, occasionally at lower concentrations
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 ± S9	0.2-2000 µg/plate	MON 79672 (Roundup Ultramax); 74.7% monoammonium glyphosate salt; 68.2% glyphosate	Negative ± S9	Lope (2008)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 ± S9	0.617-50 µL/plate ± S9	SC-0224, 19.2% purity	Negative ± S9	Majeska (1982)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	TA strains: 10 - 5000 µg/plate (+S9); 3.33-3330 µg/plate (-S9); E. coli: 33.3-5000 µg/plate (+/- S9)	MON 78239 36.6% glyphosate (44.9% potassium salt of glyphosate)	Negative	Mecchi (2003a)	Increase in revertants seen in TA98 and TA1535 -S9 on first trial, not conc-dep; however no increase in revertants seen in repeat in those strains; overall negative.

Table F.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Formulations.						
Test/Endpoint	**Test System**	**Concentrations**	**Test Material/ Concentration**	**Results**	**Reference**	**Comments**
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	TA strains: 3.33-3330 µg/plate (+S9); 1.0-1000 µg/plate (-S9); E. coli: 33.3-5000 µg/plate (+/- S9)	MON 78634 65.2% w/w glyphosate (71.8% w/w as monoammonium salt of glyphosate)	Negative	Mecchi (2003b)	
Bacterial Reverse Mutation	*S. typhimurium* TA 98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	10 - 5000 µg/plate (+/-S9)	MON 79864 38.7% glyphosate acid (wt %)	Negative	Mecchi (2008a)	Inhibited growth seen at ≥2000 -S9
Bacterial Reverse Mutation	*S. typhimurium* TA 98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	33.3-5000 µg/plate	MON 76313 30.9% glyphosate acid	Negative	Mecchi (2008b)	
Bacterial Reverse Mutation	*S. typhimurium* TA 98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	10-5000 µg/plate (+/-S9)	MON 76171 31.1% glyphosate	Negative	Mecchi (2008c)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA 98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	10-5000 µg/plate (+/-S9)	MON 79991 71.6% glyphosate acid	Negative	Mecchi (2009a)	
Bacterial Reverse Mutation	*S. typhimurium* TA 98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	10-5000 µg/plate (+/-S9)	MON 76138 38.5% glyphosate	Negative	Mecchi (2009b)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA97a, TA98, TA100, and TA1535 ± S9	1-5000 µg/plate	MON 77280 646.4 g/L salt equivalent	Negative	Perina (1998)	

Table F.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Formulations.						
Test/Endpoint	**Test System**	**Concentrations**	**Test Material/ Concentration**	**Results**	**Reference**	**Comments**
Bacterial Reverse Mutation	*S. typhimurium* TA98 and TA100 ± S9	0-1440 µg/plate (calculated as glyphosate IPA salt)	Roundup, 480 g/L glyphosate isopropylamine salt	Negative – S9, Equivocal +S9	Rank *et al.* (1993)	Stat significant increase at 360 µg/plate for TA98 (-S9) and 720 µg/plate for TA100 (+S9). Not significant at higher concentrations and were not replicated. Effects occurred at close to toxic levels.
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	500-5000 µg/plate;	495 g/L glyphosate isopropylamine salt; 371.0 g/L (equivalent of glyphosate acid)	Negative ± S9	Silvino (2011)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	1.5-5000 µg/plate	MON 8709 495 g/L glyphosate isopropylamine salt; 371.0 g/L (equivalent of glyphosate acid)	Negative ± S9	Silvino (2011)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, and TA1537 ± S9	15-5000 µg/plate	MON 76313 468 g/L glyphosate isopropylamine salt (351 g/L glyphosate acid equivalent)	Negative ± S9	Silvino (2012)	Cytotoxic at 5000 µg/plate for some strains
Bacterial Reverse Mutation	*S. typhimurium* TA97a, TA98, TA100 and TA1535 ± S9	1-5000 µg/plate	Glifos formulation (glyphosate isopropylammonium salt , Berol 907 and water)	Negative ± S9	Vargas (1996)	Cytotoxic at the two upper concentrations

Table F.1. *In vitro* Test for Gene Mutations in Bacteria: Glyphosate Formulations.

Test/Endpoint	Test System	Concentrations	Test Material/ Concentration	Results	Reference	Comments
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537± S9	3.16-316 µg/plate	FSG 3090-H1 360 g/L	Negative ± S9	Uhde (2004)[1]	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100 ± S9	0.01-100 µg/plate	64% (glyphosate Isopropylammonium salt)	Negative ± S9	Wang *et al.* (1993)	
Bacterial Reverse Mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 *uvrA* ± S9	All strains: 33.3-5000 µg/plate (+S9); 10-3330 µg/plate (-S9)	MON 78910 30.3% glyphosate acid	Negative ± S9	Xu (2006)	Cytotoxic ≥1000 µg/plate (-S9)

[1] Study was cited in Kier and Kirkland (2013). Supplementary information about the study was provided online including test guideline, test material purity, control chemicals and summary data tables.

Table F.2. *In Vitro* Tests for Chromosome Damage in Mammalian Cells- Glyphosate Formulations						
Test/Endpoint	**Test System**	**Concentrations**	**Test Material/ Concentration**	**Results**	**Reference**	**Comments**
***In vitro* Chromosomal Aberration using fluorescent in situ hybridization (FISH)**	Bovine lymphocytes (from two 6-8 month old calves) -whole chromosome (1) painting probe	28-1120 µM 24 h exposure	62% Isopropylamine salt of glyphosate (38% inert ingredients)	Negative.	Holeckova (2006)	Small but significant increase in polyploidy seen at 56µM No positive control reported.
***In vitro* Cytokinesis Block Micronucleus Assay (with FISH analysis)**	TR146 cells (human-derived buccal epithelial cell line)	0, 10, 15 and 20 mg/L; 20 minute exposure.	Roundup Ultra Max (450 g/l glyphosate acid)	Positive Increase in MN at all test concentrations	Koller *et al*. (2012)	No apoptosis observed at any conc. Necrosis reported at 20 mg/L. Increase in NB and NPB seen at all concentrations

MI= mitotic index. FISH= fluorescent in situ hybridization, MN= micronuclei; NB= nuclear buds; NPB= nucleoplasmic bridges.

Table F.3. *In Vivo* Tests for Chromosomal Aberrations in Mammals- Glyphosate Formulations.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Bone Marrow Chromosomal Aberration	Swiss albino mice (males only) Vehicle: DMSO	Intraperitoneal injection; sampling 24, 48 and 72 h	0, 25 and 50 mg/kg (5/dose)	Roundup (>41% isopropylamine glyphosate)	Positive Increase in MN at all time points at both doses	Prasad *et al.* (2009)	Significant decrease in mitotic index seen at all doses and time points
Bone Marrow Chromosomal Aberration	C57BL mice (males only) Vehicle: water	Oral administration; sampling 6, 24, 48, 72, 96 and 120 h	0.05, 0.01, 0.5 and 1.0% (8/dose)	Roundup	Negative	Dimitrov *et al.* (2006)	
Bone Marrow Chromosomal Aberration	New Zealand white rabbits (males only) Vehicle:	Drinking water for 60 days	0, 750 ppm (5/dose)	Roundup	Positive	Helal and Moussa (2005)	

BM= bone marrow, SC= spermatocyte.

Table F.4. *In Vivo* Tests for Micronuclei Induction in Mammals- Glyphosate Formulations.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Bone Marrow Micronucleus Test	Swiss CD1 mice (males only)	Intraperitoneal injection; 2 injections of half the dosage of 135 mg/kg 24 h apart; sampling at 6 and 24 h	0, 450 mg/kg roundup, equiv. to 135 kg glyphosate (3/dose)	Roundup, 30.4% glyphosate	Positive	Bolognesi *et al.* (1997)	Stat significant increase in MN at 6 and 24 h
Bone Marrow Micronucleus Test	C3H mice (males only) Vehicle: water	Intraperitoneal Injection (single treatment); sampling after 24, 48 and 72 h	0, 90 mg/kg	Not reported	Negative	Chruscielska *et al.* (2000)	
Bone Marrow Micronucleus Test	Swiss mice (males and females) Vehicle: water	Intraperitoneal Injection (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 50, 100 and 200 mg/kg	480g/L Isopropylamine salt of glyphosate	Negative	Grisolia (2002)	
Bone Marrow Micronucleus Test	CD-1 mice (males and females)	Intraperitoneal injection; sampling 24, 48 and 72 h	0, 140, 280, and 555 mg/kg	Roundup (31% glyphosate salt)	Negative	Kier (1992)	Some deaths observed at high dose (HD), ↓PCE/NCE ratio at HD at 48 h in males.
Bone Marrow Micronucleus Test	Swiss albino mice (males and females)	Intraperitoneal Injection (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 212.5, 425 and 637.5 mg/kg	MON 77280 646.4 g/L glyphosate salt equivalent	Negative	Monma (1998)	Doses tested corresponded to 25%, 50% and 75% LD50

Table F.4. *In Vivo* Tests for Micronuclei Induction in Mammals- Glyphosate Formulations.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Bone Marrow Micronucleus Test	NMRI-Bom mice	Intraperitoneal Injection (single treatment); sampling after 24 h	0, 133 and 200 mg/kg (4/sex/dose)	Roundup, 480 g glyphosate isopropylamine salt per liter	Negative	Rank *et al.* (1993)	BM toxicity indicated by %PCE decreased at 200 mg/kg
Bone Marrow Micronucleus Test	Swiss albino mice (males only[2]) Vehicle: water	Oral gavage (two treatments, 24 h apart); sampled at 18 and 24 h after last dose	0, 2000 mg/kg	MON 8709494.7 g/L salt of isopropylamine (371.0 glyphosate acid)	Negative	Claro (2011)	OECD 474 Guideline No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	C57BL mice (males only) Vehicle: water	Oral administration; sampling 6, 24, 48, 72, 96 and 120 h	0.05, 0.01, 0.5 and 1.0% (1%=1080 mg/kg) (8/dose)	Roundup	Negative	Dimitrov *et al.* (2006)	Toxicity seen in 1.0% dose group
Bone Marrow Micronucleus Test	Crl:CD-1(ICR) BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 78239 (36.6% glyphosate)	Negative	Erexson (2003a)	EPA Guideline (84-2) No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	Crl:CD-1(ICR) BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 78634 (65.2% glyphosate)	Negative	Erexson (2003b)	EPA Guideline (84-2) No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	Crl:CD-1(ICR) BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 78910 (30.3% glyphosate)	Negative	Erexson (2006)	EPA Guideline (84-2) No significant signs of toxicity observed in main study.

Table F.4. *In Vivo* Tests for Micronuclei Induction in Mammals- Glyphosate Formulations.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Bone Marrow Micronucleus Test	NMRI mice (males and females) Vehicle: 0.8% hydroxypropylmethyl cellulose	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 mg/kg (5/sex/dose)	TROP M (Glyphosate 480); 358.4 g/L glyphosate acid; 483.6 g/L IPA salt	Negative	Flügge (2010c)[1]	OECD Guideline 474 No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	Swiss mice (males only[2]) Vehicle: water	Oral gavage (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 2000 mg/kg (6/dose)	A17035A 289.7 g/L glyphosate	Negative	Negro Silva (2009)[1]	OECD Guideline 474 No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	Swiss mice (males only[2]) Vehicle: water	Oral gavage (2 treatments, 24 h apart); sampling after 24 h (last treatment)	0, 2000 mg/kg (6/dose)	Glyphosate SL (499.35 g/L glyphosate)	Negative	Negro Silva (2011)[1]	OECD Guideline 474 No significant signs of toxicity observed in main study
Bone Marrow Micronucleus Test	Hsd:CD-1(ICR) mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 79864 (38.7% glyphosate)	Negative #	Xu (2008a)	EPA Guideline (84-2) /OECD 474 No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	CD-1(ICR)BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 76171 (31.1% glyphosate)	Negative	Xu (2008b)	EPA Guideline (84-2) /OECD 474 No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	CD-1(ICR)BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 79991 (71.6% glyphosate)	Negative	Xu (2009a)	EPA Guideline (84-2) /OECD 474 No significant signs of toxicity observed in main study.

Table F.4. *In Vivo* Tests for Micronuclei Induction in Mammals- Glyphosate Formulations.							
Test/Endpoint	**Test System**	**Route of Administration**	**Doses**	**Test Material Purity**	**Results**	**Reference**	**Comments**
Bone Marrow Micronucleus Test	CD-1(ICR)BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 76138 (38.5% glyphosate)	Negative	Xu (2009b)[1]	EPA Guideline (84-2) /OECD 474 No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	Hsd:CD-1(ICR)BR mice (males only[2]) Vehicle: water	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 (mg/kg) (5/dose)	MON 76313 (30.9% glyphosate)	Negative	Xu (2009c)[1]	EPA Guideline (84-2) /OECD 474 No significant signs of toxicity observed in main study.
Bone Marrow Micronucleus Test	CD rats (males and females) Vehicle: 0.8% hydroxypropylmethyl cellulose	Oral gavage (single treatment); sampling after 24 and 48 h (high dose only)	0, 500, 1000, and 2000 mg/kg (5/sex/dose)	757 g/kg granular formulation (69.1% glyphosate acid)	Negative	Flügge (2010e)[1]	OECD Guideline 474 No significant signs of toxicity observed in main study

[1] Study was cited in Kier and Kirkland (2013). Supplementary information about the study was provided online including test guideline, test material purity, control chemicals and summary data tables.

[2] Only males tested; report indicated that there were no difference between sexes seen in range finding study.

BM= bone marrow, CA= chromosomal aberrations, MN= micronucleated erythrocytes, NCE= normochromatic erythrocytes, PCE=polychromatic erythrocytes.

Table F.5. Other Assays for Detecting DNA Damage- Glyphosate Formulations.

Test/Endpoint	Test System	Route of Administration	Doses/ Concentrations	Test Material/ Concentration	Results	Reference	Comments
Bacterial SOS Chromotest	*Escherichia coli* PQ37 strain	NA (*in vitro*)	0.25µg/sample	Roundup BIO formulation;	Positive	Raipulis *et al.* (2009)	
DNA Adducts ^{32}P-postlabeling	Swiss CD1 mice (males and females) Liver and kidney evaluated	Intraperitoneal injection	0, 400, 500 and 600 mg/kg, corresponding to 122, 152 and 182 mg/kg glyphosate salt	Roundup (30.4% isopropylammonium salt of glyphosate)	Positive (liver and kidney)	Peluso *et al.* (1998)	
DNA oxidative damage: 8-OHdG formation	Swiss CD-1 mice (males) liver and kidney evaluated	Intraperitoneal injection (single dose); sampling 4 and 24 h after injection	900 mg/kg corresponding to 270 mg/kg glyphosate (3/dose)	900 mg/kg corresponding to 270 mg/kg glyphosate	Kidney: positive at 8 and 24 h Liver: negative	Bolognesi *et al.* (1997)	
Single-cell gel electrophoresis (SCGE) assays-COMET assay	TR146 cells (human-derived buccal epithelial cell line). Alkaline conditions	NA (*in vitro*)		Roundup Ultra Max (450 g/l glyphosate acid)	Induced DNA migration at >20 mg/L	Koller *et al.* (2012)	Also measured multiple cellular integrity parameters to assess cytotoxicity. Formulation was more toxic than technical. Significant increase in LDHe at all concentrations tested. Cytotoxic ≥ 60 mg/L
Sister Chromatid Exchange (SCE)	Bovine lymphocytes	NA (*in vitro*)	28 - 1112 µM;; ±S9; sampling at 24 and 48 h	62% Isopropylamine salt of glyphosate	Positive	Sivikova & Dianovsky (2006)	

Table F.5. Other Assays for Detecting DNA Damage- Glyphosate Formulations.

Test/Endpoint	Test System	Route of Administration	Doses/ Concentrations	Test Material/ Concentration	Results	Reference	Comments
Sister Chromatid Exchange (SCE)	Human lymphocytes (2 donors)	NA (*in vitro*)	250, 2500 and 25000 µg/mL	Roundup; Isopropylamine salt of glyphosate (purity not stated)	Stat. significant increase ($p<0.001$) at 250 µg/mL in both donors, and in one donor at 2500 µg/mL	Vigfusson and Vyse (1980)	No growth seen at highest concentration (25 mg/mL)
Sister Chromatid Exchange (SCE)	Human lymphocytes	NA (*in vitro*)	-S9: 0, 0.1 and 0.33 mg/mL; 72 h exposure	Roundup, 30.4% glyphosate	Positive	Bolognesi *et al.* (1997)	Stat significant increase in SCE/cell at ≥ 0.1 mg/mL
Alkaline elution assay- DNA single strand breaks	Swiss CD-1 mice (males) liver and kidney evaluated	Intraperitoneal injection (single dose); sampling 4 and 24 h after injection	900 mg/kg corresponding to 270 mg/kg glyphosate (3/dose)	900 mg/kg corresponding to 270 mg/kg glyphosate	Positive (Increased elution rate) at 4 hours in liver and kidney At 24 h, returned to control levels	Bolognesi *et al.* (1997)	Return to control values at 24 h may indicate DNA repair or reflect rapid elimination of compound

h= hour, NA= not applicable, SCE= sister chromatid exchange, LDHe= extracellular lactate dehydrogenase

Appendix G

The following studies were considered during the systematic review, but were excluded from the analysis.

Amer S.M. et al (2006). In vitro and in vivo evaluation of the genotoxicity of the herbicide glyphosate in mice. Bulletin of the National Research Centre (Cairo) 31 (5): 427-446.

Aboukila, R.S. et al. (2014). Cytogenetic Study on the Effect of Bentazon and Glyphosate Herbicide on Mice. Alexandria Journal of Veterinary Sciences, 41: 95-101.

Majeska (1982d) MRID 00126616

Majeska (1982e) MRID 00126614

Majeska (1982f) MRID 00126615

Australian Pesticides and Veterinary Medicines Authority (APVMA)

A Review of the Earth Open Source (EOS) Report “Roundup and Birth Defects: Is the Public Being Kept in the Dark?”

July 2013

Australian Government

Australian Pesticides and Veterinary Medicines Authority

A REVIEW OF THE EARTH OPEN SOURCE (EOS) REPORT

"ROUNDUP AND BIRTH DEFECTS: IS THE PUBLIC BEING KEPT IN THE DARK?"

This Report was prepared for the APVMA by

Scitox Assessment Services

Canberra, ACT, Australia

July 2013

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GLOSSARY OF TERMS AND ABBREVIATIONS

AChE	acetylcholinesterase
ADD/ADHD	attention deficit disorder / attention deficit and hyperactivity disorder
ADI	acceptable daily intake
AMPA	aminomethyl sulphonic acid
APVMA	Australian Pesticides and Veterinary Medicines Authority
BNMN	binucleated cells with micronuclei
BVL	(German) Bundesamt fur Verbraucherschutz und Lebensmittelsicherheit
bw	body weight
CFR	conditional fecundability ratio
ChE	cholinesterase
CI	confidence interval
DNA	deoxyribonucleic acid
DoHA	Department of Health and Ageing
DSEWPaC	Department of Sustainability, Environment, Water, Population and Communities
EOS	Earth Open Source
EU	European Union
g	gram
GBHF	glyphosate-based herbicide formulation
GC	gas chromatography
GD	gestation day
GIT	gastro-intestinal tract
g/L	grams per litre
GLP	good laboratory practice
GM	genetically modified
h	hour
ha	hectare
hAR	human androgen receptor
hER	human oestrogen receptor
HC	historical control
HCL	hairy cell leukaemia
IC50	concentration inhibiting a chemical reaction by 50%
IP	intraperitoneal route of administration
IPA	isopropylamino
IV	intravenous route of administration
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	kilogram
km	kilometre
L	litre
lb/gal	pounds per gallon
LC50	lethal concentration to 50% of test cells or animals
LD50	lethal dose to 50% of test animals
LH	luteinising hormone
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	limit of quantification
µg	microgram

M	molar (concentration)
mg	milligram
mg/kg bw/d	milligrams per kilogram bodyweight per day
mL	millilitre
min	minute
mM	millimolar (concentration)
MM	multiple myeloma
mo	month
MRL	maximum residue limit or level
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NEDI	national estimated daily intake
ng	nanogram
NHL	non-Hodgkin's lymphoma
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NTP	US National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OR	odds ratio
PND	post natal day
PO	oral route of administration
POEA	polyoxyethylene tallow amine
ppb	parts per billion
ppm	parts per million
RA	retinoic acid
RBC	red blood cell
RNA	ribonucleic acid
RR	relative risk
s	second
SCE	sister chromatid exchange
StAR	steroidogenic acute regulatory protein
TTP	time to pregnancy
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
wk	week
yr	year

1. SUMMARY

In June 2011, Earth Open Source (EOS) published an article titled "Roundup and Birth Defects: Is the public being kept in the dark?" in which the organisation made a number of claims about the safety of the herbicide glyphosate and products containing it. These were said to include:

- Developmental malformations affecting the skull, face, brain and spinal cord in frog and chicken embryos at concentrations lower than used in agricultural and garden spraying;
- Endocrine disruption, reproductive toxicity and a range of developmental malformations in humans and experimental animals;
- Damage to DNA and genetic material in laboratory animals, humans a variety of *in vitro* test systems;
- Cancer of the testis in rats, skin cancer in mice, and blood system cancers in humans; and
- Neurotoxicity, including the development of Parkinson's disease in humans.

EOS was also highly critical of the European Union's review of glyphosate (EU, 2002 and 1998); challenged the design, conduct and scientific independence of industry-funded toxicology studies; and questioned some of the scientific principles normally applied to the assessment of hazard and risk from chemicals.

Given the widespread use of glyphosate in Australia for weed control in agricultural, home garden and other settings, the APVMA has investigated[1] the claims made in the EOS article and created this web-based publication to facilitate communication of its findings with the public and other stakeholders. The APVMA has:

- evaluated the key published studies cited in the EOS article together with some newer related publications and archived toxicology studies;
- examined the EU review of glyphosate and compared its findings with those of similar reviews prepared by the Australian DoHA, the US EPA (1993) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 2004a,b);
- assessed the scientific merit of the EOS arguments and the research upon which they are based; and
- considered whether there are implications for the registration of products containing glyphosate in Australia.

The APVMA's findings are summarised in this section of the publication. More detailed evaluations and scientific discussions of each main issue are presented in Sections 2 to 5 and Appendices 1–5.

1.1 The association between glyphosate / glyphosate-based herbicides and developmental malformations

[1] The work was performed by Mark Jenner of Scitox Assessment Services, Canberra, ACT.

As stated by EOS, Paganelli et al (2010) have shown that glyphosate and a glyphosate-based herbicide formulation (GBHF) cause malformations including microphthalmia and microcephaly (abnormally small eyes and head) in toad and chicken embryos. However, the routes of administration (incubation with, or injection into toad embryos, and injection into chicken eggs) are not relevant to humans and other mammals, whose foetuses can only become exposed to chemicals if they are absorbed by their mother and transferred across the placenta from her blood circulation.

In 1996 the APVMA reviewed glyphosate products because of evidence of toxicity to amphibians when applied in and around aquatic areas. Toxicity was attributed to polyethoxylated tallow amine (POEA) surfactants in some glyphosate products. The APVMA consequently strengthened label warnings and restricted the use of glyphosate products around waterways and water bodies to reduce the risk of aquatic contamination (see http://www.apvma.gov.au/products/review/completed/glyphosate_history.php), until less toxic formulations could be developed and registered. Today, over a third of registered glyphosate products contain low toxicity surfactants, and can be used in or around waterways (see http://www.apvma.gov.au/news_media/community/2010-13_glyphosate_au.php). Nevertheless, the APVMA will refer Paganelli's findings to the Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC) for consideration.

Eight developmental toxicity studies with glyphosate in rats and seven in rabbits have been reviewed by pesticide regulatory agencies and scientific organisations including the APVMA, the US EPA, the EU and the JMPR. These and additional studies have also been evaluated by Kimmel et al (2013). The reviews have concluded that at high oral doses, glyphosate causes toxicity to mother rats and foetuses but is not a teratogen (ie, does not cause foetal malformations). The APVMA is satisfied that the German BVL has not misused historical control (HC) data in its evaluations, despite the EOS claim to this effect.

The lowest NOEL for maternal and foetal toxicity in rats was 300 mg/kg bw/d (1000-times the Australian ADI for glyphosate) and the lowest LOEL in foetuses was 1000 mg/kg bw/d. In rabbits, visceral abnormalities including heart dilation and intraventricular septal defect were reported in six of nine developmental toxicity studies. By the most conservative interpretation, these effects were confined to ~~a~~ doses of 450 and 500 mg/kg bw/d. The lowest NOEL for foetal toxicity in rabbits was 100 mg/kg bw/d, or 333-times higher than the Australian ADI. The margins between women's dietary exposure to glyphosate and the NOELs in laboratory animals are even higher; following a dietary survey of pregnant Australian women and analysis of composite food samples they provided, McQueen et al (2012) estimated that maternal dietary exposure to glyphosate is 0.001 mg/kg bw/d. This dose is 0.33% of the ADI, and is also only 5% of the National Estimated Dietary Intake (NEDI) of 0.02 mg/kg bw/d.

Dallegrave et al (2003) have reported skeletal abnormalities in foetal rats whose mothers were treated at 500–1000 mg/kg bw/d during gestation with an herbicide containing 36% glyphosate and 18% POEA surfactant. However, the study has been criticised for reporting deficiencies and anomalies, and its results may have been affected by non-standard methods used to fix and prepare foetuses for skeletal examination (Williams et al, 2012). The APVMA notes that POEA is not a

developmental toxin and has a NOAEL of 300 mg/kg bw/d in foetal rats (Holson, 1990).

The APVMA has investigated EOS' claims that agricultural use of glyphosate is causing adverse reproductive outcomes in exposed human populations. However, the published body of epidemiological research has produced inconsistent, equivocal or weak evidence of reproductive harm. In particular, most epidemiology studies rely on self-reported exposure information, do not measure exposure, and cannot demonstrate causal associations between glyphosate and reproductive harm. Many studies are also affected by confounding variables including exposure to other possible risk factors and the use of, or potential exposure to, other chemicals.

Furthermore, evidence suggests that exposure of glyphosate product users to glyphosate contained in herbicide products is relatively low, possibly due the low dermal absorption rate of glyphosate, which the EU (2002) has estimated to be less than 3%. In a urinary biomonitoring study of American farming families, the maximum absorbed doses from a single mixing / loading / application event were 0.004 and 0.00004 mg/kg bw in the farmers and spouses, respectively (Acquavella et al, 2004 and JMPR, 2004b). These values represent 1.3 and 0.013% of the Australian ADI for glyphosate.

Therefore, the APVMA is satisfied that glyphosate does not pose a risk of developmental toxicity through public or occupational exposure, despite EOS's claim to this effect.

1.2 The association between glyphosate / glyphosate-based herbicides, endocrine disruption and reproductive toxicity

Numerous single- and multi-generation studies have been performed with glyphosate in rats at daily doses of up to 1500 mg/kg. Despite thorough and systematic investigation of relevant parameters, they have yielded no evidence that glyphosate is toxic towards the male or female reproductive systems. No biologically significant effects occurred in a 13-week US National Toxicology Program (NTP) reproduction toxicity study in rats and mice at dietary doses of up to 5000 and 7500 mg/kg bw/d in the respective species. Furthermore, no effects indicative of endocrine disruption have been found in short-term repeat-dose, subchronic and chronic toxicity studies with glyphosate in laboratory animals, and glyphosate has negligible or weak effects on steroid hormone receptors and biosynthesis *in vitro*.

Little reliance can be placed on the study of Yousef et al (1995), which EOS claims to have demonstrated sperm damage in rabbits. When administered for six weeks, glyphosate may have caused fully or partially reversible decreases in ejaculate volume and the viability and activity of sperm, but the study used low numbers of animals and deficient experimental methods, was markedly affected by variation within the control group, and was poorly reported. It is even unclear what doses of glyphosate were administered.

Although EOS has described glyphosate as causing testicular cancer in rats, independent assessments of the relevant study (Lankas et al, 1981) by Australia, the WHO and the US EPA have concluded that the tumours were not treatment-related. Furthermore, neither testicular tumours nor other forms of cancer have developed in eight other carcinogenicity studies with glyphosate in mice or rats, respectively at doses of up to *ca* 5000 and 1200 mg/kg bw/d. Mink et al (2012) have reviewed the epidemiological literature (7 cohort studies and 14 case-control studies) to evaluate

whether exposure to glyphosate is associated causally with cancer risk in humans. They found no consistent pattern of positive associations to indicate a causal relationship between total cancer (in adults or children) or any site-specific cancer and exposure to glyphosate. This provides strong evidence that glyphosate does not pose a carcinogenicity hazard to humans.

The APVMA anticipates that glyphosate's potential to cause endocrine disruption will be clarified in the near future, as the active has been tested according to US EPA Series 890 Test Guidelines following its selection for Tier 1 screening under the EPA's Endocrine Disruption Screening Program. As at June 2013, all data have been received by the EPA and are currently under review (see http://www.epa.gov/scipoly/index.html). So far three abstracts have been published, demonstrating a lack of potential to interact with oestrogen and androgen receptors in vitro, inhibit steroidogenesis in vitro, affect thyroid-mediated developmental endpoints in the amphibian metamorphosis assay, or cause endocrine disruption in the Hershberger and uterotrophic assays in rats (Levine et al 2012, Webb et al 2012, Saltmiras and Tobia 2012).

There is experimental evidence in support of EOS's assertion that glyphosate-based herbicide formulations (GBHFs) cause reproductive toxicity in drakes and, in male rats, interfere with the maturation of the reproductive organs during puberty. In some studies (Oliviera et al 2007, Romano et al 2010) GBHFs were administered directly to the test animals, while other studies (Dallegrave et al 2007, Romano et al 2012) involved maternal exposure to GBHFs during pregnancy and/or lactation. However, the observed effects have been inconsistent, including increases and decreases in blood testosterone levels and sperm production, and delaying and hastening of the onset of puberty. Furthermore, most of the relevant studies are deficient in aspects of their design and reporting, have used novel, unvalidated test methods, and/or may have been subjected to interference by experimental artefacts. None of the studies have identified which component(s) of the test GBHFs caused the reported effects.

In vitro, some GBHFs have caused anti- androgenic and oestrogenic activity, changes in the expression of hormonally-regulated genes, inhibition of aromatase (an enzyme that converts testosterone to oestradiol), cell injury and death. However, many *in vitro* studies have used cancer cells or other novel test systems, and a 2009 Canadian PMRA assessment concluded that their findings are not representative of the exposure of live animals and humans (see http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_fact-fiche/glyphosate/reconsideration-reexamen-eng.php). Furthermore, surfactants (including POEA) are a likely cause of cellular toxicity and interference with *in vitro* assays of hormonal regulation. Few studies have identified or controlled for the surfactants and other adjuvants present in test formulations, creating uncertainty as to which chemicals are causing the reported effects, and their mode of action.

Therefore, the APVMA believes it is premature to characterise GBHFs as endocrine disruptors.

1.3 Evidence for the genotoxicity and carcinogenicity of glyphosate / glyphosate-based herbicides

Only a small minority of the genotoxicity studies with glyphosate and GBHFs have yielded positive findings, some of which were inconsistent with negative results in other studies examining the same end-point. Interpretation of several published studies is hindered by methodological failings or inadequately-detailed reporting.

Many instances of positive findings could also be explained by cytotoxicity, ie, generalised toxicity against the test cells, tissues or organs, as opposed to direct effects on genetic material. When the activity of glyphosate and GBHFs was compared under the same experimental conditions, the active constituent was usually inactive or much less active than the formulations. Where studies were performed with GBHFs without examining the individual ingredients, it is unknown whether the findings were caused by glyphosate, surfactants or other adjuvants, or depended on interaction between the various formulation components. A recent, comprehensive review of published and sponsored regulatory genotoxicity studies (Kier and Kirkland, 2013) has concluded that glyphosate and typical GBHFs do not appear to present significant genotoxic risk under normal conditions of human or environmental exposures. Studies of genetic injury within human populations have not yielded consistent evidence of a causal association between glyphosate exposure and genotoxicity. Therefore, weight and strength of evidence supports the view that glyphosate is not genotoxic.

Between them, the Australian DoHA, the US EPA, the EU and the JMPR have reviewed four dietary carcinogenicity studies with glyphosate in mice and six similar studies in rats, performed over dose ranges of 11 – *ca* 5000 and 4 – *ca* 1200 mg/kg bw/d in the respective species. Although the incidence of testicular tumours was increased in glyphosate-treated rats in one study (Lankas, 1981), the reviewing agencies agreed that by reference to HC data, the tumours were not related to treatment. Furthermore, tumours did not develop in the testis – or any other organs or tissues – in the remaining carcinogenicity studies.

A GBHF has been found to promote skin tumours when applied dermally to mice at 25 mg/kg bw/d (George et al, 2010), but carcinogenesis depended on prior treatment with a tumour initiator chemical, without which there was no development of cancer. The study did not demonstrate which component(s) of the product caused the promoting activity. The finding is of limited relevance to persons preparing GBHFs for use because the tumour promoting activity was relatively weak, and to achieve an equivalent level of exposure, operators would have to be exposed three times weekly for over a decade at doses unattainable while wearing the required protective clothing and equipment.

The Australian DoHA (2005) and the JMPR (2004b) have assessed nine epidemiological studies performed from 1999 onwards, including those cited by EOS as showing associations between glyphosate and blood system cancers. Some researchers have found increased odds of developing non-Hodgkin's lymphoma, multiple myeloma or hairy cell leukaemia in persons who have used or been exposed to glyphosate. However, the evidence has been inconsistent both between and within studies, whose outcomes are potentially confounded by inaccurate exposure data and exposure to other pesticides and environmental agents. A recent review (Mink et al, 2012) of epidemiological studies relevant to cancer end-points considered seven cohort studies and 14 case—control studies; there was no consistent pattern of positive associations to indicate any causal relationship between total cancer (in adults or children) or any site-specific cancer and exposure to glyphosate.

Currently, the weight and strength of evidence does not support the conclusion that glyphosate causes cancer in either laboratory animals or humans.

1.4 Neurotoxicity of glyphosate / glyphosate-based herbicides

Glyphosate does not have the same biological properties as organophosphate insecticides, and an extensive battery of neurotoxicology and general toxicity studies in laboratory animals has found no evidence that glyphosate inhibits cholinesterase activity, or causes neuropathy or other disorders in the nervous system. The largest and most comprehensive study of pesticide applicators (Kamel et al, 2007) has found no association between the use of glyphosate and Parkinson's disease.

1.5 Human exposure to glyphosate

During the assessment process for pesticides that may leave residues in food, chemicals are assigned an Acceptable Daily Intake (ADI), which is the level of intake of a chemical that can be ingested daily over a lifetime without any appreciable risk to health.

The current ADI for glyphosate, set by the Australian DoHA in 1985, is 0.30 mg/kg bw/d, based on a NOEL of 30 mg/kg bw/d (the highest administered dose) in a three-generation reproduction study in rats. There is a 100-fold safety factor between the pivotal NOEL and the ADI, comprised of a ten-fold component to account for extrapolation from animals to humans and a further ten-fold component to account for variation in sensitivity within the human population. The toxicological studies cited by EOS do not demonstrate any need to revise the ADI.

By comparison with the ADI, the actual level of exposure for Australians is probably much lower. Based on the consumption of food commodities for which the APVMA has set Maximum Residue Limits (MRLs), the National Estimated Daily Intake (NEDI) of glyphosate is 0.02 mg/kg bw/d, or only six percent of the ADI. Even this value may be conservative. Following a dietary survey of pregnant Australian women and analysis of composite food samples they provided, McQueen et al (2012) have estimated that maternal dietary exposure to glyphosate is 0.001 mg/kg bw/d. This dose is 0.33% of the ADI, and is also only 5% of the NEDI of 0.02 mg/kg bw/d. Internationally, the JMPR (2004a) estimated theoretical maximum daily intake for glyphosate is 1% of the WHO ADI of 0–1.0 mg/kg bw/d.

Evidence suggests that exposure of glyphosate product users is also relatively low. This may be due to the relatively low dermal absorption rate, which the EU (2002) assessment estimated to be less than 3% for glyphosate and no more than 1% for glyphosate trimesium. In a biomonitoring study of American farming families, Acquavella et al (2004) detected glyphosate in the urine of 60% of farmers, 4% of their spouses and 12% of their children on the day of application. According to the JMPR (2004b) assessment, the maximum systemic (absorbed) doses from a single mixing / loading / application event were 0.004, 0.00004 and 0.0008 mg/kg bw in the farmers, spouses and children, respectively. These values represent 1.3%, 0.013% and 0.27% of the Australian ADI for glyphosate.

1.6 Overseas assessment activity

The US EPA and the Canadian PMRA initiated routine scheduled re-registration reviews of glyphosate in 2009 and 2010, respectively. Both these regulators will use the reviews to consider new research on glyphosate, relating to potential effects on environmental and human health. The EPA will assess studies on the immunotoxicity and acute and subchronic neurotoxicity of glyphosate, the ecotoxicity of products containing the surfactant POEA, and the ecological risk posed by aminomethyl

phosphonic acid (AMPA, a degradation product of glyphosate). The review is scheduled for completion in 2015 (US EPA, 2009). In addition to the re-registration review, the EPA is also evaluating glyphosate under the Endocrine Disruptor Screening Program. The Canadian review, targeted for completion in 2014, will include health and environmental risk assessments of the POEA/glyphosate combination (see http://www.hc-sc.gc.ca/alt_formats/pdf/pubs/pest/decisions/rev/rev2010-02-eng.pdf).

Conclusions

1. The APVMA currently has no data before it suggesting that glyphosate products registered in Australia and used according to label instructions present any unacceptable risks to human health, the environment and trade.
2. The weight and strength of evidence shows that glyphosate is not genotoxic, carcinogenic, or neurotoxic.
3. Glyphosate causes malformations in toad and chicken embryos treated by incubation and/or injection, but these findings are not predictive of a developmental hazard to humans because of the routes of administration used. Studies in birds and/or rats have reported that some glyphosate-based herbicide formulations (GBHFs) cause foetal skeletal abnormalities, toxicity to the male reproductive system and interference with the maturation of the male reproductive organs during puberty. However, the relevant studies were affected by flawed design, methodology and / or reporting, and the claimed effects on puberty have been inconsistent in different studies.
4. Glyphosate is not a teratogen in rats and rabbits treated via oral administration and has not shown reproductive toxicity in multi-generation dietary studies in rats. Epidemiological studies have found no consistent or convincing evidence of reproductive dysfunction in human populations reportedly exposed to glyphosate. Glyphosate is therefore extremely unlikely to cause reproductive or developmental toxicity in humans under normal conditions of exposure.
5. The potential for glyphosate to cause endocrine disruption will be clarified by the current review under the US EPA's Endocrine Disruptor Screening Program. In studies published so far, glyphosate has shown a lack of activity in the Hershberger and uterotrophic assays in rats or in tests for interaction with oestrogen and androgen receptors, inhibition of steroidogenesis, or interference with metamorphosis in amphibians. At present, there is no scientific justification for classifying glyphosate as an endocrine disruptor.
6. Surfactants present in the test GBHFs may have confounded the results of *in vitro* studies of their effects on hormonal regulation and cellular toxicity. Furthermore, the relevance of some test systems to human hazard and risk assessment is unproven.
7. Most studies with GBHFs have not identified which of their chemical constituents caused the reported effects on cells and laboratory animals, or characterised their mode of action.
8. The toxicological studies cited by EOS do not demonstrate a need to revise the current Australian ADI of 0.3 mg/kg bw/d for glyphosate. The available evidence indicates that there are very wide margins between the ADI and the

actual intake of glyphosate via food and from exposure while preparing and applying glyphosate products.

9. The APVMA will monitor the US and Canadian reviews of glyphosate and consider any new information that emerges.

2. MAIN BODY OF THE REVIEW

2.1 The association between glyphosate / glyphosate—based herbicides and developmental malformations

2.1.1 Effects in toad and bird embryos

According to EOS, Roundup causes developmental malformations in toad and chicken embryos at doses "much lower than those used in agricultural spraying" and "ten times lower than the MRL". These claims are based on an article by Paganelli et al (2010; see Appendix 3), who treated African clawed toad (*Xenopus laevis*) embryos with glyphosate (360 or 500 pg by intracellular injection) or a 480 g/L Roundup formulation (present in the incubation medium at a 5000-fold dilution, or 96 mg glyphosate/L). The test compounds decreased the expression of genes that regulate embryonic development, impaired the formation of neurons (nerve fibres) and the neural crest, and also caused microphthalmia and microcephaly (abnormally small eyes and head).

Incubation with Roundup at 4000- and 3000-fold dilutions caused increases in retinoic acid (RA) signalling activity within toad embryos, whereas co-treatment with a RA-receptor antagonist blocked increases in RA signalling and prevented microcephaly. The study authors also found that injecting Roundup into chicken eggs (20 µL of 3500- or 4500-fold dilutions, equivalent to 2.7 or 2.1 µg glyphosate/egg) caused microphthalmia and microcephaly in the embryos. However, they did not investigate whether the malformations occurred in response to stimulation of RA signalling, as in *Xenopus*.

APVMA comment

Retinoic acid, a metabolite of vitamin A, has a pivotal role in the development of the central nervous system and causes microcephaly, microphthalmia and neural tube defects including spina bifida when administered in excess to pregnant laboratory animals (Maden, 2002). Therefore, in principle, Paganelli's study suggests that glyphosate and glyphosate-based herbicides may have the potential to cause developmental malformations by a mechanism involving RA.

However, caution should be exercised in extrapolating from findings in amphibians and birds to predicting risks for humans. The absorption, distribution, excretion and toxicokinetics of chemicals in pregnant mammals are fundamentally different to those in organisms whose development occurs in the external environment. Furthermore, the experimental routes of administration used by Paganelli (incubation or injection) do not reflect the likely routes of human exposure (oral, dermal, or inhalational) or the protective effect of the placental barrier (BVL, 2010).

Above all, as discussed later in this Section, glyphosate has been tested in numerous developmental studies over a 20—year period in rats and rabbits without causing malformations of the head and neural tube, even at doses high enough to be toxic to the mother and foetus.

2.1.2 Effects in laboratory animals

The major theme of the EOS article is that glyphosate has shown teratogenic activity in industry-sponsored developmental toxicity studies in rats and rabbits, with effects on foetuses including mortality, reduced ossification (bone formation) and increased incidences of skeletal and visceral abnormalities. Furthermore, EOS claims that these findings were wrongly dismissed in the EU (1998) review of glyphosate performed by the German Bundesamt fur Verbraucherschutz und Lebensmittelsicherheit (BVL). In particular, EOS criticises the use of historical control (HC) data to assist in deciding whether foetal malformations and anomalies were related to treatment, or occurred by chance. EOS was concerned that HC data introduced variability into the analysis and obscured the teratogenic effects of glyphosate.

EOS's comments on the BVL evaluations of specific studies can be summarised as follows:

- The BVL evaluation of Tasker et al (1980a) did not consider that an increased incidence of foetal malformations in rats at the highest dose (3500 mg/kg bw/d) was treatment-related, because the incidence lay within the HC range. EOS regards this as unjustifiable, due to the findings of malformations in other studies with glyphosate. EOS also criticises the BVL's definition of sternebral unossification as a variation, rather than a malformation.

- EOS disagrees with the BVL assessment of Suresh (1993a), a developmental study in rabbits at 20, 100 and 500 mg/kg bw/d in which there was an increase at all doses in major visceral anomalies, including dilated heart. Suresh concluded that the NOEL for maternotoxicity was 20 mg/kg but there was no NOEL for foetal visceral malformations. The BVL dismissed the biological significance of the foetal findings, and set the NOEL at 100 mg/kg bw/d based on comparison with HC data.
- The BVL evaluation of Brooker et al (1991b; a gavage study in rabbits at 50, 150 and 450 mg/kg bw/d) was criticised for dismissing an increased incidence of foetal heart malformations at the high dose by reference to HC data.
- EOS criticises the BVL's assessment of Bhide and Patil (1989; a developmental study in rabbits at 125, 250 and 500 mg/kg bw/d), which assigned a NOAEL of 250 mg/kg for developmental toxicity based on embryo- and foetal lethality and visceral and skeletal malformations at the high dose. EOS believes that heart, lung and kidney malformations were increased at all doses, while rudimentary 14th rib was increased at 250 and 500 mg/kg.
- EOS does not concur with the BVL evaluation of an anonymous (1981) oral feeding study in rabbits, in which increased foetal mortality at 50.7 and 255 mg/kg bw/d was not attributed to treatment because the doses were "far below those at which foetal effects were found in the gavage studies."

APVMA comments

The mammalian toxicology of glyphosate has been reviewed by several national and international pesticide regulatory agencies and scientific organisations, including the

APVMA[2], the US EPA, the EU and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). Between them, these agencies have evaluated eight developmental toxicity studies with glyphosate in rats and seven in rabbits. Kimmel et al (2013) and Williams et al (2012) have also reviewed developmental studies with glyphosate in laboratory animals.

2.1.2.1 Effects in rats

The German BVL assessed six rat developmental studies for the EU and/or JMPR. These are summarised in Appendix 1. There was a wide span of doses, ranging from 22 to 3500 mg/kg bw/d. According to the BVL, maternotoxicity was seen as clinical signs and reduced bodyweight gain at ≥1000 mg/kg, with maternal deaths at 3500 mg/kg. Effects on foetuses comprised increased incidences of wavy ribs, unossified sternebrae[3], and incompletely ossified finger / toe bones, cranial centre and vertebral arches at ≥1000 mg/kg; with increased mortality and depressed litter and mean foetal bodyweights at 3500 mg/kg. Overall, the lowest NOEL for maternal and foetal effects in rats was 300 mg/kg bw/d, a dose 1000-times higher than the Australian ADI for glyphosate.

After closely examining the German evaluations for the EU and JMPR, the APVMA supports the BVL's conclusions, including those relying on HC data[4]. Indeed, it is possible to rebut EOS's claim that the BVL incorrectly dismissed the treatment-relatedness of dwarfism and bent tail seen at 3500 mg/kg bw/d in Tasker et al (1980a). The US EPA, Australian DoHA and Kimmel et al (2013) have also evaluated this study, and independently reached the same conclusions as the BVL. The DoHA (1985) attributed the malformations to genetic factors because all dwarf foetuses were in one litter, all those with bent tails were confined to another litter, and the control and 3500 mg/kg groups had the same number of litters with malformed foetuses.

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[2] Human health risk assessments are performed for the APVMA by the Department of Health and Ageing (DoHA).

[3] According to the OECD (2008) Guidance Document on Mammalian Reproductive Toxicity Testing and Assessment, there is no generally accepted classification of malformations (permanent structural changes that may adversely affect survival, development or function) and variations (divergence beyond the usual range of structural constitution, which may not adversely affect survival or health). The nomenclature used by study laboratories and regulatory agencies may therefore vary, in part because there is a continuum between normal and abnormal development, because some observations are classified as malformations in one species and variations in another, or due to the use of different nomenclature conventions by different organisations. The highly authoritative DevTox website (http://www.DevTox.org), whose terminology and classification system was developed by a series of international harmonisation workshops, does not classify sternebral unossification as either a malformation or variation.

[4] Besides identifying the effects of the test compound on animals, the major purpose of regulatory toxicology studies is to establish the doses at which the effects do or do not occur. This is most commonly done by comparing findings from groups of animals treated over a range of doses with those from an untreated group of the same species and genetic background, housed under the same conditions as the test groups. These untreated animals are usually referred to as "study" or "concurrent" controls. In addition to presenting data from the test groups and study controls, reports may also include "historical control" (HC) data from other studies performed in animals from the same supplier and genetic background at the same laboratory. HC mean values and ranges are sometimes used during evaluation to clarify the biological significance of differences between the study controls and groups of animals treated with the test compound. HC data can also provide information about whether a study control group's results are atypical compared with those observed in other control groups. The use of HC data generated within a five-year span around the study under review is accepted internationally under the OECD (2008) guidelines.

Furthermore, based on the available evaluation reports, neither dwarfism nor bent tail occurred at any dose in the other rat studies, or in rabbits.

However, there are possibly significant findings in Dallegrave et al (2003), a developmental toxicity study in which pregnant rats were dosed orally from GD 6–15 with a Roundup formulation containing 360 g/L glyphosate and 18% w/v polyethoxylated tallow amine (POEA)[5]. The doses were equivalent to 500, 750 or 1000 mg glyphosate/kg bw/d. Based on increased mortality in dams at the highest dose, the apparent NOEL for maternotoxicity was 750 mg/kg bw/d but this is uncertain because Dallegrave et al did not report clinical signs, even in dams which died. The test formulation did not affect foetal survival or growth, but from 500 mg/kg upwards caused skeletal abnormalities including ossification deficits, absent and wavy ribs, absent vertebrae, and divided sternebrae and supraoccipital and interparietal bones.

The fact that the test formulation caused malformations at half the lowest foetal LOEL in rat studies with glyphosate active constituent (1000 mg/kg bw/d; see above) suggests that formulation adjuvants caused or contributed to the effects. When Holson (1990) administered POEA to pregnant rats by gavage on GD 6–15 at 15, 100 and 300 mg/kg bw/d, there was significant maternal toxicity at 300 mg/kg while decreased food consumption and mild clinical signs occurred in dams at 100 mg/kg. The maternal NOEL was 15 mg/kg bw/d. Foetal growth and development were unaffected, so the NOEL for developmental toxicity was 300 mg/kg bw/d. In Dallegrave et al (2003), by comparison, rat dams were exposed to POEA in the test formulation at *ca* 250, 375 or 500 mg/kg bw/d, exceeding the maternal LOEL of the pure surfactant by 2.5 to 5-fold. Furthermore, Dallegrave's mid and high dose dams received more POEA than administered in Holson's study (Williams et al 2000; Williams et al, 2012).

Williams et al (2012) have also noted anomalies in the numbers of foetuses, corpora lutea and implantations reported by Dallegrave et al (2003), and commented that Dallegrave used a non-standard method for fixing and protein-digesting foetuses prior to skeletal examination, which may have created areas that appeared to be incompletely ossified. Given the reporting and methodological issues identified in Dallegrave et al (2003), and because there are no other known developmental toxicity studies with GBHFs that can be compared with Dallegrave's study, the APVMA can not reach any further conclusions on Dallegrave's findings.

2.1.2.2 Effects in rabbits

Six of the nine known developmental studies with glyphosate in rabbits have been assessed by the German BVL for the EU and/or JMPR. Two other sponsored regulatory studies have been assessed by Kimmel et al (2013), and a further study (Stauffer Chemical Co, 1983b) was evaluated by the Australian DoHA. The doses spanned from 10 to 500 mg/kg bw/d. Evidence of maternotoxicity was fairly consistent between studies, but the threshold doses for each effect varied widely. Clinical signs and bodyweight depression occurred at $\underline{\geq}$40 mg/kg, with increased maternal mortality and abortion at $\underline{\geq}$100 mg/kg and decreased food consumption and

[5] POEA (also known as polyoxyethylene tallow amine and polyoxyethyleneamine; CAS Registry no. 61791-26-2) is a mixture of polyethoxylated long chain alkylamines synthesised from animal-derived fatty acids (Williams et al, 2000).

bodyweight gain at ≥150 mg/kg. Due to the varying LOELs, maternal NOELs lay between 20–250 mg/kg bw/d.

Four gavage studies did not demonstrate any effects on foetuses at the highest doses administered (100 mg/kg bw/d in Stauffer Chemical Co, 1983b; 300 mg/kg in Hojo, 1995; 350 mg/kg in Tasker et al, 1980b and 400 mg/kg in Coles and Doleman, 1996). In four gavage studies there was fetotoxicity, seen as bodyweight depression and reduced skeletal ossification at 300 mg/kg, increased mortality at ≥450 mg/kg and extra 13^{th} rib or unilateral 14^{th} rib at 500 mg/kg bw/d.

Visceral abnormalities occurred in six studies. These included heart or ventricular dilation and cardiomegaly, the incidences of which were elevated at 20, 100 and 500 mg/kg bw/d in Suresh (1993a). By reference to HC data, the BVL concluded that the effects were biologically significant only at the high dose, and set the foetal NOEL at 100 mg/kg bw/d. Intra-ventricular septal defect (either alone or combined with other cardiac abnormalities) was reported in Brooker et al (1991b), Bhide and Patil (1989), Hojo (1995) and Moxon (1996). Brooker et al observed incidences of 3.6% and 5.3% at 150 and 450 mg/kg bw/d, compared with 0.6% among study controls. However, given that the incidences lay within the HC range (0.7–5.9%), the BVL did not ascribe the finding to treatment at either dose. Septal defect was increased at 125, 250 and 500 mg/kg bw/d in Bhide and Patil (incidences were 0.9, 0.8 and 2.6% vs zero among controls). The BVL evaluator reasoned that the finding was unlikely to have been caused by glyphosate but could not exclude a relationship to treatment at 500 mg/kg bw/d. The APVMA concurs with this view, especially in the absence of HC data from the study laboratory. Also in Bhide and Patil, but no other study, there were elevated incidences of absent kidney (0.9, 1.8, 1.6 and 7.7% at 0, 125, 250 and 500 mg/kg bw/d) and postcaval lung lobe (0, 0.9, 1.6 and 5.1% in the respective groups). Again, the BVL attributed the findings to treatment at 500 mg/kg but not at lower doses.

Hojo (1995) reported one foetus affected by interventricular septal defect and hypoplasia of the pulmonary artery at 100 mg/kg bw/d, but no cardiac abnormalities at 10 or 300 mg/kg. Coles and Doleman (1996) observed a foetus with a heart and great vessel defect at 200 mg/kg bw/d but no cases at 50 or 400 mg/kg. Moxon (1996) found three foetuses having heart defects involving septation, one each at 0, 100 and 300 mg/kg bw/d. In these latter three studies, it is clear that the cardiovascular abnormalities were unrelated to treatment.

Overall, the range of foetal NOELs in rabbits was 100–400 mg/kg bw/d, overlapping the lowest foetal LOEL of 300 mg/kg bw/d. The margin between the *lowest* foetal NOEL and the Australian ADI is 333. Examining the dose-effect relationship in the rabbit gavage studies, the most sensitive end-points are foetal bodyweight and skeletal ossification, which were depressed at 300 mg/kg. If cardiac dilation, ventricular septal defect and major visceral malformations (including missing lung lobes and kidney) were indeed caused by glyphosate, by any reasonable interpretation they are confined to the 450 and 500 mg/kg groups. The margin between the doses causing these effects and the Australian ADI is 1500.

The final issue in rabbits involves a seriously-deficient study report of increased foetal deaths occurring at 50.7 and 255 mg/kg bw/d in a developmental study by dietary administration (Anon, 1981). The BVL assigned a NOEL of 10.5 mg/kg bw/d but highlighted the inconsistency between these particular findings and the results in the gavage studies, in which foetal mortality was not enhanced below 300 mg/kg bw/d.

Based on the comparative weight and strength of evidence, this comment is entirely reasonable.

2.1.3 Epidemiological evidence

According to EOS, a report commissioned by the state government of Chaco, Argentina (CPICA, 2010), found an increase of nearly four-fold in the rate of malformations over a decade, coinciding with the expansion of agriculture into the region and a corresponding rise in the use of agrochemicals, including glyphosate.

EOS, Paganelli et al (2010) and Carrasco (2011) cite Benitez-Leite et al (2009) as finding that Paraguayan women exposed to herbicides during pregnancy were more likely than unexposed women to deliver offspring with malformations. These included microcephaly or anencephaly (small head or absence of a cranium), facial defects, myelomeningocele (protruding brain), cleft palate, synotia (ears extended below the jaw), polydactyly (too many fingers / toes) and syndactyly (fused digits). The specific risk factors identified were living near treated soy fields, dwellings located <1 km from treated fields, storage of pesticides in the home, and contact with pesticides (Carrasco, 2011).

EOS also claims that Savitz et al (1997) found high levels of premature births and miscarriages in female members of Canadian farming families that used pesticides, including glyphosate.

APVMA comments

According to the BVL (2010), Mulet (2011) and Saltmiras et al (2011), the database studied by Benitez-Leite et al was small and confined to children born in one hospital. Benitez-Leite et al suspected a relationship between malformations and pesticide (*not specifically herbicide*) exposure but did not provide evidence of maternal exposure to glyphosate, or even mention glyphosate in their article. The association between "living near treated fields" and congenital malformations was weak, with an odds ratio (OR) $1/6^{th}$ of the reported association between malformations and pesticide storage at home.

The "Ontario Farm Family Health Study" (Savitz et al, 1997) has been assessed by the JMPR (2004b), the Australian DoHA (2005), Mink et al (2011) and Williams et al (2012). In a cross-sectional study of 1898 couples and 3984 pregnancies, Savitz et al examined the association between pregnancy outcome and the father's exposure to pesticides during the three months before conception. The study relied on mail questionnaires, with telephone interviews of non-respondents. Couples were asked to provide information on all pregnancies (of which over $1/3^{rd}$ had occurred over 10 years previously) and farm activities and pesticide use over the previous five years. Not all reports of adverse pregnancy outcomes were confirmed from medical or other records, and the study was uncontrolled for maternal age, smoking and previous history of spontaneous abortion.

There were no statistically significant associations with the use of glyphosate alone. There were slightly increased odds ratios (OR) but no statistically significant associations between miscarriage and paternal use of herbicides *and* glyphosate on crops (17 exposed cases, OR = 1.5; 95% Confidence Interval = 0.8–2.7) or in the yard (13 exposed cases, OR = 1.4; 95% CI = 0.7–2.8). Based on five exposed cases, the

OR for pre-term delivery and use of herbicides *and* glyphosate on crops was 2.4 but the risk estimate was of low precision (the 95% CI was 0.8–7.9). There was no association between the use of farm chemicals and small-for-gestational age births or sex ratio.

DoHA questioned the apparent association between miscarriage and herbicide/glyphosate application due to the small number of cases and the imprecision of the risk estimate, noted that the study authors had not directly tested for association between glyphosate use and reproductive effects, and observed that the study was further weakened by the lack of quantitative exposure assessment and data on the time spent using pesticides. The JMPR assessment commented that the claimed associations were weak, were not controlled for confounding factors including other pesticides, and did not meet generally accepted criteria for determining causal relationships.

Sanin et al (2009) undertook a retrospective cohort study of time to pregnancy (TTP) among 2592 fertile women living in five regions of Colombia, between which there was variation in the use of glyphosate-based herbicides. Glyphosate was not used in the region with lowest risk of prolonged time to pregnancy (TTP). The region with greatest risk (fecundability[6] OR of 0.15; 95% CI = 0.12–0.18) was a sugar cane-growing district with a prolonged history of use of glyphosate and other chemicals. Glyphosate was applied to illegal crops in two of three other regions with enhanced risk, but not in the third, an organic agriculture area. The study authors concluded that the observed differences in TTP remained unexplained.

Numerous other epidemiological studies have examined datasets for associations between glyphosate and adverse reproductive outcomes, but found little evidence that glyphosate is causing ill health within human populations. Furthermore, many of these studies are weakened by shortcomings including survey methods prone to inaccurate or biased recall of pesticide exposures; lack of quantitative information on the timing, duration and extent of exposures; and the absence of appropriate controls for smoking habit, maternal age and previous reproductive history. The following publications were included in a review by Mink et al (2011) of research published over a twelve year period:

- Rull et al (2006) pooled data from two Californian case-control studies evaluating neural tube defects and residential proximity to areas where pesticides were applied; mothers were considered “exposed” if any crop within 1 km had been treated with to glyphosate. Based on 45 exposed cases and 33 exposed controls, ORs of 1.4–1.5 were found depending on the regression model used for analysis. In each instance, the 95% CIs included 1.0.
- In a case-control study performed in an agricultural region of Spain, Garcia et al (1998) observed no significant association between congenital malformations and the fathers’ exposure to glyphosate during the three months prior to conception or the first trimester of pregnancy (OR = 0.94; 95% CI = 0.37–2.3).

[6] Fecundability is the probability that conception will occur in a given population of couples during a specific time interval.

- In a population of 2110 Ontario farmers' wives from the Ontario Farm Family Health Study, Arbuckle et al (2001) reported a borderline significant association between pre-conception exposure to glyphosate and spontaneous abortion (33 exposed cases; OR = 1.4; 95% CI = 1.0–2.1), but no significant association with post-conception exposure (22 exposed cases; OR = 1.1; 95% CI = 0.7–1.7). Arbuckle and co-workers considered their investigation as "exploratory" and noted many limitations to their study, including the potential for inaccurate classification of pesticides and timing of exposure relative to conception. They also cautioned that the results should be interpreted with care and confirmed in further investigations.

- To investigate whether reported pesticide use by men or women was associated with delayed pregnancy, Curtis et al (1999) measured the conditional fecundability[7] ratio (CFR)[8] in 2012 planned pregnancies among the Ontario Farm Family Health Study farming couples. The CFR for women who had used glyphosate (regardless of men's use) was depressed (0.61; 95% CI = 0.30–1.3) but there was no statistical significance. Fecundability was slightly elevated (CFR = 1.3; 95% CI = 1.07–1.56) in men who had used glyphosate but whose wives had not. The study authors attributed this finding to uncontrolled factors or chance.

- Self-reported glyphosate exposure during pregnancy was *inversely* associated with gestational diabetes (OR = 0.61; 95% CL = 0.26–1.48) in a cross-sectional analysis of data from the Agricultural Health Study by Suldana et al (2007).

- Self-reported use of glyphosate was associated with a small, statistically non-significant increase in birthweight in the most recent offspring of 700 women in the US Agricultural Health Study (Sathyanarayana et al, 2010).

- Garry et al (2002) conducted a cross-sectional analysis of pesticide applicators and their families. Parent-reported ADD / ADHD in children was associated positively and significantly with use of glyphosate, with 6/14 affected children having parents who had exposure to glyphosate or Roundup (OR = 3.6; 95% CI = 1.35–9.65). ADD / ADHD diagnosis was not confirmed by a clinician, however.

A further review of the scientific literature (Williams et al, 2012) concurred with the conclusion of Mink, i.e., that no consistent effects of glyphosate exposure have been found on reproductive health or offspring development in either humans or animals.

[7] Conditional fecundability is the probability of conception per unit time conditional on a woman being susceptible at the beginning of that time interval.
[8] The ratio of conditional fecundability of the exposed and unexposed groups. A CFR <1.0 indicates a reduced probability of conception in the exposed group.

2.2 The association between glyphosate / glyphosate-based herbicides, endocrine disruption and reproductive toxicity

According to the EOS article:

- Romano et al (2010) have shown that a Roundup formulation was a potent endocrine disruptor in male rats and caused disturbances in reproductive development during puberty. Adverse effects (including delayed puberty and reduced testosterone production) were found at and above the lowest dose of 5 mg/kg.
- Dallegrave (2007) observed adverse reproductive effects in the male offspring of female rats treated with a Roundup formulation at 50, 150 or 450 mg/kg during pregnancy and lactation. The effects, which occurred in the absence of maternotoxicity, included dose-related decreases in serum testosterone level at puberty, decreased sperm number and daily sperm production in adulthood, an increased percentage of abnormal sperm, and sperm cell degeneration.
- Glyphosate active constituent causes sperm damage in rabbits (Yousef et al, 1995).
- When administered to rats for two years at 3, 10 and 32 mg/kg bw/d, glyphosate caused testicular tumours (Lankas, 1981). Although the effect did not occur in a second rat carcinogenicity study at 100, 410 and 1060 mg/kg bw/d, EOS argues that effects related to endocrine hormones can be more potent at low doses than higher ones.

Based on the following evidence, EOS proposes that glyphosate and GBHFs cause reproductive toxicity by mechanisms involving endocrine disruption:

- Glyphosate-based herbicides perturb hormone levels in female catfish and decrease egg viability (Soso et al, 2007) and mediate anti-androgenic and anti-oestrogenic activity in human cells at concentrations as low as 5.0 ppm (Gasnier et al, 2009).
- Roundup reduces production of progesterone in mouse cells *in vitro* by inhibiting expression of a regulatory protein (Walsh et al, 2000).
- Glyphosate disrupts oestrogen-regulated gene expression in human cells (Hokanson et al, 2007) and is toxic to human placental cells, an effect enhanced in the presence of Roundup adjuvants (Richard et al, 2005). Richard et al are said to have shown that Roundup inhibits aromatase (the enzyme responsible for oestrogen production), and proposed this as an explanation for increased premature births and miscarriages reported in female members of farming families using glyphosate (Savitz et al, 1997 and Arbuckle et al, 2001; see previous Section).
- Glyphosate and Roundup damage or kill human umbilical, embryonic and placental cells at concentrations below those recommended for agricultural use, and may interfere with human reproduction and embryonic development (Benachour et al, 2007; Benachour and Seralini, 2009).

APVMA comment

2.2.1 Reproductive effects of glyphosate in vivo

Between them, the German BVL (for the EU and JMPR), Australian DoHA and US EPA have assessed no fewer than eight single- or multi-generation reproduction studies with glyphosate in rats, most of which involved dietary administration. The various agency evaluations are summarised in Appendix 3. The overall dose range was 3 – *ca* 1500 mg/kg bw/d. The toxicological end-points examined included oestrus cycling, mating performance, pregnancy rate and gestation length; litter size and sex ratio; the growth rate, attainment of post-natal developmental landmarks and onset of puberty in pups; and histology of the reproductive organs and analysis of sperm and oocytes in adults. If glyphosate was capable of interfering with the sexual development and reproductive performance of either males or females, the studies would have revealed these effects.

There were few indications of reproductive toxicity. In the parental generations, toxicity was seen as depressed bodyweight or bodyweight gain from doses of *ca* 670 mg/kg bw/d upwards; and, in one study only, histological abnormalities in the salivary glands occurred at $\geq$200 mg/kg. Parental NOELs ranged from 10 to *ca* 700 mg/kg bw/d. Pup bodyweight or bodyweight gain was depressed at $\geq$670 mg/kg, while in one study, litter size was reduced at *ca* 1500 mg/kg bw/d. NOELs in pups varied from 10 to *ca* 800 mg/kg bw/d. The Australian ADI for glyphosate (0.3 mg/kg bw/d) is based on the three-generation dietary study of Schroeder and Hogan (1981), in which there were no treatment-related effects on the parental of filial generations at the highest dose of 30 mg/kg bw/d.

Lower threshold doses for toxicity were seen with glyphosate trimesium in a two-generation study by Stauffer Chemical Co (1983a, assessed by DoHA, 1991). A NOEL of 7.5 mg/kg bw/d was assigned for parental animals and offspring based on reduced bodyweight gain, food consumption and plasma protein levels in adults and depressed pup bodyweight and relative spleen weight at $\geq$40 mg/kg. The only effect on reproductive parameters was a reduction in litter size, which occurred at the highest dose of 100 mg/kg bw/d.

For the EU and JMPR reviews, the BVL also assessed a 13—week US National Toxicology Program study in rats (Chan and Mahler, 1992). Caudal epididymal sperm concentrations declined by *ca* 20% at 25 000 and 50 000 ppm glyphosate in the diet (calculated glyphosate intake *ca* 2500 and 5000 mg/kg bw/d). However, all values were within the HC range and no effects occurred on caudal, epididymal and testicular weights, sperm motility, total spermatid heads/testis and total spermatid heads/gram caudal tissue. Compared with controls, oestrus cycle length was prolonged from 4.9 to 5.4 days at 50 000 ppm. The EU and JMPR regarded this finding as having unknown biological significance, if any. An identical study in male and female mice did not find any evidence of reproductive toxicity or endocrine modulation at up to 50 000 ppm in the diet (7500 mg/kg bw/d), the highest dietary concentration tested.

In an unreliable and poorly-reported study, Yousef et al (1995) administered glyphosate orally to male rabbits for six weeks at 1% or 10% of the LD_{50}. The study authors did not identify the dosing interval, or the doses in terms of mg/kg bw. Semen quality was assessed at weekly intervals for six weeks prior to treatment, during the dosing period, and a further six weeks after treatment to study reversibility of effects.

Glyphosate was claimed to have caused fully or partially reversible decreases in ejaculate volume, sperm viability and sperm activity. However, the results are likely to have been affected by methodological deficiencies, and effects on sperm concentration and morphology are uninterpretable due to major, unexplained variations over time within the control group.

2.2.2 Evidence of endocrine modulation in other studies

Even though they are not specifically designed to test for endocrine disruption, the short-term repeat-dose, subchronic and chronic *in vivo* toxicology studies required by the APVMA and other regulatory agencies can detect modulation of endocrine system activity. Chemicals affecting endocrine target sites initiate direct or compensatory biochemical or cellular responses which are observable by assessment of the weight, gross pathology and histopathology of endocrine organs and tissues. In fact, these studies have some advantages over *in vitro* screening assays, as they assess a variety of endocrine-sensitive endpoints in live animals capable of metabolic activation and/or detoxification of xenobiotic chemicals, and use extended exposure periods encompassing various stages of endocrine development (Williams et al, 2000).

There have been no findings in these subchronic or chronic toxicity studies indicating that glyphosate produces any endocrine-modulating effects. Negative results also were obtained in a dominant lethal mutation study in mice at 2000 mg/kg bw PO (Wrenn, 1980). While this latter test is typically used to assess genetic toxicity, substances that affect male reproductive function through endocrine modulating mechanisms can also produce effects in this type of study (Williams et al, 2000).

2.2.3 Testicular carcinogenicity

A carcinogenicity study by Lankas (1981) has been reviewed by the Australian DoHA (1985), the WHO (1994) and the US EPA (1993). The German BVL did not evaluate this study for the JMPR, but the EU review includes a summary of the WHO assessment. Rats were treated with glyphosate in the diet for 26 months to achieve intakes of *ca* 3, 10 and 31 mg/kg bw/d in males and 3.4, 11 and 34 mg/kg bw/d in females. The incidence of testicular interstitial (Leydig) cell tumours at termination was 0/15 among controls and 2/26, 1/16 and 4/26 at the low-, mid- and high-doses respectively. The total incidence for all males was 0/50, 3/50, 1/50 and 6/50. The BVL evaluator did not attribute the finding to treatment, noting that Leydig cell tumours are common in ageing rats, that the incidence at 31 mg/kg "only slightly exceeded the historical control range," and that no such effect had been observed in several more recent rat studies at much higher doses. In the absence of treatment-related effects, the NOEL was set at 31 mg/kg bw/d.

The WHO (1994), US EPA (1993) and DoHA (1985) all agreed that the tumours were not treatment—related because their incidence lay within the HC range. This interpretation was supported by data shown in the Australian assessment, showing that the incidences of Leydig cell tumours in glyphosate-treated rats were not different to those in male controls from concurrent studies at the same laboratory (4/65, 3/11, 3/26, 3/24 and 3/40).

Furthermore, testicular tumours have not occurred in any of the other carcinogenicity studies with glyphosate in rats or mice at doses of up to 4800 and 1200 mg/kg bw/d, respectively. Despite EOS's claim that endocrine-mediated effects are specifically *low dose* phenomena, doses of between 4 and 12 mg/kg bw/d (within the range given by

Lankas) have failed to cause any testicular effects in two carcinogenicity studies in mice or in three similar studies in rats. Therefore, the weight of evidence does not support EOS's assertion that glyphosate is a testicular carcinogen.

2.2.4 Effects of glyphosate-based herbicide formulations

Notwithstanding the mainly negative findings on glyphosate in carcinogenicity and reproductive toxicity studies in laboratory animals, the APVMA has initiated an independent assessment of publications cited by EOS, and other relevant articles obtained from the scientific literature. Three of these publications describe studies of the effects of GBHFs on the reproductive physiology of rodents and birds, while the remainder cover experiments in isolated cells. The detailed assessments are presented in Appendix 4.

2.2.4.1 Findings in birds

Oliviera et al (2007) observed a 90% reduction in plasma testosterone levels in sexually mature drakes gavaged orally with Roundup (480 g/L glyphosate isopropylamine salt, no other constituents identified) for 15 days at 5 or 100 mg/kg bw/d. This occurred in conjunction with decreased androgen receptor expression within testicular (Sertoli) cells and histological abnormalities in the testis (reduction in seminiferous tubule epithelium and interstitial tissue), epididymal region, proximal efferent ductules (vacuolisation and increased lipid in the epithelium) and epididymal duct (collapsing and folding). As most of these effects were present in birds receiving the lowest dose of 5 mg/kg bw/d, a NOEL was not demonstrated. The study did not investigate whether there were any associated effects on the behaviour or reproductive performance of the birds, define the mechanism by which the effects occurred, or identify the causative component(s) of the test formulation.

2.2.4.2 Findings in rats

Dallegrave et al (2007) performed a single generation reproduction study in rats with a Roundup product (360 g/L glyphosate and 18% POEA surfactant) at maternal oral doses equivalent to 0, 50, 100 and 450 mg glyphosate/kg bw/d. The test formulation was administered to the dams throughout pregnancy and lactation, until the offspring reached 21 days of age. Male pups were then evaluated when 65 or 140 days old. There was no NOEL because of decreased sperm production, an increased incidence of abnormal sperm, and depression in blood testosterone concentration at and above the lowest dose.

In a post-natal development study, Romano et al (2010) treated weanling rats orally with a Roundup product containing 648 g/L glyphosate isopropylamine salt plus unidentified "inert ingredients". The doses were 0, 5, 50 and 250 mg glyphosate/kg bw/d, administered from 23 to 53 days of age. Treated males displayed reduced serum testosterone levels and thinning of the seminiferous tubule germinal epithelium, suggesting diminished production of sperm. Male puberty was delayed at 50 and 250 mg/kg. There was no NOEL.

The APVMA's independent assessment notes that the studies by Dallegrave et al (2007) and Romano et al (2010) appear to have demonstrated evidence of reproductive toxicity. However, both studies are affected by flaws in their design, methodology and / or reporting. Neither research group identified which constituent(s) in the test formulations mediated the reported effects. Also, while there

is a biologically plausible association between delayed puberty, deficiency in circulating testosterone level and inhibited sperm production, the studies did not identify the mechanism involved.

The situation is complicated by a pre / post-natal development experiment by Romano et al (2012), which yielded markedly different findings despite using the same rat strain and Roundup product as did the 2010 study. In the 2012 report, reproductive physiology and behaviour were investigated in male rat pups whose mothers had been dosed orally from GD 18 to PND 5, at 50 mg glyphosate/kg bw/d. The pups were then reared without further exposure until evaluation at 60 days of age. Compared to controls, puberty occurred earlier in the test group; serum testosterone, oestradiol and LH concentrations were doubled; sperm production was enhanced; and males showed a greater preference for the company of female rats despite an increase in the delay before mating. Based on these findings, Romano et al concluded that glyphosate is a potential endocrine disruptor.

However, DeSesso and Williams (2012; see Appendix 4), have questioned several aspects of the study's design and conduct, and observed that the average age and bodyweight of test animals at puberty lay within the range shown by concurrent controls and controls in Romano et al (2010). DeSesso and Williams also note that surfactants likely to be present in the test formulation inhibit steroid production in Leydig (testicular) cells (Levine et al, 2007) and could have affected the study outcome.

2.2.4.3 Findings in vitro

According to the JMPR (2004b), glyphosate had no oestrogenic activity in assays for activation of rainbow trout oestrogen receptors in yeast or vitellogenin production in a trout liver cell culture system (Petit et al, 1997). The incubation concentrations of glyphosate were not given.

A Roundup formulation was reported as having dose-dependently inhibited progesterone synthesis in mouse MA-10 (Leydig tumour) cells (IC_{50} of 24 µg/mL) (Walsh et al, 2000). The putative mechanism involved preventing the expression of steroidogenic acute regulatory (StAR) protein, a mitochondrial phosphoprotein that transfers cholesterol to cytochrome P450scc, the enzyme that initiates steroid hormone biosynthesis. Glyphosate active constituent, by contrast, had no such effect over the concentration range tested (0–100 µg/mL). However, Levine et al (2007) replicated the effect on progesterone synthesis in the same experimental model using 'blank' Roundup formulation (without glyphosate), and demonstrated that inhibition arose from damage to mitochondrial membranes by the surfactant.

In MCF-7 human breast adenocarcinoma (oestrogen sensitive) cells exposed for 18 hours to a GBHF at 0.00023 – 0.23%, significant changes occurred in the activity of three out of 1550 oestrogen-regulated genes. There was a 2.2-fold increase in the activity of HIF1 (which primes cells for the initiation of apoptosis) and *ca* 50% reductions in expression of CXCL12 (a lymphocyte chemoattractant) and EGR1 (which has a range of activities potentially affecting apoptosis and tumour vascularisation) (Hokanson et al, 2007). However, the study did not demonstrate any alteration of the physiology, survival or growth of the test cells, or establish whether the effects on gene expression would have implications for the survival, development and function of other mammalian cells, tissues, foetuses or adult animals.

Furthermore, the formulation component that altered gene expression levels was not identified.

As reported by EOS, a Roundup formulation inhibited aromatase (CYP19, an enzyme which converts androgens to oestrogens) in human plancental cancer (JEG3) cells (Richard et al, 2005; assessed by DoHA, 2005). However, as the DoHA evaluation observed, the use of human placental cancer cells (rather than normal placental cells) was not a valid basis for any conclusion that glyphosate or its products cause reproductive effects in humans, particularly given the weight of evidence from laboratory animals that glyphosate is not a reproductive toxin. Williams et al (2012) have pointed out that the concentrations of Roundup causing aromatase inhibition (0.2–2.0%) in Richard et al's study were cytotoxic and much higher than physiologically relevant; by contrast, pure glyphosate had no effect in the assay system at up to 0.8%, the highest concentration tested. The French Ministry of Agriculture and Fish (2005) has also evaluated Richard et al (2005), and concluded that the study was of no value for human health risk assessment.

Roundup formulations also inhibited aromatase in human embryonic kidney (HEK293) (Benachour et al, 2007) and hepatoma (HepG2) cells (Gasnier et al, 2009). By contrast, glyphosate inhibited aromatase weakly or had no effect on its activity. Roundup formulations had anti-oestrogenic activity at human oestrogen receptors (hER) α or β, and anti-androgenic activity at human androgen receptors (hAR) (Gasnier et al, 2009). However, the potencies of Roundup formulations correlated poorly with the concentration of glyphosate they contained; furthermore, glyphosate itself had no anti-oestrogenic activity at hER α or β and, at most, weak anti-androgenic activity at hAR.

Benachour and Seralini (2009) studied the cytotoxicity of glyphosate, its metabolite AMPA, four Roundup products and the surfactant POEA in three human cell lines (umbilical cord vein endothelial [HUVEC] cells, JEG3 and HEK293). Based on inhibition of mitochondrial respiration, the least potent cytotoxin was AMPA, glyphosate had intermediate potency, and POEA was the most potent (the respective EC50s were $\geq$40 000, *ca* 10 000 and 3–30 ppm). All the product concentrates were more toxic than glyphosate alone, having EC50s of 30 – 9000 ppm. Their potency was not dependent on the concentration of glyphosate they contained, suggesting that other formulation components were biologically active. AMPA and POEA caused necrotic cell death, glyphosate caused cell death via apoptosis, while the Roundup formulations mediated cell death via both necrosis and apoptosis.

Cytotoxicity experiments with isolated rat testicular cells *in vitro* have shown that germ cells are relatively resistant to glyphosate and Roundup Bioforce, Leydig cells are resistant to glyphosate but sensitive to the product at concentrations of $\geq$0.10% in solution, and Sertoli cells are sensitive to glyphosate at $\geq$0.01% and the product at 0.10% (Clair et al, 2012). Notwithstanding the decreases in circulating testosterone levels observed *in vivo*, neither the active nor the formulation inhibited 3β-hydroxysteroid dehydrogenase activity (an index of testosterone synthesis) in cultured Leydig cells exposed for 24 hours at up to 0.10%. Testosterone concentration in the cell incubation medium declined by *ca* 1/3rd in response to glyphosate and Roundup at 0.0001%, but not at higher concentrations. There was no explanation for this paradoxical concentration-response relationship.

2.3 Evidence for the genotoxicity of glyphosate / glyphosate-based herbicides

EOS contradicts the EU review's conclusion that glyphosate is not genotoxic, citing evidence that:

- Roundup increases the frequency of gender-linked recessive lethal mutations in fruit flies (Kale et al, 1995), DNA adducts in the livers and kidneys of mice (Peluso et al, 1998) and sister chromatid exchanges in human lymphocytes (Vigfusson and Vyse, 1980);
- Mice injected with glyphosate and Roundup show an increased frequency of chromosome damage and increased DNA damage in bone marrow, liver and kidney (Bolognesi et al, 1997);
- GBHFs cause DNA damage in human cells (Gasnier et al, 2009);
- In sea urchin embryos, GBHFs and AMPA (the environmental degradation product of glyphosate, aminomethylsulphonic acid) alter cell cycle checkpoints by interfering with DNA repair (Marc et al, 2002; 2004a,b; Belle et al, 2007) and cause inhibition of RNA transcription and delayed hatching (Marc et al, 2005); and
- An epidemiology study in Ecuador found more extensive DNA damage in people living in an area that was aerially sprayed with glyphosate compared with those living 80 km away (Paz-y-Mino et al, 2007).

APVMA comment

The genotoxicity of glyphosate, its metabolite AMPA and GBHFs (with and without surfactants including POEA) has been reviewed by Williams et al (2000), Kier and Kirkland (2013) and the Australian DoHA (1985, 1991, 1992 and 2005), US EPA (1993), WHO (1994) EU (1998) and JMPR (2004b). In addition to assays for gene mutation in bacteria and cultured mammalian cells, the investigated end-points included tests for DNA damage and repair *in vitro* and chromosomal aberrations (clastogenicity) *in vitro* and *in vivo*. All the reviews agreed that the vast majority of studies within the highly extensive database had clearly negative outcomes, and concluded that glyphosate, AMPA and GBHFs do not present a genotoxicity hazard. Furthermore, POEA is not mutagenic (Stegeman and Li, 1990; Williams et al, 2000).

The JMPR and/or EU reviews (both performed by the German BVL) covered four of the studies cited by EOS (2011) as demonstrating genotoxic activity. However, as outlined below, the BVL concluded that the findings were also consistent with cytotoxicity (cellular injury or death not caused by damage to genetic material), and commented that assessment of these data was complicated by a lack of information on product composition, reporting limitations, and by the use of some test systems which were of uncertain relevance for the assessment of risk to humans.

Kale et al (1995) obtained positive results in a test for lethal mutations in fruit flies (*Drosophila melanogaster*) after larvae were treated with a Roundup product (41% glyphosate IPA salt with POEA surfactant) or Pondmaster (41% glyphosate IPA salt with alkyl sulphate surfactant). Dosing conditions were not specified but the test insects were exposed to concentrations close to the LC_{50}. The BVL considered that it

would have been very difficult for the investigators to distinguish between deaths from lethal mutations and deaths from the anticipated high toxicity.

Using a ^{32}P-postlabelling assay, Peluso et al (1998) found a weak, dose-related increase in DNA adducts in the liver and kidney of mice injected IP with 400, 500 and 600 mg/kg of a Roundup product containing 30.4% glyphosate IPA salt with alkyl sulphate surfactant. No adducts were seen with glyphosate IPA alone at 130 or 270 mg/kg, or in a control group. While agreeing that the finding was an indication of possible DNA damage, the BVL regarded the biological significance as equivocal because DNA adducts can occur naturally or arise from increases in endogenous metabolite levels, as well as from direct interaction with chemicals. The BVL also questioned the relevance of IP administration to normal exposure conditions, and criticised the absence of any positive control group, individual animal data and information on the DNA adducts' structure.

Vigfusson and Vyse (1980) observed a weak but statistically significant increase in the frequency of sister chromatid exchanges (SCEs) in human lymphocytes incubated with a Roundup product (composition unspecified) at 250 and 2500 µg/mL. The BVL observed inconsistencies in the results, in that a dose response occurred in cells from only one of the two donors, and the statistically increased values from one donor lay below the control values from the other.

Bolognesi et al (1997) examined the effects of glyphosate and a Roundup product (30.4% glyphosate IPA salt with alkyl sulphate surfactant) on several end-points:

> i) A SCE assay in cultured human lymphocytes from two female donors was positive with glyphosate at 1–6 mg/mL and Roundup at 100 and 330 µg/mL. The formulation was cytotoxic at higher concentrations. The BVL criticised the statistical analysis, as data from the donors were pooled and individual values were not provided.
>
> ii) A weakly positive alkaline elution assay for single-strand DNA breaks and formation of alkali-labile sites in DNA suggested possible transient DNA damage in the liver and kidney of mice, four hours after IP injection with glyphosate or Roundup at 300 and 900 mg/kg respectively. The BVL noted that IP injection was an inappropriate route because the test chemicals could be directly cytotoxic to the tissues within the peritoneal cavity. Furthermore, the outcome was inconsistent with three other studies in which glyphosate did not cause cytogenetic damage, mutation or DNA adduction in mice treated IP at up to 1000 mg/kg bw.
>
> iii) One day after treatment as described in (ii), measurement of 8-hydroxydesoxyguanosine (OHdG) adducts revealed evidence of increased oxidative metabolism / injury in the liver (with glyphosate only) and kidney (with Roundup only). The BVL suggested that the finding may elucidate a mechanism of toxicity but is not evidence of genotoxicity.
>
> iv) In a bone marrow micronucleus assay, groups of three male mice received two IP doses of glyphosate (150 mg/kg) or Roundup (225 mg/kg) at 24—hour intervals, and were killed for assessment six and 24 hours after the final dose. A weakly positive response was obtained with Roundup at both time points, and glyphosate at 24 hours. With respect to glyphosate, the BVL highlighted the inconsistency between the positive outcome and other micronucleus assays, which were negative in rats treated at up to 1000 mg/kg IP and in mice

receiving up to 5000 mg/kg PO. Furthermore, Bolognesi's assay did not comply with the relevant OECD Test Guideline, as the treated groups contained fewer than the recommended five animals and only one dose was tested, precluding the assessment of dose-response. It was unclear when the control mice were killed, weakening the validity of the statistical comparison. The BVL also commented that the formulation (although not the active) may have caused cytotoxicity in the bone marrow, as evidenced by a decrease in the ratio between polychromatic and normochromatic erythrocytes. Cytotoxicity may therefore have affected the frequency of chromosomal aberrations. There was apparently no data on the mutagenicity of the alkyl sulphate surfactant present in the tested Roundup product.

Using the Comet assay, Gasnier et al (2009) measured single- and double-stranded DNA breakage and alkali-labile DNA damage in HepG2 liver cancer cells *in vitro* after 24 hours of incubation with Roundup Grands Travaux, a product containing glyphosate at 400 g/L together with unidentified adjuvants (see assessment in Appendix 3). The test cells were exposed at 1, 2.5, 5, 7.5 and 10 ppm. The pro-mutagen benz[a]pyrene (50 µM) was used as positive control. The test product had no effect at the two lowest concentrations but caused a dose-dependent increase in DNA strand breaks at 5, 7.5 and 10 ppm (50, 60 and 75% breakage compared with 35% in negative controls and 95% in positive controls). However, Gasnier et al also reported that the test product was cytotoxic against HepG2 cells at concentrations of 5 ppm upwards, with an LC50 of 12 ppm. It is therefore possible that the increased DNA strand breakage seen at 5–10 ppm was secondary to cellular injury or death, rather than arising directly from damage to DNA by the test product. Furthermore, it is unclear which component(s) of Roundup Grands Travaux was biologically active, as the effects of glyphosate or adjuvant(s) alone were not tested.

The Australian DoHA (2005) assessment found that Marc et al (2005) had demonstrated that Roundup (diluted to glyphosate concentrations of up to 4 mM) delayed RNA synthesis, transcription of the hatching enzyme and hatching of sea urchin embryos by *ca* two hours. There was only a marginal effect on cell division indicating the delay was not due to any cell-cycle effect. Pure glyphosate at up to 8 mM had only a weak effect on hatching (a delay of 30 min). Marc et al also reported that POEA was "highly toxic to the embryos leading to irreversible damage" but provided no supporting data. The DoHA considered the sea urchin model as being of "dubious" value for human health risk assessment, given that glyphosate had already been tested by validated methods.

In an investigation of associations between genotoxic risk and aerial application of glyphosate-based herbicides for control of illicit crops, Bolognesi et al (2009) performed a cytogenic biomonitoring study on agricultural workers in Colombia. In areas where glyphosate was sprayed, blood samples were taken prior to application and then at five days and four months post-application. Chromosomal damage and cytotoxicity in lymphocytes were evaluated by cytokinesis-block micronucleus assay. Compared with Santa Marta, where organic coffee is grown without pesticides, the baseline frequency of binucleated cells with micronuclei (BNMN) was significantly greater in subjects from four other regions. However, only gender, region and older age were associated with baseline BNMN frequencies, and glyphosate was *not* used in one of the two regions where the highest frequencies of BNMN were found. In three regions, a significant increase in BNMN frequency occurred five days after glyphosate was applied, which reversed in one of these regions within four months

post-application. The study authors concluded that genotoxic damage associated with glyphosate application was small and transient, and the genotoxic risk was low.

2.4 Carcinogenicity of glyphosate / glyphosate-based herbicides

2.4.1 Evidence from studies in laboratory animals

The EOS article claims that glyphosate is carcinogenic, based on an increase in testicular tumours in rats treated via their diet for two years at 3, 10 and 32 mg/kg bw/d. However, pesticide regulatory agencies have not classified glyphosate as a carcinogen because the effect did not occur at higher doses in another two-year rat study. EOS argues that endocrine effects are more potent at low doses than higher doses, and so regulators should re-classify glyphosate as a carcinogen. EOS also claims that George et al (2010) have demonstrated that glyphosate induces cancer in mouse skin.

APVMA comment

2.4.1.1 Carcinogenicity via the oral route

The study in which testicular tumours occurred (Lankas, 1981) has been reviewed by the Australian DoHA (1985), WHO (1994) and US EPA (1993). The German BVL did not evaluate this study for the JMPR (2004b), but the EU review includes a summary of the WHO assessment. Rats were treated with glyphosate for 26 months at dietary doses of *ca* 3, 10 and 31 mg/kg bw/d in males and 3, 11 or 34 mg/kg bw/d in females. The incidence of testicular interstitial (Leydig) cell tumours at termination was 0/15 among controls and 2/26, 1/16 and 4/26 at the three respective doses. The total incidence for all males was 0/50, 3/50, 1/50 and 6/50. The BVL did not attribute the finding to treatment, noting that Leydig cell tumours are common in ageing rats, that the incidence at 31 mg/kg "only slightly exceeded the historical control range," and that no such effect had been observed in several more recent rat studies at much higher doses. In the absence of treatment-related effects, the NOEL was set at 31 mg/kg bw/d.

The WHO (1994), US EPA (1993) and DoHA (1985) all agreed that the tumours were not treatment-related because their incidence lay within the HC range. This interpretation was supported by data shown in the Australian assessment, showing that the incidences of Leydig cell tumours in glyphosate-treated rats were not different to those in male controls from concurrent studies at the same laboratory (4/65, 3/11, 3/26, 3/24 and 3/40).

Furthermore, glyphosate has not caused cancer in the testis – or at other sites – in any of the other dietary carcinogenicity studies assessed the Australian DoHA (1985, 1991 and 1992), US EPA (1993), EU (1998) and JMPR (2004b). The database comprises:

- A 20-month study in mice at *ca* 11.3 – 45 mg/kg bw/d (Indian Institute of Toxicology, undated);
- A 22-month study with glyphosate trimesium in male and female mice treated at 11.7 – 991 and 16.0 – 1341 mg/kg bw/d respectively (Stauffer Chemical Co, 1987a);
- Two-year studies in mice at 100 – 1000 mg/kg bw/d (Atkinson et al, 1993a) and 157 – 4841 and 190 – 5874 mg/kg bw/d in males and females, respectively (Knezevich and Hogan, 1983); and

- Two-year studies in rats at 89 – 940 and 113 – 1183 mg/kg bw/d in males and females respectively (Stout and Ruecker, 1990); 10 – 1000 mg/kg bw/d (Atkinson et al, 1993b); 121 – 1214 and 145 – 1498 mg/kg bw/d in males and females (Brammer, 2001); 6.3 – 595 and 8.6 – 886 mg/kg bw/d in males and females (Suresh, 1996); and at 4.2 – 41.8 and 5.4 – 55.7 mg/kg bw/d in males and females (glyphosate trimesium salt; Stauffer Chemical Co, 1984).

Despite EOS's argument that endocrine-mediated effects are specifically *low dose* phenomena, dietary doses of between 4 and 16 mg/kg bw/d (which lie within the range given by Lankas, 1981) have failed to cause any testicular effects in two mouse and three rat carcinogenicity studies. Therefore, the weight of evidence does not support the EOS assertion that glyphosate is a testicular carcinogen.

2.4.1.2 Dermal carcinogenicity

George et al (2010) tested Roundup Original (a product containing 360 g/L glyphosate and 15% POEA) in a mouse two-stage initiation / promotion model of skin cancer. Following a single dermal dose of the tumour initiator DMBA (7,12-dimethyl benz[a]anthracene) mice were treated dermally, three times per week for 32 weeks, with Roundup (25 mg/kg bw) or a positive control chemical (the tumour promoter TPA (12-*O*-tetradecanoyl-phorbol 13-acetate) at 5 µg/mouse). Skin cancers (squamous cell papillomas) were present on eight/20 Roundup-treated mice and 20/20 positive controls at termination. By contrast, tumours did not develop on untreated (negative control) animals or further mice that received a single dose of DMBA without a promoter; or 32 weeks' treatment with Roundup or TPA without prior initiation; or one dose of Roundup followed by TPA for 32 weeks.

Before discussing the significance of George et al's findings, we must briefly consider the biological basis for the two-stage initiation / promotion model they utilised. This experimental model has been developed in light of the *multistage model of carcinogenesis*[9], the current scientific explanation of how cancers are formed from normal cells. In their experimental design, George et al used a single dose of DMBA to initiate skin tumours and repeated doses of TPA to promote them. Tumours did not develop on animals that received the initiator without subsequent promotion, or on mice treated with the promoter without prior initiation. When substituted for DMBA, Roundup did not behave as a tumour initiator, as tumours did not form on mice treated subsequently with TPA. Furthermore, Roundup was not a complete carcinogen, since tumours did not develop on animals that received it without prior initiation. However, Roundup did behave as a tumour promoter on mice that had already received DMBA.

[9] As described by Derelanko (2002), the development of a single cell into malignant tumours is believed to occur in three stages, the first of which is **initiation** (a normal cell changes irreversibly – usually by genetic alteration – in a way that allows unrestricted division; however, initiated cells may remain latent for months or years, during which they are indistinguishable from normal). The subsequent stage, **promotion**, involves prolonged and repeated exposure to a promoting agent which causes the initiated cell to undergo clonal expansion and form a pre-cancerous focus. Promoters, which do not interact directly with DNA, are believed to act via a variety of mechanisms most often resulting in increased cellular replication. The final step is **progression**, in which the pre-cancerous focus becomes transformed into a malignant tumour, a process characterised by changes in the number and arrangement of chromosomes, an increased rate of replication, and invasiveness.

Because George et al did not apply pure glyphosate or POEA to the test animals, their study could not identify which component(s) of Roundup Original was responsible for the promoting activity. Therefore, EOS's assertion that "glyphosate induces cancer in mouse skin" is not strictly correct. Furthermore, while single doses of Roundup and TPA induced similar changes in dermal protein expression, it remains unclear whether the formulation and positive control shared a common mode of action (see assessment in Appendix 3).

However, the most important issue raised by this study is whether Roundup Original or other GBHFs are likely to pose a dermal carcinogenicity hazard to persons preparing them for application. In this regard, several factors require consideration:

- The weight of evidence suggests that neither glyphosate nor POEA are genotoxins, either alone or in combination. Furthermore, glyphosate has been shown not to be carcinogenic via the oral route in ten studies in two laboratory species.
- Roundup Original was not a complete carcinogen in the mouse initiation / promotion model. Tumour initiation was a prerequisite for the eventual development of dermal cancers. Therefore, this and similar products would not be expected to promote tumour formation on human skin in the absence of prior initiation.
- Roundup Original was a markedly less potent promoter than the positive control, TPA. George et al applied the formulation at a 150-fold higher dose than TPA (25 mg/kg bw compared with 5 µg/mouse, equivalent to *ca* 0.17 mg/kg assuming a 30 g bodyweight). Despite this, Roundup promoted tumour formation more slowly than did the positive control. Tumours first appeared after 130 days on Roundup-treated mice, compared with 52 days on those receiving TPA. Fewer, smaller tumours developed on Roundup-treated mice than on those receiving TPA. Moreover, tumour formation occurred on all positive control mice, compared with 40% of those receiving Roundup.
- Tumour promotion is reversible, requires prolonged and repeated exposure to the promoter, and the promoted cell population depends on the continued presence of the promoter (Derelanko, 2002). On mice, tumours did not appear until 130 days of treatment with Roundup Original. Assuming a lifespan of 80 years, humans would have to be exposed to Roundup for three days per week for *ca* 14 years to achieve the equivalent of 130 days of the *ca* 730-day mouse lifespan. Few herbicide mixer / loaders, if any, would experience such prolonged uninterrupted exposure, especially in situations where GBHFs have a seasonal pattern of use.
- Mice received Roundup Original at 25 mg/kg bw/d, which is equivalent to 1500 mg/d for a 60 kg human. The mass of Roundup formulation that must be handled per day to attain a dermal dose of 1500 mg can be estimated using the US EPA (2012) Exposure Surrogate Reference Table. Based on monitoring studies of operators mixing and loading liquid pesticide concentrates under field conditions, this nominates a mean unit dermal exposure of 0.083 mg/kg handled for persons wearing a single clothing layer and gloves[10]. Therefore, to

[10] Label Safety Directions for liquid glyphosate-based professional strength products require users to wear PPE including coveralls and gloves, consistent with recommendations in the Handbook of First Aid Instructions and Safety Directions (DoHA, 2012).

attain a dermal exposure of 1500 mg, 1500 ÷ 0.083 = 18 072 kg of the product would have to be handled, which is at least ten times higher than could be achieved in a working day.

2.4.2 Evidence from human populations

Citing human epidemiology studies by De Roos et al (2005), Hardell and Eriksson (1999), Hardell et al (2002) and Eriksson et al (2008), EOS claims that there is an association between exposure to glyphosate / GBHFs and the blood system cancers multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL).

APVMA comment

In 2005, the Australian DoHA evaluated epidemiological evidence of associations between use of glyphosate and cancer.

- According to the DoHA, McDuffie et al (2001) found no significant association between previous use of Roundup and the occurrence of Non-Hodgkin's Lymphoma (NHL) among Canadian men (119 test and 301 control), although the study did suggest an association between increased risk of NHL and the use of multiple pesticides.
- The Agricultural Health Survey, a prospective cohort study of 57 311 licensed pesticide applicators in Iowa and North Carolina (De Roos et al, 2005a) found no association between glyphosate exposure and NHL. Based on 22 of 32 cases[11], mixing or using glyphosate products was claimed to be associated with an elevated risk of multiple myeloma (MM), with an odds ratio of 2.6 (95% Confidence Interval = 0.7–9.4), although the lower CI of 0.7 limited the strength of the finding. There was also a possible relationship between the risk of MM and the cumulative exposure days (years of glyphosate use X days per year) but not intensity-weighted exposure (years of glyphosate use X days X intensity level). However, when Sorahan (2012) re-analysed the complete dataset of 32 cases, the relative risk for ever using glyphosate was only 1.1 (95% CI = 0.5–2.4) when adjusted for age. Additional adjustment for education, smoking, alcohol use, family history of cancer and use of 10 other pesticides had little effect (OR = 1.2; 95% CI = 0.5–2.9). This demonstrates that glyphosate use is not associated with increased risk of MM.
- De Roos et al (2005b) found a possible association between NHL and the use of glyphosate in a pooled analysis of 650 males participating in case-control studies performed by the US National Cancer Institute during the 1980s. An OR of 2.1 (95% CI = 1.1–4.0) was detected by logistic regression, but the association was weaker (OR = 1.6; 95% CI = 0.9–2.8) when analysed by hierarchical regression.
- In a study of 515 cases and 1141 controls, Hardell et al (2002) obtained elevated risk of NHL or hairy cell leukaemia (HCL) among men who had used glyphosate. However, the DoHA considered the finding as equivocal because of the small sample size (8 cases and 8 controls), inconsistency between the odds ratios obtained by univariate analysis (3.04; 95% Confidence Interval =

[11] De Roos et al reduced the dataset from 32 to 22 MM cases by excluding subjects with missing data for several variables (Sorahan, 2012).

1.08–8.52) and multivariate analysis (1.85; 95% CI = 0.55–6.20), and the wide breadth of the 95% confidence intervals.

In a follow-up study (see assessment in Appendix 3), Eriksson et al (2008) examined exposure to pesticides as a risk factor for NHL in 910 cases and 1016 controls. Univariate analysis revealed a significant association between NHL and exposure to glyphosate (29 cases and 18 controls; OR = 2.02; 95% CI = 1.10–3.71), exposure to glyphosate with a latency of >10 years between exposure and diagnosis (OR = 2.26; 95% CI = 1.16 – 4.40) and exposure to glyphosate for >10 days (17 cases and 9 controls; OR = 2.36; 95% CI = 1.04–5.37). However, NHL was not associated with exposure to glyphosate with a latency of 1–10 years between exposure and diagnosis (OR = 1.11; 95% CI = 0.24 – 5.08) and was, at most, only weakly associated with exposure to glyphosate for <10 days (12 cases and 9 controls; OR = 1.69; 95% CI = 0.70 – 4.07). Multivariate analysis did not demonstrate any association between NHL and glyphosate exposure (OR = 1.51; 95% CI = 0.77–2.94).

Of the epidemiology studies assessed in Australia, three have suggested an association between glyphosate use or exposure and NHL, but obtained inconsistent results depending on the type of statistical analysis performed. Two other studies have searched for but did not find any such association. Possible associations between glyphosate and HCL and MM were observed in one study each, although the association with MM has subsequently been discounted following a re-analysis of the data.

When weighing up the significance of these results, it is worth taking account of the limitations in the design of the studies, which (with the exception of De Roos, 2005a) collected exposure data in questionnaires relying on the accuracy of the respondent's memory. This would result in recall bias, misclassification of pesticide exposure, and increased uncertainty regarding the actual level of exposure. Epidemiological studies of this type are also potentially confounded by exposure to multiple pesticides and by established risk factors for haematopoietic system cancers, such as immunosuppression and Epstein-Barr virus (DoHA, 2005).

The JMPR (2004b) review of glyphosate reached similar conclusions from its assessment of epidemiology studies by Hardell and Eriksson (1999), Nordstrom et al (1998) and McDuffie et al (2001), commenting that the claimed associations between glyphosate and lymphopoietic cancers were weak, were not controlled for confounding factors including other pesticides, and did not meet generally accepted criteria for determining causal relationships.

2.5 Neurotoxicity of glyphosate / glyphosate-based herbicides

The EOS article describes glyphosate as an organophosphate, and asserts that it has shown a range of neurotoxic effects. These include neurobehavioural disorders in the children of pesticide applicators (Garry et al, 2002), Parkinson's disease in a man who accidently sprayed himself (Barbosa et al, 2001), biochemical abnormalities in rat brain cells including depletion of the neurotransmitters serotonin and dopamine (Anadon et al, 2008) and loss of mitochondrial trans-membrane potential (Astiz et al, 2009), and synergistic toxicity with diazinon towards neuroblastoma (nerve cancer) cells *in vitro* (Axelrad et al, 2003).

APVMA comment

Glyphosate is an organic chemical containing a phosphorus atom, but does not exhibit the same biological activity as organophosphate insecticides. In fact, there is a substantial body of evidence from laboratory animal studies that glyphosate does not affect cholinesterase (ChE) activity in the brain or blood, or cause acute, delayed or chronic toxicity to the nervous system.

In an acute neurotoxicity study with glyphosate trimesium in rats gavaged at 645, 968 and 1290 mg/kg bw, the mid and high doses caused behavioural depression, hypothermia and deaths but no inhibition of brain or RBC ChE activity. Glyphosate trimesium did not depress ChE activity in a two-year dietary study in rats at up to 42 (males) / 56 (females) mg/kg bw/d (Stauffer Chemical Co, 1984), in a two-generation rat reproduction study at dietary doses up to *ca* 100 mg/kg bw/d (Stauffer Chemical Company, 1983) or in dogs gavaged at up to 50 mg/kg bw/d for 12 months (Stauffer Chemical Co, 1987b) (DoHA, 1991).

The JMPR (2004b) review of glyphosate included BVL evaluations of acute (single oral dose) and 13–week (dietary administration) neurotoxicity studies in rats, performed according to OECD Test Guideline 424 (Horner, 1996a,b). Despite the occurrence of general toxicity, there was no behavioural or histological evidence of toxicity to the central or peripheral nervous systems at the respective highest doses of 2000 mg/kg bw and 1547 mg/kg bw/d. Similarly, glyphosate displayed no acute delayed neurotoxicity when tested in chickens by OECD Test Guideline 418 at an oral dose of 2000 mg/kg bw (Johnson, 1996). There was no treatment-related depression in brain acetylcholinesterase (AChE) activity or neuropathy target esterase activity in the brain or spinal cord.

The EU review of glyphosate included BVL assessments of two 21-day oral repeat-dose neurotoxicity studies in chickens, performed with glyphosate at up to 1000 mg/kg bw/d (Bhide, 1987) and Glycel 41 SL at doses up to an equivalent of 1600 mg glyphosate/kg bw/d (Bhide, 1988d). Both studies investigated behaviour, spinal cord and sciatic nerve histology, plasma ChE activity, haematology and clinical chemistry. Slight ataxia (loss of touch sensation) occurred in 1/3 high dose hens on day 18 of Bhide (1987), but otherwise there was no behavioural or histological evidence of neurotoxicity, and no depression in ChE activity. The EU concluded there was no primary neurotoxic effect. The Australian DoHA (1992) assessment of Bhide (1987) agreed that there was no neurotoxicity or neurological change in the spinal cord or peripheral nerves.

The case report of Parkinson's disease in a man following exposure to glyphosate (Barbosa et al, 2001) is inconsistent with previous findings in animals and humans,

and insufficient to prove a causal relationship (JMPR, 2004b). In a review of published epidemiological studies, Mink et al (2011) cite a case-control study (Weschler et al, 1991) reporting an unadjusted OR of 4.04 for Parkinson's disease and use of Roundup at home, based on 19 cases (14 exposed) and 22 controls (9 exposed). However, the strength of the association is questionable due to the small sample size and variability in the data (the 95% CI of 0.91–19.3 was very wide and included 1.0). Furthermore, there was no association between glyphosate exposure and Parkinson's disease in a much larger cohort study of pesticide applicators and their spouses (Kamel et al, 2007), either at enrolment (relative risk of 1.1 in 79 640 subjects) or follow—up (RR of 1.0 in 56 009 subjects).

Garry et al (2002) conducted a cross-sectional analysis of pesticide applicators and their families. Parent-reported ADD / ADHD in children was associated positively and significantly with use of glyphosate, with 6/14 affected children having parents who had exposure to glyphosate or Roundup (OR = 3.6; 95% CI = 1.35–9.65). ADD / ADHD diagnosis was not confirmed by a clinician, however (Mink et al, 2011). The biological significance of findings by Anadon et al (2008), Astiz et al (2009) and Axelrad et al (2003) is unknown, and it is uncertain whether these studies are indicative of any hazard to humans.

The APVMA will monitor the scientific literature for future developments in this area, ensure that relevant research reports are reviewed, and take action if required.

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APPENDIX 1: ASSESSMENTS OF DEVELOPMENTAL STUDIES IN RATS

Summary Table: Developmental toxicity studies in rats – Percentage incidences of foetal anomalies and malformations

Reference	Assessor	Treatment-related Findings	Dose (mg/kg bw/d) Green = foetal NOEL Red = foetal LOEL			
Brooker et al (1991a)	**BVL for JMPR & EU**		**0**	**300**	**1000**	**3500**
		Wavy ribs	CC: 0.6	0	1.8	19.7
		Reduced ossification of cranial centre(s)	CC: 1.9	1.4	7.2	6.9
		Reduced ossification of sacro-caudal vertebral arches	CC: 1.9	5.6	10.2	10.4
		Unossified sternebrae	CC: 13.7	28.5	17.6	33.8**
		Skeletal anomalies (all)	CC: 11.7 HC: 21.9-27.2	22.6	28.4*	35.7**
Tasker et al (1980a)	**BVL for EU**		**0**	**300**	**1000**	**3500**
		Foetal malformations (all)	CC: ? HC: ?	?	?	?
		Unossified sternebrae	CC: ?	?	?	?
	Australia DoHA		**0**	**300**	**1000**	**3500**
		Unossified sternebrae	CC: ?	?	?	?
	US EPA		**0**	**300**	**1000**	**3500**
		Unossified sternebrae	CC: ?	?	?	?
Moxon (1996a)	**BVL for JMPR**		**0**	**250**	**500**	**1000**
		None	NR	NR	NR	NR
Suresh (1991)	**BVL for EU**		**0**		**1000**	
		None	NR		NR	
Bhide (1986)	**BVL for EU**		**0**	**100**	**500**	
		None	NR	NR	NR	
	Australia DoHA		**0**	**100**	**500**	
		None	NR	NR	NR	
Anon (1981)^	**BVL for EU**		**0**	**22**	**103**	**544/558**
		None	NR	NR	NR	NR
Stauffer Chemical Co (1982)^^	**Australia DoHA**		**0**	**30**	**100**	**333**
		None	NR	NR	NR	NR

Statistical significance vs concurrent control group: *p < 0.05 **p<0.01
CC = Concurrent control group mean HC = Historical control group range NR = None reported
? = No incidence data provided in assessment. ^Glyphosate administered in the diet; otherwise gavage dosing.
^^Glyphosate trimesium

Brooker et al (1991a) [Reviewing Agency: BVL] The BVL assessed this study for both the JMPR (2004b) and EU (1998) reviews of glyphosate. There were no discrepancies between the two evaluations, although the EU report provided more data on skeletal ossification. In rats orally gavaged from GD 6 – 15 at 0, 300, 1000 or 3500 mg/kg bw/d, there were maternal deaths at 3500 mg/kg and other evidence of maternotoxicity (clinical signs and a dose-related reduction in bodyweight gain) at 1000 and 3500 mg/kg. Litter and mean foetal weights were depressed at 3500 mg/kg. The incidence of malformations was not affected by treatment but at 1000 and/or 3500 mg/kg, there were increased incidences of wavy ribs and deficits in ossification of the cranium, vertebral arches and sternebrae (see Table). The proportion of foetuses

displaying skeletal anomalies was elevated significantly at 1000 and 3500 mg/kg compared with concurrent controls. The incidence of skeletal anomalies was also increased at 300 mg/kg, but lay within the HC range (the BVL also noted that the study control incidence of skeletal variations was atypically low). The finding in this particular group was therefore considered not to be treatment-related, so the NOAEL for maternotoxicity and developmental toxicity was set at 300 mg/kg bw/d. Kimmel et al (2013) set maternal and foetal NOAELs of 1000 mg/kg bw/d in an evaluation of this study, without commenting on the skeletal anomalies.

Tasker et al (1980a) [Reviewing Agencies: BVL, US EPA and Australian DoHA] According to the BVL evaluation for the EU, glyphosate was administered by gavage to rats over GD 6 – 19 at 0, 300, 1000 or 3500 mg/kg bw/d. Maternal toxicity (mortality, clinical signs and reduced bodyweight gain), enhanced foetal mortality, depressed foetal bodyweight and a higher incidence of unossified sternebrae occurred at 3500 mg/kg. At this dose there was also an increased number of foetuses with malformations (which the BVL did not describe). However, since the incidence and type of malformations were similar to those from HC data, the BVL did not ascribe them to treatment. No further information was provided. NOELs of 1000 mg/kg bw/d were therefore set for maternal and foetal toxicity.

The US EPA (1993) assessment of Tasker et al (1980a) agreed with the BVL evaluation. In the presence of maternotoxicity at 3500 mg/kg bw/d, foetal developmental effects were assessed as increased numbers of foetuses and litters with unossified sternebrae, and decreased mean foetal bodyweight. The NOAEL for maternal toxicity and developmental toxicity was set at 1000 mg/kg bw/d.

The Australian DoHA (1985) evaluated Tasker et al (1980a) in greater detail than the other two agencies but agreed with their principal findings. At 3500 mg/kg, signs of maternal toxicity comprised decreased bodyweight gain; diarrhoea, soft stools, reduced activity and rales in all dams from half way through the dosing period, and six maternal deaths. Dams receiving 300 and 1000 mg/kg showed no reaction to treatment. The NOEL for maternotoxicity was therefore set at 1000 mg/kg bw/d. Increases in the number of foetuses with unossified sternebrae (a developmental variation), dwarfism and bent tail were noted at 3500 mg/kg. However, all dwarf foetuses were in one litter, all foetuses with bent tail were from another litter, and the control and 3500 mg/kg groups had the same number of litters containing malformed foetuses. HC data indicated there were five bent tails out of 5008 foetuses, all confined to one litter out of 383. The DoHA therefore attributed dwarfism and bent tail to genetic factors and in the absence of foetal malformations at 1000 or 300 mg/kg, set a NOEL of 1000 mg/kg bw/d fetotoxicity. Assessments of this study by Williams et al (2012) and Kimmel et al (2013) made the same conclusions.

Moxon (1996a) [Reviewing Agency: BVL] This study, in which dams were orally gavaged from GD 7 – 16 at 0, 250, 500 or 1000 mg/kg bw/d, was assessed for the JMPR review only. There were no treatment-related findings, and so NOELs of 1000 mg/kg bw/d were set for maternal and developmental toxicity. The BVL's conclusions have been corroborated independently by Kimmel et al (2013).

Suresh (1991) [Reviewing Agency: BVL] Rats received glyphosate at 0 or 1000 mg/kg bw/d by gavage between GD 6 and 15. There was no evidence of maternotoxicity, embryolethality or foetal malformations in the treated group, but

there was a higher incidence of delayed ossification of the caudal vertebral arch and proximal forelimb and distal hindlimb phalanges. However, delayed ossification of other parts of the skeleton, particularly the skull, was more frequently seen in the control group. As there was "no clear and consistent impact of test compound administration" on ossification, NOELs of 1000 mg/kg bw/d were set for maternal and developmental toxicity. Kimmel et al (2013) also accepted there were no treatment-related effects in this study.

Bhide (1988a) [Reviewing Agencies: BVL and Australian DoHA] The BVL and DoHA (1992) assessments of this study were closely similar. Glyphosate was administered to rats from GD 15 to LD 21 at nominal doses of 0, 50 and 100 mg/kg bw/d. The bodyweight and food consumption of dams were unaffected, and there were no treatment-related effects on litter parameters including pup bodyweight, survival or growth. No pathological examination was performed. Both agencies set the NOEL in parents and offspring at 100 mg/kg bw/d.

Bhide (1986) [Reviewing Agencies: BVL and Australian DoHA] Again, the two agencies' evaluations coincided. No treatment-related maternal or foetal effects were observed in rats gavaged with glyphosate at 0, 100 or 500 mg/kg bw/d on GD 6 to 15. NOELs for materno- and fetotoxicity were set at 500 mg/kg bw/d. The EU review classified this study as "supplementary" due to reporting deficiencies.

Anon (1981) [Reviewing Agency: BVL] Glyphosate was administered in the diet to rats over GD 6 – 18. Achieved doses were 0, 22, 103 and 544 mg/kg bw/d. There was no materno- or fetotoxicity, and no foetal malformations were recorded. At the highest dietary concentration, an additional group of rats was allowed to litter and nurse their pups until LD 28. Their achieved dose was 558 mg/kg bw/d. No treatment-related effects were observed either in the dams or pups. The EU classified this study as "supplementary" due to reporting deficiencies.

Stauffer Chemical Company (1982) [Reviewing Agency: Australian DoHA] Glyphosate trimesium was administered by gavage to pregnant rats over GD 6 – 20 at 0, 30, 100 or 333 mg/kg bw/d. At the high dose, there was maternotoxicity seen as mortality, clinical signs, reduced bodyweight gain and food consumption. The maternal NOEL was therefore 100 mg/kg bw/d. No treatment-related effects on foetal survival or development occurred, but mean foetal bodyweight was depressed at 333 mg/kg bw/d. A NOEL of 100 mg/kg bw/d was set for fetotoxicity (DoHA, 1991).

Studies with aminomethylphosphonic acid (AMPA)

In addition to developmental toxicity studies on the parent chemical, the EU review included BVL assessments of oral gavage studies in pregnant rats with the glyphosate metabolite AMPA. Following a range finding experiment which found no maternal or foetal effects at up to and including the highest dose of 1000 mg/kg bw/d **(Holson, 1991a)**, AMPA was given at 0, 150, 400 or 1000 mg/kg bw/d from GD 6 through 15 **(Holson, 1991b)**. Dams displayed hair loss and mucoid faeces at 400 and 1000 mg/kg together with transient depression in bodyweight gain and food consumption at 1000 mg/kg only. Foetal bodyweight was slightly but significantly reduced at 1000 mg/kg, but there was no evidence of developmental malformations. Accordingly, the BVL set NOELs of 150 and 400 mg/kg bw/d for maternal and foetal toxicity. Williams et al

(2012) have confirmed these findings, although reporting the maternal NOEL as 400 mg/kg bw/d. No treatment-related maternal or foetal effects occurred when AMPA was administered to pregnant rats at 0, 100, 350 or 1000 mg/kg bw/d over GD 6 – 16 **(Hazelden, 1992)**.

APPENDIX 2: ASSESSMENTS OF DEVELOPMENTAL STUDIES IN RABBITS

Developmental toxicity studies in rabbits: Incidences of foetal mortality, anomalies and malformations

Reference	Assessor	Treatment-related Findings	Dose (mg/kg bw/d) Green = foetal NOEL Red = foetal LOEL			
Brooker et al (1991b)	**BVL for JMPR & EU**		**0**	**50**	**150**	**450**
		Late embryonic deaths (Mean no./litter)	CC: 0.2 HC: 0.1-1.3	0.9	0.5	1.3**
		Postimplantation loss (%)	CC: 5.7 HC: 6.5-17.5	19.5*	15.3*	21.0**
		Malformations (all) (%)	CC: 1.8	2.9	4.5	6.3
		Intraventricular septal defect & other cardiac abnormalities (%)	CC: 0.6 HC: 0.7-5.9	1.0	3.6	5.3
	Kimmel et al (2013)		**0**	**50**	**150**	**450**
		Embryofetal deaths (Mean no./litter)	CC: 0.6	1.8*	1.5*	1.8**
		Postimplantation loss (%)	CC: 5.7	19.5*	15.3*	21.0**
		Malformations (all) (%)	CC: 1.8	2.9	4.5	6.3
		Intraventricular septal defect & other cardiac abnormalities (%)	CC: 0.6	1.0	3.6	5.3
Bhide and Patil (1989)	**BVL for EU**		**0**	**125**	**250**	**500**
		Viable implants (Mean no./litter)	CC: 7.3	8.0	8.0	5.2
		Non-viable implants (Mean no./litter)	CC: 0.07	0.13	0.27	1.4
		Ventricular septal defect (%)	CC: 0	0.9	0.8	2.6
		Postcaval lung lobe absent (%)	CC: 0	0.9	1.6	5.1
		Kidney(s) absent (%)	CC: 0.9	1.8	1.6	7.7
		Rudimentary 14th rib, unilateral (%)	CC: 0.9	0	1.6	6.4
	Kimmel et al (2013)		**0**	**125**	**250**	**500**
		Embryofetal deaths (Mean no./litter)	CC: 0.07	0.13	0.27	1.4
		Total no. foetuses with visceral malformations	CC: 1	4	5	12
		Total no. foetuses with cardiovascular malformations	CC: 0	1	1	2
		Total no. foetuses with skeletal malformations	CC: 1	0	2	5
Moxon (1996b)	**BVL for JMPR**		**0**	**100**	**175**	**300**
		Partially ossified transverse process, 7th vertebra (%)	CC: 0.7	NR	NR	5.6
		Unossified transverse process, 7th lumbar vertebra (%)	CC: 2.8	NR	NR	9.7
		Partially ossified 6th	CC: 2.8	NR	NR	11.1

Reference	Assessor	Treatment-related Findings	Dose (mg/kg bw/d) Green = foetal NOEL Red = foetal LOEL			
		sternebra (%)				
	Kimmel et al (2013)		**0**	**100**	**175**	**300**
		Postimplantation loss (%)	CC: 11.7	9.5	12.1	13.6
		Total no. foetuses with cardiovascular malformations	CC: 1	1	0	1
		Total no. foetuses with major skeletal malformations	CC: 3	0	0	1
		Total no. foetuses with minor skeletal malformations	CC: 58	82**	59	79**
		Total no. foetuses with skeletal variations	CC: 119	129	116	132**
Suresh (1993a)	**BVL for EU**		**0**	**20**	**100**	**500**
		Dilated heart (%)	CC: 0	5.1*	5.2*	17.9*
		Major visceral malformations (all) (%)	CC: 3.0	7.7	7.7	29.6
		Extra 13th rib (%)	CC: 0	1.3	2.6	3.6*
	Kimmel et al (2013)		**0**	**20**	**100**	**500**
		Embryofetal deaths (Mean no./litter)	CC: 0.90	1.38	2.00	1.67
		Postimplantation loss (%)	CC: 13.5	18.6	23.4	23.2
		Total no. foetuses with visceral malformations	CC: 4	6	6	8*
		Total no. foetuses with cardiovascular malformations	CC: 2	4	6	6
		Total no. foetuses with “seal-shaped” heart	CC: 1	0	0	0
		Total no. foetuses with “seal-shaped” heart & cardiomegaly	CC: 0	0	1	0
		Total no. foetuses with dilated heart	CC: 0	4*	4*	5*
		Total no. foetuses with dilated ventricle	CC: 1	0	1	1
		Total no. foetuses with skeletal malformations	CC: 11	5	0	1
Tasker et al (1980b)	**BVL for EU**		**0**	**75**	**175**	**350**
		None	NR	NR	NR	NR
	Australia DoHA		**0**	**75**	**175**	**350**
		None	NR	NR	NR	NR
	US EPA		**0**	**75**	**175**	**350**
		None	NR	NR	NR	NR
	Kimmel et al (2013)		**0**	**75**	**175**	**350**
		Postimplantation loss (%)	CC: 16.7	4.9	2.5	18.7
		Total no. foetuses with cardiovascular malformations	CC: 0	0	0	0
		Total no. foetuses with skeletal malformations	CC: 0	3	2	0
Anon (1981)^	**BVL for EU**		**0**	**10.5**	**50.7**	**255**
		Foetal loss (%)	0.9	0.8	6.1	7.0
Stauffer	**Australia**		**0**	**10**	**40**	**100**

Reference	Assessor	Treatment-related Findings	Dose (mg/kg bw/d) Green = foetal NOEL Red = foetal LOEL			
Chemical Co (1983b)^^	**DoHA**	None	NR	NR	NR	NR
Coles and Doleman (1996)	**Kimmel et al (2013)**		**0**	**50**	**200**	**400**
		Embryofetal deaths (Mean no./litter)	CC: 0.36	0.33	1.00*	1.40
		Postimplantation loss (%)	CC: 3.7	3.6	11.5*	12.1
		Total no. foetuses with cardiovascular malformations	CC: 0	0	1	0
Hojo (1995)	**Kimmel et al (2013)**		**0**	**10**	**100**	**300**
		Embryofetal deaths (Mean no./litter)	CC: 0.7	1.1	1.0	0.6
		Postimplantation loss (%)	CC: 7.1	13.8	8.7	6.5
		Foetuses with cardiovascular malformations (%)	CC: 0	0	1.0	0
		Foetuses with skeletal malformations (%)	CC: 0.7	3.1	4.0	5.4
		Foetuses with skeletal variations (%)	CC: 28.6	24.6	40.7*	27.7

Statistical significance vs concurrent control group: *p < 0.05 **p<0.01
CC = Concurrent control group mean HC = Historical control group range NR = None reported
^Glyphosate administered in the diet; otherwise, gavage dosing ^^Glyphosate trimesium

Brooker et al (1991b) [Reviewing Agency: BVL] The BVL assessed this study for the JMPR (2004b) and EU (1998) reviews of glyphosate. The evaluation for the EU was less detailed, but both assessments established the same NOELs / NOAELs and reached the same conclusions as to the biological significance of foetal mortality and heart malformations, based on HC data. In female rabbits orally gavaged from GD 7 – 19 at 0, 50, 150 or 450 mg/kg bw/d, there were dose-related increases in the incidence of soft / liquid faeces and inappetence and decreases in food consumption and bodyweight gain. The NOAEL for maternotoxicity was set at 50 mg/kg bw/d. Late embryonic deaths were increased significantly at 450 mg/kg, but not at the mid and low doses. At and above 50 mg/kg bw/d, total embryonic deaths and post-implantation losses were significantly higher than in the concurrent controls. Although no explicit rationale was given the BVL did not attribute embryo mortality at 50 and 150 mg/kg to treatment, possibly because the incidence of total (early + late) embryonic death was not dose-related and lay within the HC range from 21 studies performed over 1989 – 1990. The proportion of malformed foetuses was slightly increased at 150 and 450 mg/kg, due to increased incidences of interventricular septal defect and other cardiac abnormalities. However, the BVL did not consider the cardiac abnormalities to be treatment-related, as their incidences lay within the HC range in 13 studies performed in 1989. The NOAEL for developmental toxicity was set at 150 mg/kg bw/d, based on the increased incidences of late embryonic death and postimplantation loss at 450 mg/kg bw/d.

Comment: Postimplantation losses at 50 and 450 mg/kg exceeded the HC range by 2.0 and 3.5%, respectively.

Bhide and Patil (1989) [Reviewing Agency: BVL] When rabbits were gavaged with glyphosate at 0, 125, 250 or 500 mg/kg bw/d between GD 6 and 18, abortion occurred in 2/15 does from the high dose group, which also displayed depression in food consumption and bodyweight gain. A maternal NOEL of 250 mg/kg bw/d was set. Fetotoxicity, skeletal variations and visceral malformations were noted at 500 mg/kg, seen as decreased foetal viability, increased foetal non-viability and increased incidences of unilateral 14th rib, ventricular septal defect, absent kidney and absent postcaval lung lobe. A NOEL of 250 mg/kg bw/d was established for developmental toxicity. No reference was made to historical control data.

Comment: EOS's disagreement with the BVL evaluation focuses on increases in the incidences of ventricular septal defect, absent postcaval lobe and absent kidney at 125 and 250 mg/kg, even though the increases are small compared with those seen at 500 mg/kg. EOS also contends that the increase in rudimentary 14th rib at 250 mg/kg was treatment-related.

Moxon (1996b) [Reviewing Agency: BVL] This particular assessment was performed only for the JMPR review. In female rabbits orally gavaged from GD 8 – 20 at 0, 100, 175 or 300 mg/kg bw/d, the NOAEL for maternotoxicity was 100 mg/kg bw/d based on clinical signs (diarrhoea and reduced faecal output) and reduced food consumption and bodyweight gain at and above 175 mg/kg bw/d. At 300 mg/kg, mean foetal bodyweight was depressed by *ca* 8%, there were significant increases in the incidence of partially or un-ossified vertebrae and sternebrae (see Table), and slight increases in *manus* and *pes* scores[12]. The proportion of foetuses with minor skeletal defects was statistically significantly increased at the low and high doses but not at 175 mg/kg bw/d, which the BVL assigned as the NOAEL for developmental toxicity based [probably] on reduced foetal bodyweight at 300 mg/kg.

Suresh (1993a) [Reviewing Agency: BVL] Rabbits were gavaged with glyphosate at 0, 20, 100 or 500 mg/kg bw/d over GD 6 – 18. The 500 mg/kg dose caused inappetence, clinical signs, a possible depression in bodyweight gain and the death of 8/16 does. A further 4/16 does died at 100 mg/kg without displaying signs, but the BVL attributed their mortality to treatment and set the maternal NOEL at 20 mg/kg bw/d. Abortion did not occur at any dose but one doe displayed complete resorption at 500 mg/kg. At caesarean section on GD 28 there were 20 / 133, 13 / 78, 12 / 77 and 6 / 28 pregnant does / foetuses in the respective groups.

There was no treatment-related effect on external or skeletal malformations. A slight, dose-related upwards trend in the incidence of extra 13th rib was evident in the treated groups, attaining statistical significance ($p \leq 0.05$) at 500 mg/kg only. There were also eight foetuses with major visceral malformations at 500 mg/kg (significant, but p value unstated), compared with four in the control group and six at 20 and 100 mg/kg. Of these foetuses, four, four and five at 20, 100 and 500 mg/kg had dilated heart, compared with none in the control group. The percentage incidence was significant vs control ($p \leq 0.05$) at all doses; see Table. In contrast to the study author, who interpreted the lowest dose (20 mg/kg bw/d) as an effect level, the BVL reviewer assigned a NOEL of 100 mg/kg bw/d based on the increased incidence of 13th rib and heart dilation at 500 mg/kg.

[12] Pathology scores relating to the skeletal development of the hands and feet.

The BVL's rationale for the choice of NOEL was as follows:

1. The absolute number of foetuses with dilated heart was small.
2. The number of affected litters (3/13, 2/12 and 2/6 at 20, 100 and 500 mg/kg) was also low.
3. The numbers of affected foetuses or litters did not differ markedly between the treated groups.
4. The study author provided no information about the severity of heart dilation, and the consequences of such a finding in a foetus were "equivocal".
5. There was no evidence of other and much more common visceral anomalies.
6. Therefore, it was "rather unlikely" that the isolated finding of heart dilation was indeed related to treatment, but nevertheless
7. Based on the [foetal incidence data], a treatment-related effect could not be completely excluded, at least at 500 mg/kg.

Comment:

- The BVL did not identify the other major visceral malformations found in four, two, two and three foetuses at 0, 20, 100 and 500 mg/kg.
- No reference was made to HC data; hence, it is unclear whether the control group was unrepresentative of the background rates of cardiac abnormalities at the study laboratory.
- Heart dilation was classified both as a *malformation* and a major visceral *anomaly* (final paragraph of p 109 and Table B.5.6.2.2.1-1, Annex B-5). Combined with the lack of information as to the severity of the finding, this creates ambiguity as to the functional significance to the developing foetus.

Tasker et al (1980b) [Reviewing Agencies: BVL, US EPA and Australian DoHA] According to the BVL evaluation for the EU, rabbits gavaged with glyphosate at 0, 75, 175 or 350 mg/kg bw/d over GD 6 – 27 displayed clinical signs and potentially treatment-related maternal mortality at and above 175 mg/kg. The NOEL for maternotoxicity was therefore set at 75 mg/kg bw/d. There were no effects on foetal survival, growth or development, and so the foetal NOEL was set at 350 mg/kg bw/d.

The US EPA (1993) assessment differed in setting a NOAEL of 175 mg/kg bw/d for maternotoxicity. However, the EPA agreed that there was no developmental toxicity at any dose tested.

The DoHA (1985) set a NOEL for maternotoxicity at 175 mg/kg bw/d, based on diarrhoea, soft stools, nasal discharge and the death of 10/16 rabbits at 350 mg/kg. In common with the BVL and EPA, no treatment-related effects were considered to have occurred on foetal survival, growth, sex ratio or development. This assessment has been corroborated independently by Williams et al (2012).

Anon (1981) [Reviewing Agency: BVL] In this study, which the EU classified as "supplementary" due to serious reporting deficiencies, glyphosate was administered in the diet to rabbits over GD 6 – 19 at calculated actual doses of 0, 10.5, 50.7 and 255 mg/kg bw/d. There was no evidence of maternal toxicity, but foetal losses were markedly enhanced at the mid and high doses (incidences were 0.9, 0.8, 6.1 and 7.0% in the respective groups). Foetal bodyweight was not affected and no malformations were noted. The BVL assigned a NOEL of 10.5 mg/kg bw/d for fetotoxicity.

Comment: The evaluator remarked that it was unclear why "...an increase in intrauterine mortality would be elicited in a feeding study at doses far below those at which foetal effects were observed in the gavage studies. Thus, it is very doubtful whether this finding was actually related to glyphosate administration. Against the background of the data obtained in more valid, GLP-like studies, it can be concluded that the NOEL for developmental toxicity in rabbits is much higher." Presumably, the BVL reasoned that foetal exposure to glyphosate after maternal dietary dosing at 50.7 and 255 mg/kg would have been lower than attained at doses up to 350 mg/kg in the gavage studies.

Stauffer Chemical Company (1983b) [Reviewing Agency: Australian DoHA] When pregnant rabbits were gavaged with glyphosate trimesium at 0, 10, 40 or 100 mg/kg bw/d from GD 7 to 19, maternal mortality and abortion occurred at 100 mg/kg bw/d and clinical signs were observed at 40 mg/kg and above. Significant decreases in maternal bodyweight gain and food consumption were noted throughout the dosing period at 100 mg/kg, while there was depression in bodyweight during the first seven days of dosing at 40 mg/kg. The maternal NOEL was 10 mg/kg bw/d. There were no effects on foetal survival, bodyweight gain or development at any dose.

Kimmel et al (2013) [Reviewer: Scitox Assessment Services] These authors assessed seven proprietary developmental studies with glyphosate in rabbits. Five studies (Moxon, 1995b; Brooker et al 1991b, Tasker et al, 1980b; Suresh, 1993a; Bhide and Patil, 1989) had been reviewed previously by the BVL, US EPA and / or Australian DoHA (see above).

- Kimmel et al corroborated the BVL assessment of Brooker et al (1991b), describing cardiovascular malformations including intraventricular septal defect, retroesophageal right subclavian artery, dilated or narrowed aorta or pulmonary artery, and disproportionally sized ventricles, seen either alone or in combination.
- In the study of Moxon (1996b), Kimmel et al noted three foetuses (one each in the control, 100 and 300 mg/kg groups) had "heart defects involving effects on septation", together with statistically significant increases in the incidences of minor skeletal malformations at 100 and 300 mg/kg and skeletal variations at 300 mg/kg only. The NOAELs for maternal and developmental toxicity were set at 100 and 175 mg/kg bw/d, respectively, the same doses assigned by the BVL.
- Kimmel et al confirmed that there were no cardiovascular malformations or treatment-related skeletal malformations in Tasker et al (1980b), and in common with the BVL assigned a NOAEL of 75 mg/kg bw/d for maternal toxicity. Kimmel et al set a developmental NOAEL of $\geq$175 mg/kg bw/d because they considered that too few foetuses were available for adequate morphological assessment of the 300 mg/kg group.
- With respect to Suresh (1993a), Kimmel et al corroborated the BVL's reporting of maternal mortality and clinical signs but set a maternotoxicity NOAEL of 100 mg/kg bw/d. They also confirmed the BVL's stated incidences of cardiac dilation among foetuses, while adding that Suresh reported (but did not define) "seal-shaped" heart in one control foetus and one 100 mg/kg foetus, the latter also displaying cardiomegaly. Kimmel et al also clarified that two visceral malformations (single cases of liver haematoma and

absent gall bladder) seen at 500 mg/kg were unrelated to the cardiovascular system. Given that only 28 foetuses were available for examination at 500 mg/kg, Kimmel et al established the developmental NOAEL at 100 mg/kg bw/d. They commented that the observation of dilated hearts (which was unique to this study) may have been due to overly stringent inspection compared to criteria used by other laboratories.

- Kimmel et al also reviewed the study by Bhide and Patil (1989), but concluded its data were unsuitable for setting NOELs because of reporting deficiencies and inappropriate experimental methods. Nevertheless, their assessment of embryofetal mortality and malformations was consistent with the BVL's.

Two other studies in rabbits (Hojo, 1995; Coles and Doleman, 1996) have not been included in any available agency review. Hojo administered glyphosate by oral gavage at 0, 10, 100 or 300 mg/kg bw/d over GD 7 – 19 and observed hypoplasia of the pulmonary artery and ventricular septal defect in one foetus at 100 mg/kg, but no other cardiac abnormalities. No skeletal variations or malformations were ascribed to treatment. Based on clinical signs (soft / liquid faeces) at 300 mg/kg, a NOAEL of 100 mg/kg bw/d was assigned for maternal toxicity. The developmental NOAEL was $\geq$300 mg/kg bw/d.

Coles and Doleman gave oral gavage doses of 0, 50, 200 or 400 mg glyphosate/kg bw/d to pregnant rabbits from GD 7 to 19. Based on clinical signs (soft, liquid, mucoid faeces) and decreased bodyweight gain, a NOAEL for maternal toxicity was set at 200 mg/kg bw/d. Embryofetal deaths and post-implantation losses were increased at 200 and 400 mg/kg, but statistical significance was attained at 200 mg/kg only. At 400 mg/kg, the increase was due to one doe with nine late foetal deaths, which Kimmel et al considered to be of questionable biological significance. At 200 mg/kg, a heart and great vessel defect occurred in an acephalic (headless) foetus. However, there were no other cardiovascular malformations and no treatment-related skeletal malformations or variations. A NOAEL of $\geq$400 mg/kg bw/d was assigned for developmental toxicity.

After Kimmel et al aggregated the data for each dose level (excluding those from Bhide and Patil, 1989), the incidences of septum-related defects were 1/770 in controls and 6/1939 among glyphosate-exposed foetuses (i.e. 0.13 and 3.1%). Four of the six cases in treated groups occurred at the maternally toxic dose of 450 mg/kg. Septal defects were *not* observed among 747 foetuses whose mothers received 175, 200, 300, 350 or 400 mg/kg bw/d.

Cardiomegaly was seen in one foetus at 100 mg/kg (i.e. in 1/374 foetuses or 0.27% incidence), while one case of dilated ventricles occurred at 0, 100 and 500 mg/kg (i.e. 1/770, 1/374 and 1/28 fetuses in the respective groups, = 0.13, 0.27 and 3.6% incidences). Dilated heart was reported in 4/78 (5.1%), 4/374 (1.1%) and 5/28 (17.9%) foetuses at 20, 100 and 500 mg/kg. None of the 954 foetuses whose mothers received glyphosate at 150 – 450 mg/kg bw/d displayed cardiac or ventricular enlargement or dilation. The aggregated data suggest that even if they are not a reporting artefact, the cases at 20 and 100 mg/kg bw/d were not treatment-related.

Kimmel et al concluded that "there was no increase in cardiovascular malformations at doses that were not overtly toxic to the pregnant rabbits (i.e. generally at doses over 150 mg/kg [bw]/d").

Comment: Inclusion of data from Bhide and Patil (1989) in the aggregated dataset would make negligible difference to Kimmel et al's analysis of the incidences of cardiac / ventricular enlargement or dilation, since these findings were reported only by Suresh (1993a). It would add single cases of septal defects at 125 and 250 mg/kg and a further two cases at 500 mg/kg bw/d, making a total of ten affected foetuses from treated mothers[13] (one each at 100, 125, 150 and 250 mg/kg, with four at 450 and two at 500 mg/kg). In the APVMA's opinion, this pattern is most consistent with septal defects having a relationship to treatment at 450 and 500 mg/kg, but not at $\leq$250 mg/kg bw/d.

[13] Kimmel et al do not report the numbers of foetuses Bhide and Patil (1989) examined at each dose, so the incidences of septal defects in all seven rabbit studies combined are unknown.

APPENDIX 3: ASSESSMENTS OF REPRODUCTIVE TOXICITY STUDIES

A3.1 Rats

The German BVL has evaluated eight reproduction studies on glyphosate in rats, of which six were included only in the EU (1998) review, one appeared in the JMPR (2004b) review, and the remaining study was assessed in both reviews. The toxicological end-points examined included oestrus cycling, mating performance, pregnancy rate, gestation length, numbers, sexes, growth, post-natal developmental landmarks and onset of puberty in pups, bodyweights, histology of the reproductive organs and analysis of sperm and oocytes.

Moxon (2000) [Reviewing Agency: BVL] The study was performed over two generations at dietary glyphosate concentrations of 1000, 3000 and 10 000 ppm. In the JMPR review, the BVL found no effects on sexual development or fertility at up to the highest dietary concentration of 10 000 ppm (985 mg/kg bw/d). A NOAEL for parent and offspring toxicity was set at 3000 ppm (293 mg/kg bw/d) based on a reduction in bodyweight of F1A pups and a subsequent reduction in bodyweight of F1 parent males at 10 000 ppm.

Brooker et al (1992) [Reviewing Agency: BVL] This was a two-generation study performed at dietary glyphosate concentrations of 1000, 3000 and 10 000 ppm in the diet. For the EU review, the BVL based a NOEL for parental toxicity of 1000 ppm (79 and 87 mg/kg bw/d in males and females) on histological abnormalities in the parotid and submaxillary salivary glands at glyphosate dietary levels of 3000 and 10 000 ppm. A NOEL of 10 000 ppm (*ca* 797 and 881 mg/kg bw/d in males and females) was set for effects on reproduction and pups.

In the JMPR review, the BVL concluded that there had been no effects on sexual development or fertility at up to the highest dietary concentration of 10 000 ppm. A NOAEL of 3000 ppm (197 mg/kg bw/d) for parent and offspring toxicity was assigned based on increased food and water consumption in F1 females, depressed bodyweight in F1 males, and an increased incidence of cellular alteration of the salivary glands in F0 and F1 adults at 10 000 ppm[14].

Brooker et al (1991c) [Reviewing Agency: BVL] Prior to the main study (above), a one generation range finding experiment was performed on small numbers of rats at dietary glyphosate concentrations of 0, 3000, 10 000 and 30 000 ppm. The parental generation received treatment from GD 3 to PND 21, after which their offspring were treated until termination a six weeks of age. Fecundity and pup survival were unaffected, but [unquantified] reductions in pup bodyweight occurred at all doses. Hence, a NOEL was not established. The BVL discounted this finding because none of the fully comprehensive reproduction studies reviewed for the EU had found treatment-related effects on pups at up to and including 10 000 ppm.

[14] The discrepancy between the BVL's conclusions for the EU and JMPR reviews occurred because the JMPR assigns No Observed *Adverse* Effect Levels to toxicology studies, as opposed to No Observed Effect Levels (as assigned by the EU and Australia). By JMPR criteria, the histological abnormalities in the salivary glands at 3000 ppm were not classified as an adverse effect.

Reyna (1990) [Reviewing Agencies: BVL and US EPA] A two-generation study was performed at dietary levels of 0, 2000, 10 000 and 30 000 ppm. In-life and *post mortem* examinations conformed with OECD TG 416 and included histological examination of reproductive organs from all control and high dose F0 and F1 adults and one F2B weanling/sex/litter. A NOEL of 10 000 ppm (722 and 757 mg/kg bw/d for males and females, respectively) was assigned for parental and offspring toxicity. This was based on reduced bodyweight gain and soft faeces in adults receiving 30 000 ppm, and reductions in litter size and pup bodyweight gain during lactation at this same dietary level.

The US EPA (1993) evaluation of Reyna (1990) differed slightly from the EU / BVL assessment insofar as there was no mention of decreased litter size, but was otherwise closely similar. The EPA assigned a systemic NOEL of 10 000 ppm (500 mg/kg bw/d), a reproductive NOEL of 30 000 ppm (1500 mg/kg bw/d) and a developmental NOEL of 10 000 ppm (500 mg/kg bw/d). The doses appear to have been estimated, rather than having been calculated from parental food intake.

Suresh (1993b) [Reviewing Agency: BVL], In this two-generation study compliant with OECD TG 416, there were no treatment-related effects on the parents or offspring at the highest administered dietary level of 10 000 ppm, equivalent to *ca* 700 – 800 mg/kg bw/d. The BVL therefore set a NOEL of 10 000 ppm.

Antal (1985) [Reviewing Agency: BVL] Similarly, the BVL assessed this three-generation study as having demonstrated no effects of treatment at the highest dietary concentration of 5000 ppm in the diet, or 462 and 502 mg/kg bw/d in males and females. A NOEL of 5000 ppm was therefore assigned for parental and reproductive toxicity.

Bhide (1988b and 1988c) & Schroeder and Hogan (1981) [Reviewing Agencies: BVL, US EPA and Australian DoHA] These three studies were performed at very low doses, and the BVL / EU regarded them as providing supplementary information only. No treatment-related effects occurred in the parental generations or offspring in a three-generation study at dietary feeding levels of 0, 75, 150 and 300 ppm, equivalent to *ca* 15 mg/kg bw/d at the high dose (Bhide, 1988b); during a single-generation study by oral gavage at 0, 5 and 10 mg/kg bw/d prior to mating, through pregnancy and up to PND 21 (Bhide, 1988c); or in a three-generation dietary study at 0, 3, 10 and 30 mg/kg bw/d (Schroeder and Hogan, 1981).

The DoHA (1985) and the US EPA (1993) also assessed Schroeder and Hogan (1981) as having demonstrated no treatment-related effects on the parental or filial generations, and set a NOEL of 30 mg/kg bw/d. This NOEL forms the basis for the current Australian ADI for glyphosate, of 0.30 mg/kg bw/d. A DoHA (1992) evaluation reached the same conclusions as the BVL with regard to the studies by Bhide (1988b and 1988c).

Stauffer Chemical Company (1983a) [Reviewing Agency: Australian DoHA] In a two-generation study with glyphosate trimesium in rats at dietary concentrations of 0, 150, 800 and 2000 ppm (equivalent to *ca* 7.5, 40 and 100 mg/kg bw/d), the only adverse effect on reproductive indices was a reduction in litter size at 2000 ppm. A NOEL of 150 ppm was assigned for the parental animals and offspring based on reduced bodyweight gain, food consumption and plasma protein and albumin levels in

adults and depressed pup bodyweight and relative spleen weight at and above 800 ppm (DoHA, 1991).

APPENDIX 4: STUDY ASSESSMENTS PERFORMED BY MARK JENNER, SCITOX ASSESSMENT SERVICES

A4.1 Effects of a glyphosate-based herbicide formulation on gene expression in vitro

Hokanson et al (2007): In a study of the effects of glyphosate on the expression of oestrogen-regulated genes, MCF-7 human breast adenocarcinoma (oestrogen sensitive) cells were exposed to an unidentified home garden herbicide containing 15% glyphosate (no additional details provided) with or without 3.0 X 10^{-10} M 17β-estradiol (oestrogen). Cells were incubated for 18 hours with the herbicide at final glyphosate concentrations of 0.23, 0.023, 0.0023, or 0.00023%. Following purification of cellular RNA and generation of cyanine 3- and 5-labelled anti-sense RNA, the activity of 1550 genes was then measured by DNA microarray analysis using RZPD chips.

According to the study authors, 680 of the 1550 investigated genes were dysregulated by exposure to the herbicide. However, they did not state by how much the affected genes' activity differed from control levels, or at what glyphosate concentrations. The study authors listed a sub-set of 29 genes whose activities were up- or down-regulated by greater than 2-fold, of which seven were tested further by quantitative real-time PCR to corroborate the results of DNA microarray analysis.

Only three of the 1550 genes fulfilled the criteria for significant dysregulation, when appraised by both methods. In the presence of glyphosate at 0.00023%, DNA microarray analysis indicated that HIF1 was up-regulated by 2.2-fold, while CXCL12 and EGR1 were down-regulated to 0.46 and 0.49 of control activity. qrtPCR expression analysis showed that HIF1 was up-regulated by over two-fold whereas CXCL12 and EGR were down-regulated by over 50%. For each gene, cell treatment with oestrogen alone yielded expression levels that were intermediate between those observed in control cells and cells exposed to oestrogen and herbicide combined.

According to the study authors, the HIF1 gene primes cells for the initiation of apoptosis under hypoxic conditions, and therefore plays a key role in cell death resulting from cerebral and myocardial ischemia. They raise the possibility that elevated levels of HIF1 [protein] may initiate apoptosis in the absence of hypoxia, promoting a variety of hypoxia-initiated patho-physiological states including ischemia of the myocardium, brain and retina; pulmonary hypertension, pre-eclampsia and intrauterine [foetal] growth retardation.

The CXCL12 gene product (also known as stromal cell-derived factor 1 and pre-beta cell growth-stimulating factor) is a lymphocyte chemoattractant, may be involved in lymphocyte activation, and is reportedly critical for the mobilisation of cells of the haematopoietic tissues into peripheral blood. Hokanson et al suggest that altered [decreased] levels of CXCL12 may contribute to disruption of immune surveillance and basal extravasation of mono- and lymphocytes.

Among the biological effects attributed to EGR1 are regulating the expression of transforming growth factor beta-1, involvement in the suppression of [cellular] growth and transformation, and the regulation of apoptosis, endothelial cell growth, neovasculatisation, tumour initiated angiogenesis and tumour growth. The study authors consider that [decreased] levels of EGR1 may potentially affect the rate of

initiation of apoptosis and alter the level of vascularisation associated with tumour formation.

Comment

This paper is of limited value: it does not identify which components of the glyphosate-based herbicide formulation are responsible for altering gene expression, does not identify any mode of action of those components, does not provide evidence that the observed changes in gene expression are anything other than homeostatic regulation, and does not establish that the effects observed in MCF-7 cancer cells in vitro would be representative of those that would occur in non-cancerous mammalian cells (especially within tissues or at the whole animal level). Other than retardation of foetal growth, the postulated effects of HIF1, CXCL12 and EGR dysregulation have not been reported in toxicology studies in laboratory animals, and there appears to be no justification for extrapolating from the study's findings to predicting adverse effects on human health.

Mink et al (2011) have reviewed epidemiological studies relevant to some of the non-cancer end-points that Hokanson et al speculate may be affected. In the study populations, there was no statistically and/or biologically association between exposure to glyphosate and retinal degeneration (Kirrane et al, 2005), myocardial infarction (Dayton et al, 2010 and Mills et al, 2009) or depressed birthweight (Sathyanarayana et al, 2010). Furthermore, epidemiological evidence of associations between glyphosate exposure and cancer is weak and conflicting (DoHA, 2005). A recent review (Mink et al, 2012) of epidemiological studies relevant to cancer end-points considered seven cohort studies and fourteen case-control studies looking at possible associations between glyphosate and one or more cancer outcomes; there was no consistent pattern of positive associations to indicate any causal relationship between total cancer (in adults or children) or any site-specific cancer and exposure to glyphosate.

A4.2 Cytotoxicity of glyphosate, AMPA and glyphosate-based herbicides in vitro

Benachour et al (2007): Human embryonic kidney (HEK) 293 and human choriocarcinoma-derived placental JEG3 cells were exposed for 1 – 72 hours *in vitro* to Roundup Bioforce (360 g/L glyphosate acid present as 480 g/L glyphosate isopropylamine salt, no other constituents identified; Monsanto, Anvers, Belgium) at up to 2% in the incubation medium, or glyphosate at equivalent concentrations (up to 42 mM). Cell viability was measured by the MTT assay, based on the cleavage of MTT by the mitochondrial enzyme succinate dehydrogenase (SDH). When the effects of the test formulation and glyphosate were compared, glyphosate solutions were adjusted to *ca* pH 5.8, the pH of a 2% Roundup solution.

Roundup Bioforce showed greater concentration- and time-dependent cytotoxicity against both cell lines than glyphosate at equivalent concentrations, suggesting that adjuvants in the formulation were contributing to cellular injury. JEG3 cells were more resistant to Roundup Bioforce than HEK293 cells, but both types were of similar susceptibility to glyphosate.

Table 4.1: EC50s* (% in serum-containing medium) of Roundup Bioforce and equivalent concentrations of glyphosate for viability of HEK293 and JEG3 cells.

Test compound	1 h	24 h	48 h	72 h
	HEK293 cell line			
Roundup Bioforce	1.4	0.8	0.7	0.05
Glyphosate	>>2.0	1.7	1.7	1.5
	JEG3 cell line			
Roundup Bioforce	>>2.0	1.3	0.4	0.2
Glyphosate	>>2.0	1.8	1.5	1.5

*EC50 (not the LD50 as claimed by the study authors[15]) = the concentration required to cause a 50% decrease in mitochondrial SDH activity. As the data were provided in graph form, all values are approximate.

Effects of Roundup Bioforce and glyphosate on the activity of aromatase (CYP19; an enzyme catalysing the conversion of androgens to oestrogens) were measured in HEK293 cells transfected with human aromatase cDNA, human placental cell microsomes and equine testicular microsomes. The HEK293 cells were exposed to the test compounds for 24 hours at up to 0.2% Roundup or 1% glyphosate, while microsomes had a 15-minute exposure period at up to 10% Roundup or 2% glyphosate. The assay quantified the release of tritiated water from [1β-^{3}H]-androstenedione.

Both the formulation and active constituent weakly inhibited aromatase activity *in vitro.* Under pH-adjusted conditions at 37 ºC, glyphosate had IC50s of *ca* 1.0% and 0.8% against aromatase in placental microsomes and HEK293 cells, respectively. Over its tested concentration range (0.01 – 0.2%), Roundup Bioforce inhibited aromatase by *ca* 20% in HEK293 cells. Roundup Bioforce had an IC50 of *ca* 4% against aromatase activity in human placental and equine testis microsomes, at 25 ºC and physiological pH.

Comment

The concentrations of Roundup and glyphosate required for cytotoxicity and aromatase inhibition were similar to those present in herbicidal spray mixtures (1 – 2% formulation or 21 – 42 mM glyphosate), orders of magnitude higher than would be attained within cells or tissues in vivo under physiological conditions. Over the more biologically relevant concentration range 0.001 – 100 µM, glyphosate has no effect on steroid hormone production in the H295R steroidogenesis assay, developed by the OECD as an in vitro screening assay for endocrine disrupting chemicals (Hecker et al, 2010). Given that surfactants inhibit aromatase activity by disrupting mitochondrial membranes (Levine et al, 2007), the reported effects of Roundup Bioforce in HEK293 cells and microsomes are likely to be experimental artefacts. Another confounding factor would have been the pH of the incubation medium, which was below the physiological range during the cell viability assays.

Benachour and Seralini (2009) evaluated the *in vitro* cytotoxicity of glyphosate (Sigma-Aldrich), the glyphosate metabolite AMPA (Sigma-Aldrich), four glyphosate-based herbicide products (see table below), and the surfactant polyethoxylated tallow amine (POEA; a component of some glyphosate formulations) to human umbilical

[15] Cellular viability was not quantified, so it could not be confirmed that the "LD50" actually corresponded to the death of half the population of exposed cells.

cord vein endothelial cells (HUVEC)[16] and the human choriocarcinoma-derived placental (JEG3) and human embryonic kidney (HEK293) cell lines.

Table 4.2: Glyphosate-based herbicides studied in Benachour & Seralini (2009) All products were manufactured by Monsanto, Anvers, Belgium

Product Name (Abbreviation used in evaluation)	Glyphosate concentration (g/L)
Roundup Express (R7.2)	7.2
Roundup Bioforce* (R360) Roundup Extra 360*	360
Roundup Grands Travaux (R400)	400
Roundup Grands Travaux Plus (R450)	450

*The study authors treated both products as being the same formulation.
No further information on product composition was provided.

Cells were exposed for 24 hours in serum-free medium to each individual test compound at 14 concentrations ranging from 10 ppm to 20 000 ppm (0.001% to 2%). Cells were also exposed to POEA at 1 and 5 ppm, and AMPA at 4, 6, 8 and 10%. Using sub-toxic concentrations of glyphosate, AMPA and POEA, evidence of additive or synergistic toxicity was sought in HEK293 and JEG3 cells exposed to combinations of POEA 1 ppm + glyphosate or AMPA 5000 ppm, and glyphosate 4000 ppm + AMPA 1000 ppm. HUVEC cells were exposed to POEA 1 ppm + glyphosate or AMPA 500 ppm, and glyphosate 400 ppm + AMPA 100 ppm.

After incubation, cytotoxicity was assessed by the following criteria: *Adenylate kinase (AK) activity* in the incubation medium, as a biomarker of cytoplasmic membrane rupture (assumed to result from cellular necrosis, either primary or secondary after apoptosis); *Intracellular succinate dehydrogenase (SDH) activity*, assayed by the MTT test as a measure of mitochondrial respiration rate; and *Intracellular caspase 3/7 activity*, as indicators of apoptosis. Results from the cytotoxicity assays were presented in graphical form alone, and therefore only approximate quantitative values are available.

Results

Cytotoxicity, assessed by impact on mitochondrial respiration rate: In all three cell types, the concentration of glyphosate causing a 50% decrease in SDH activity (ie, the EC50, and not the LD50 as claimed by the study authors[17]) was *ca* 10 000 ppm. The metabolite AMPA was markedly less toxic, having EC50s of *ca* 40 000, 100 000 and >100 000 ppm in HEK293, JEG3 and HUVEC cells, respectively. By contrast, POEA was highly cytotoxic, demonstrating a lowest EC50 of *ca* 3 ppm (see following table). All Roundup formulations were more toxic than the active constituent. Moreover, their EC50s were not linearly proportional to the concentration of glyphosate in the products or incubation medium. This is consistent with other formulation components being cytotoxic and/or potentiating the toxicity of the active constituent.

[16] HUVEC cells were chosen because *in vivo*, they form a permeable barrier between the blood and the underlying tissues and would be exposed directly to circulating chemicals, for which they may be a target.
[17] Cellular viability was not quantified, so it could not be confirmed that the "LD50" actually corresponded to the death of half the population of exposed cells.

Table 4.3: Concentrations of glyphosate and other test compounds causing a 50% decrease in intracellular succinate dehydrogenase activity in HUVEC, JEG3 and HEK293 cells

Test compound	Approx EC50 (ppm)	Glyphosate concentration (ppm) in medium at the EC50
Glyphosate	10 000	10 000
AMPA	≥40 000	-
POEA	3 – 30	-
R 7.2	6000 – 9000	42 – 63
R360	2000 – 3000	720 – 1080
R400	30	12
R450	100	45

Cell membrane integrity: AMPA, POEA and the Roundup formulations caused increases in extracellular AK activity, consistent with leakage or rupture of cell membranes. By contrast, cells exposed to glyphosate alone released little or no AK, even in the presence of marked depression in mitochondrial respiration. The study authors interpreted this as evidence that glyphosate does not mediate cell death by necrosis, in contrast to AMPA, POEA and Roundup formulations.

Interactions between glyphosate, AMPA and POEA, assessed by effects on cell membrane integrity: Combinations of glyphosate + POEA, glyphosate + AMPA and AMPA + POEA (see above) were clearly more cytotoxic to HUVEC and HEK293 cells than the individual chemicals, causing about 2-fold and 4 to 8-fold more extensive release of AK from the two respective cell types. However, for reasons unknown, additive or synergistic toxicity was not observed in JEG3 cells.

Apoptosis: At incubation concentrations of 50 ppm and above, glyphosate and R360 induced transient but marked increases in intracellular caspase 3/7 activity within HUVEC cells. The effect was first observed after 6 hours of exposure. After 12 hours, caspase activity peaked at 20 – 30 times control levels. Reversibility was well advanced by 18 hours and complete at 24 hours. Similar but much weaker responses occurred in HEK293 and JEG3 cells, within which caspase 3/7 activity increased by no more than 2 or 3-fold. These cell lines were markedly less sensitive than HUVEC cells, requiring glyphosate and R360 concentrations of at least *ca* 10 000 and 1000 ppm, respectively, for induction of caspase activity. Cell death, loss of adhesion, shrinkage and fragmentation were confirmed microscopically in all cell types after 24 hours exposure to 50 ppm R400. DAPI staining revealed DNA condensation in HUVEC, HAK293 and JEG3 cells exposed to glyphosate or R360 at 5000 ppm.

No findings were presented on the influence of AMPA and POEA on caspase activity or cell morphology.

Comment

The French Agency for Food Safety (AFSSA, 2009) has reviewed Benachour and Seralini (2009), commenting that:

- During exposure to the test compounds, cells were incubated for 24 hours in medium without serum, which could lead to disturbance of their physiological state.

- The glyphosate tested in the study was glyphosate acid, whereas glyphosate isopropylamine salt was present in the commercial formulations tested. No precise information regarding pH was given, except at the highest concentrations [where the pH was adjusted to 5.8].
- Cytotoxicity and induction of apoptosis may have been due to pH and / or variations in osmotic pressure at the highest concentrations tested.
- Surfactant effects and increased osmolality are known to increase membrane permeability, causing cytotoxicity and induction of apoptosis.
- The test cells were exposed at extremely high concentrations of the test compounds under physiologically abnormal conditions.

A4.3 Cytotoxicity, anti-estrogenic and anti–androgenic activity, and genotoxicity of glyphosate and glyphosate-based herbicides in vitro

Gasnier et al (2009) assessed the activity of glyphosate and four glyphosate-based herbicides (R7.2, R360, R400 and R450; see above evaluation of Benachour and Seralini (2009)) in the HepG2 human hepatoma or MDA-MB453-kb2 cell lines. The following end-points were investigated:

Cytotoxicity: *Intracellular SDH activity*, *extracellular AK activity* and *intracellular caspase 3/7 activity* were measured in HepG2 cells as described by Benachour and Seralini (2009). Cell viability was also assessed by the *Alamar Blue assay* and the *neutral red assay*, following 24 hours of exposure to the test compounds over the range 10 – 20 000 ppm.

Anti-oestrogenic activity:
(a) *The activity of aromatase,* the enzyme responsible for converting androgens to oestrogens, was measured in HepG2 cells after 24 hours of exposure to "non-toxic" concentrations of glyphosate or R7.2, 360, 400 and 450. The assay was based on the release of tritiated water from [1β-^{3}H]-androstenedione. *Aromatase mRNA levels* were also assayed, by semi-quantitative reverse transcriptase-PCR.

(b) *Activity at human oestrogen receptors* was measured in HepG2 cells transfected with hERα and hERβ and then incubated with 17β-estradiol (at 10^{-8}M) and glyphosate or R7.2 (each at up to 3000 ppm), R360 (up to 2000 ppm), R400 (up to 10 ppm), R450 (up to 30 ppm) or the positive control ICI 182x780 (at 10^{-8}M).

Anti-androgenic activity was measured in MDA-MB453-kb2 human breast cancer cells (which possess a high level of androgen receptor) incubated for 24 hours with glyphosate (up to 1500 ppm) or R7.2, (up to 2000 ppm), R360 (up to 500 ppm), R400 (up to 2 ppm) or R450 (up to 40 ppm) plus DHT (4×10^{-10} M). The positive control was nilutamide (10^{-6} M).

Genotoxicity: Single- and double-stranded DNA breakage and alkali-labile DNA damage were investigated in HepG2 cells after 24 hours of exposure to R400 at 1, 2.5, 5, 7.5 and 10 ppm, using the single-cell gel electrophoresis (Comet) assay. Benz[a]pyrene (50 µM) was used as positive control. It is unclear whether glyphosate or other Roundup formulations were tested.

Results

Cytotoxicity: SDH and AK activity and the Alamar Blue assay yielded fairly consistent results in the experimental system employed. As shown in the following table, the absolute and relative cytotoxic potencies of glyphosate and Roundup formulations against HepG2 cells were similar to those described by Benachour and Seralini (2009) against other human cell lines *in vitro*. Again, Roundup formulations were moderately – markedly more toxic than the active constituent, and their relative potency was not proportional to the concentration of glyphosate they contained.

Table 4.4: LOECs or EC50s of glyphosate and Roundup formulations against indices of cytotoxicity in HepG2 cells.

Test compound	Alamar Blue assay		SDH inhibition		AK activity
	LOEC (ppm)	EC50* (ppm)	LOEC (ppm)	EC50* (ppm)	LOEC (ppm)
Glyphosate	10 000	27 800	10 000	18 000	>20 000
R 7.2	2000	3600	8000	8600	8000
R360	1000	2200	5000	6500	3000
R400	5	12	50	55	50
R450	50	60	80	170	60

*Reported as LC50

At 60 ppm, R450 formulation induced apoptosis in HepG2 cells, seen as a 156% increase in caspase 3/7 activity following 24 hours exposure ($p<0.05$ vs control) and a 765% increase after 48 hours ($p<0.01$). No further data on apoptotic activity were presented.

Anti-oestrogenic activity: Over the range 600 – 3000 ppm, glyphosate had no statistically significant effects on aromatase transcription and activity in HepG2 cells, and was also devoid of anti-oestrogenic activity at hERα and β.

By contrast, intracellular aromatase activity was significantly ($p<0.05$ or <0.01) inhibited in the presence of Roundup formulations. R7.2 caused *ca* 75% inhibition at 8000 ppm. R360, R450 and R400 caused no more than *ca* 50% inhibition of aromatase activity, but maximal inhibition occurred at lower concentrations ($\geq$800, 50 and $\geq$10 ppm respectively). The mode of inhibition was not elucidated but is unlikely to have depended on inhibition of DNA transcription, because aromatase mRNA levels were generally increased in Roundup-exposed cells.

All Roundup formulations dose-dependently inhibited oestrogen-dependent transcription in HepG2 cells. R7.2 and R360 were the least potent, with IC50s of *ca* 1500 – 2500 ppm, whereas R400 and R450 had *ca* 100 – 500 times greater potency (see following table). Anti-oestrogenic potency was not correlated with the concentration of glyphosate present in the formulations or cell incubation medium.

Anti-androgenic activity: Roundup formulations dose-dependently inhibited androgen-dependent transcription in MDA-MB453-kb2 cells. R7.2 and R360 were the least potent, with respective IC50s of *ca* 800 and 300 ppm, whereas R400 and R450 had *ca* 10 – 100 times greater potency (see following table). Anti-androgenic potency was independent of glyphosate concentration.

The study authors claimed that glyphosate "was clearly anti-androgenic at sub-agricultural and non-cytotoxic dilutions". This is, however, open to question: androgen receptor-mediated transcriptional activity was depressed by *ca* 30% at the lowest glyphosate concentration tested (100 ppm?), 45% at 500 ppm but only 20% at 1500 ppm (data were presented graphically, so all values are approximate). Although

the difference from control was statistically significant ($p<0.01$) at all three concentrations, the lack of dose-dependency and failure to attain 50% inhibition are remarkable, inconsistent with the behaviour of the Roundup formulations, and seem inconsistent with a receptor-mediated phenomenon. Furthermore, results obtained with the positive control were not presented.

Table 4.5: IC50s of Roundup formulations against human steroid receptors, expressed as ppm formulation (upper line) and µM glyphosate (lower line) in the cell incubation medium

Receptor	R7.2	R360	R400	R450
hERα	2030 ppm 86.5 µM	1450 ppm 3088 µM	6.0 ppm 14.2 µM	20 ppm 53.2 µM
hERβ	2460 ppm 105 µM	1600 ppm 3407 µM	3.0 ppm 7.1 µM	ND ND
hAR	770 ppm 32.8 µM	310 ppm 660 µM	0.9 ppm 2.1 µM	20 ppm 53.2 µM

hERα = human oestrogen receptor α **hERβ** = human oestrogen receptor β
hAR = human androgen receptor ND = No data

Genotoxicity: R400 caused a dose-dependent increase in DNA strand breaks[18]. Compared with the negative control (35% breakage, with 15% class 1, 10% class 2 and 10% class 3 breaks), there was *ca* 50% total breakage at 5 ppm (comprising 25% class 1, 11% class 2 and 15.5% class 3 breaks), 60% breakage at 7.5 ppm and 75% breakage at 10 ppm (*ca* 13% class 1, 27% class 2 and 36% class 3 breaks). The NOEC was 2.5 ppm. The positive control caused 95% total breakage, of which *ca* 70% consisted of class 3 breaks.

However, there results were not necessarily caused by genotoxic activity. In the Alamar Blue assay (the most sensitive index of cytotoxicity), R400 was toxic against HepG2 cells at concentrations of 5 ppm upwards, with an EC50 of 12 ppm. It is therefore possible that the increased DNA strand breakage seen at 5 – 10 ppm arose from cellular injury or death, rather than from direct damage to DNA.

Comment

The study did not demonstrate whether the observed inhibition of aromatase and steroid receptor-mediated transcription was caused by glyphosate or other components in the test products. If surfactants were present, it is highly probable that they contributed to these effects, given that surfactants interfere with *in vitro* assays for aromatase activity and steroidogenesis (US EPA, 2009; Levine et al, 2007; & DeSesso and Williams, 2012).

Clair et al (2012): The study authors measured the cytotoxicity of glyphosate and a glyphosate-based herbicide, and investigated their effects on testosterone production and oestrogen and androgen receptor mRNA levels in rat testicular cells *in vitro.* The test compounds were laboratory-grade glyphosate (Sigma-Aldrich, Saint-Quentin Fallavier, France) and Roundup Bioforce (360 g/L glyphosate acid; no other information provided). Stock solutions of glyphosate (7.6 g/L) or 2% Roundup (= 7.6 g glyphosate/L) were prepared in cell culture medium and diluted as required.

[18] Class 1 = minimum damage, Class 2 = medium and Class 3 = maximum

Leydig, Sertoli and germ cells were isolated and purified from the testes of 70-day-old Sprague-Dawley rats. Leydig cells were incubated for 1 – 48 hours with Roundup at 0.005 – 1.0% in solution or equivalent concentrations of glyphosate. The other cell types appear to have been exposed to the same range of concentrations for 24 or 48 hours. Cytotoxicity was assessed by measurement of adenylate kinase (AK) activity (an index of cytoplasmic membrane rupture) in cell supernatants using the ToxiLight bioassay. To measure the extent of apoptosis, intracellular caspase 3 / 7 activity was quantified by the Caspase-Glo assay, and nuclear DNA was visualised *in situ* by DAPI fluorescence staining.

In Leydig cells that had been exposed for 24 hours to glyphosate or Roundup at 0.0001 – 0.10%, 3β-hydroxysteroid dehydrogenase (3β-HSD) activity was measured as an index of testosterone synthesis, and the testosterone concentration in the cell culture medium was quantified by RIA. mRNA expression of aromatase, AR, HERα and HERβ was measured by real-time PCR.

Results

Cytotoxicity (cell lysis): In Leydig cells, glyphosate caused no increase in AK activity over the concentration and time range tested, suggesting a lack of necrosis associated with cytotoxicity. By contrast, cytotoxicity was evident after one hour of exposure to Roundup at ≥0.10%. The peak effect (*ca* 3-fold increase in AK activity vs unexposed controls) occurred from 3 – 24 hours at concentrations between 0.50 and 1.0% ($p<0.005$ or 0.001).

Germ cells were resistant to injury by glyphosate (no increase in AK activity seen) and comparatively insensitive towards Roundup, which caused a maximum of *ca* 20% increase in AK activity at 24 hours at 0.50% ($p<0.001$) and at 48 hours at 0.005% ($p>0.05$).

Glyphosate was cytotoxic towards Sertoli cells, eliciting *ca* 2-fold increases in AK activity at 24 hours at 0.01 and 0.05% ($p>0.05$). Roundup also injured Sertoli cells by 24 hours, but the peak effect (a 2-fold increase in AK activity) occurred at 0.10% ($p<0.05$).

Apoptosis: In the time course experiment with Leydig cells, the only evidence of caspase activation was seen after six hours exposure to Roundup at 0.05%, which elicited a *ca* 15% increase in activity ($p<0.01$). Over the 0.1% - 1.0% concentration range, by contrast, Roundup caused concentration-dependent *decreases* in caspase activity from one hour onwards, with almost complete loss of activity after 12 – 48 hours' exposure at ≥0.5% ($p<0.001$). Roundup caused a similar effect in Sertoli and germ cells after 24 hours of exposure.

In contrast to the formulation, glyphosate did activate caspase in Leydig cells. Relatively weak (10 – 20%) and inconsistent increases in activity were observed from six hours onwards at concentrations of 0.005% and above. In germ cells, 0.005 and 0.01% glyphosate increased caspase activity by *ca* 20% after 24 hours exposure ($p<0.01$), while 20 – 40% increases ($p<0.01$ to 0.001) in activity were evident at 48 hours over the concentration range 0.50 – 1.0%. Glyphosate did not, however, mediate any consistent effect on caspase activity in Sertoli cells.

Morphological evidence of apoptosis (compaction of chromatin and DNA within the nucleus) was observed in Leydig cells exposed for 24 hours to Roundup at 0.05 and 1.0%, or glyphosate at 1.0%. However, there was no comment as to whether nuclear condensation also occurred in Sertoli or germ cells.

Testosterone: Neither Roundup nor glyphosate influenced the 3β-HSD activity in Leydig cells exposed for 24 hours at 0.0001 – 0.10%. Testosterone concentration in the cell incubation medium was depressed by *ca* 1/3rd ($p<0.01$) by glyphosate and Roundup at 0.0001%, but not at or above 0.005%.

Expression of aromatase, AR, HERα and HERβ in Leydig cells: Aromatase mRNA levels increased by *ca* 7.5-fold ($p<0.005$) in response to a 24-hour exposure to glyphosate at 0.001%, but rose by only 2-fold at 0.005 and 0.01% (non-significant). A non-significant, three-fold increase in aromatase mRNA occurred following exposure to Roundup at 0.001%, but at 0.005 and 0.01% there was no effect. Aromatase activity and oestrogen levels were not measured. Neither glyphosate nor Roundup had any effect on androgen or oestrogen receptor mRNA levels under the experimental conditions.

A4.4 Developmental and reproductive effects of glyphosate-based herbicide in amphibians and birds

Paganelli et al (2010) performed studies on neural crest development in three experimental systems:

(i) *Xenopus laevis* embryos, which were exposed from the 2-cell stage onwards to Roundup Classic (a Monsanto product containing 48% w/v of an unspecified glyphosate salt; no other constituents were identified) at 3000-, 4000- and 5000-fold dilutions in their incubation medium. The final concentrations of glyphosate were 717, 536 and 430 µM at the respective dilutions. Neurula stage embryos were fixed and examined at the by immunofluorescence following *in situ* hybridisation with antisense RNA probes. Retinoic acid (RA) activity was measured by chemiluminescence in neurula-stage embryos that had been injected with RAREZ reporter plasmid prior to Roundup exposure as described. For rescue experiments RAREZ-injected embryos were incubated with Roundup at 4000-fold dilution until the blastula stage, then exposed to the RA receptor antagonist Ro 41-5253 at 1.0 µM until assay of RA activity.

(ii) Two-cell *Xenopus* embryos were injected with 360 or 500 pg of glyphosate into one or both cells (producing intracellular concentrations of 8 – 12 µM) together with 10 ng of the visual marker Dextran Oregon Green. They were then incubated until sibling controls had reached the desired developmental stage, fixed, and examined visually or by immunofluorescence following *in situ* hybridisation as described above.

(iii) Fertilised chicken eggs were injected with 20 µL of 3500- or 4500-fold dilutions of Roundup Classic and incubated at 38℃ until fixation, *in situ* hybridisation and immunofluorescence examination as described for *Xenopus* embryos. Control embryos were treated similarly after injection of 20 µL of water.

Effects on neural crest markers, rhombomere formation and primary neuron differentiation: Compared with sibling controls, Roundup Classic at 5000-fold dilution impaired neural crest formation in 87% of *Xenopus* embryos (n = 30), seen as down-regulation of the neural crest marker *slug* and zinc finger transcription factor *krox-20* in the r3 rhombomere. Neuron formation was suppressed, as evidenced by decreased numbers of primary motor, inter- and sensory neurons in 83% of treated embryos. Similar effects occurred in 70 – 80% of embryos injected unilaterally with 500 pg glyphosate. On their injected side these displayed abolition of *slug* expression, reduced *krox-20* expression in r3 and r5, and decreased numbers of primary motor,

inter- and sensory neurons. The study authors considered the Roundup-exposed and glyphosate injected embryos to be equivalent (although not identical) phenotypes. They did not present any results obtained at the 360 pg/cell dose or the 1/4000 or 1/3000 dilutions.

Effects on the development of the head and dorsal midline: In 85% of 1:5000 Roundup-exposed neurula-stage *Xenopus* embryos, there was reduced expression of *shh* (a gene whose expression is responsible for resolving the brain and retina into two separate hemispheres) and *pax6* (responsible for eye formation). After incubation was prolonged to the tailbud stage, *ca* 90% of treated embryos displayed a decrease in anterior *shh* expression with concomitant microphthalmia, microcephaly, shortening of the anterior-posterior (A-P) axis and delayed migration of neural crest cells into the eyes, genital ridges and pharyngeal arches. Bilateral injection of 360 pg glyphosate also reduced *shh* expression and induced microphthalmia and microcephaly in the majority of treated embryos. In older (tadpole stage) embryos, Roundup exposure caused microphthalmia and a generalised reduction of cranial cartilage structures; most unilaterally-injected embryos showed these effects on the treated side, while bilateral injection caused cyclopia in 3/8 embryos. The study authors did not provide any data obtained at the 500 pg/cell dose or the 1/4000 or 1/3000 dilutions.

Effects on retinoic acid signalling: A highly significant ($p<0.0001$) dose-dependent increase in RA signalling activity occurred in Roundup-exposed *Xenopus* embryos at 4000- and 3000-fold dilutions. The magnitude of the effect was intermediate between the activity seen after addition of exogenous RA at 0.50 and 5.0 µM. However, there was no apparent response to Roundup at 1:5000, which Paganelli et al attribute to a lack of sensitivity of the RAREZ reporter plasmid. Assuming a linear response of the luminescence system, the study authors estimated that the endogenous concentration of RA in *Xenopus* embryos is *ca* 0.2 µM. The RA receptor antagonist Ro 41-5253 blocked the signalling increase mediated by 1:4000 Roundup, and prevented 1:5000 Roundup from inhibiting *shh* activity and causing microcephaly. No data were presented on the influence of Ro on RA signalling or embryo phenotype at other dilutions.

Effects in chicken embryos: Roundup caused concentration-dependent reduction in *pax6* expression and in the size of the optic vesicles, loss of the r3 and r5 domains and decrease in *shh* expression in midline cells, accompanied by microcephaly and loss of *shh* expression in the pre-chordal mesoderm.

Comment

The study authors suggest that the similarity between the phenotypes observed in Roundup-incubated and glyphosate-injected *Xenopus* embryos indicates that neural crest development is disrupted by the active constituent, rather than adjuvants present in the formulated product. Noting (a) similarities between the effects of Roundup and glyphosate with those of excess retinoic acid (RA) concentrations in *Xenopus*, mice and humans; (b) increased RA signalling levels in *Xenopus* embryos in response to Roundup; and (c) the effectiveness of the anti-retinoid Ro in preventing the developmental effects of Roundup in *Xenopus*, Paganelli et al hypothesise that glyphosate is a developmental toxin with a mode of action involving enhancement of RA signalling activity.

Given their belief that (d) glyphosate inhibits aromatase, a cytochrome P450 enzyme; and (e) retinoid activity is regulated by degradation of RA by CYP26, the study

authors further hypothesise that glyphosate increases RA signalling by inhibiting the activity of CYP26 responsible for maintaining normal RA distribution by specific territorial degradation.

Williams et al (2012) have noted that in this study

- The glyphosate solution was not pH-adjusted, and so the effects may be attributable to its acidic nature;
- The injection route of exposure was inappropriate and irrelevant to risk assessment; and
- The observations require further substantiation using appropriate methods before consideration in risk assessment.

Oliviera et al (2007): Adult drakes in breeding season (6/group) were gavaged with Roundup (360 g/L glyphosate, present as 480 g/L glyphosate isopropylamine salt; no other formulation constituents identified; Monsanto do Brasil Ltda, Sao Paulo, Brazil) in water at 5.0 or 100 mg/kg bw/d for 15 days. The study authors did not specify whether the dose levels applied to the active constituent, or the product. A control group received water only.

After the treatment period, the birds were anaesthetised and perfused intracardially with 2.5% gluteraldehyde. Fixed testes and epididymides (5/group) were then weighed, examined morphometrically and examined histochemically to investigate lysosomes and lipids within the epididymal region. Androgen receptor (AR) expression was studied by immunohistochemistry, with confirmation of antibody specificity by SDS-PAGE / Western blotting. Plasma testosterone and oestradiol concentrations were measured in three birds/group by RIA.

Results

Body and organ weights: There was no treatment-related effect on bodyweight. Relative testicular weights were depressed by *ca* 13% at both doses, but the difference from control was not statistically significant. Data on absolute testis weight were not presented.

Hormones: Plasma testosterone levels were reduced by *ca* 90% at both doses ($p<0.05$). A significant ($p<0.05$) *ca* 30% decline in plasma oestradiol occurred at 5.0 mg/kg, but there was no such effect at 100 mg/kg.

Tissue histology: Within the *testis*, Roundup at 5.0 and 100 mg/kg respectively induced slight but statistically significant ($p<0.05$ vs control) reductions in the volumetric proportion of seminiferous tubule epithelium (by 4 and 5%) and interstitial tissue (by 12 and 10%), together with 20 and 22% increases in the lumen volume ($p<0.05$). Spermatogenesis appeared to be normal, however.

Within the *epididymal region*, there were dose-related trends towards reduced volumetric proportions of proximal efferent ductules and connecting duct, together with increases in the proportion of rete testis, distal efferent ductules and connective tissue. These features attained statistical significance ($p<0.05$) at 100 mg/kg but not the low dose.

In the *proximal efferent ductules* of treated birds, qualitative morphological alterations (increased epithelial lipid content and epithelial vacuolisation caused by increased numbers of lysosomes) were found, together with increases of 11 and 7% in epithelial

height and 41 and 105% in lysosomal area at 5.0 and 100 mg/kg respectively (all $p<0.05$ vs control).

The morphology of the *epididymal duct* was also affected. Birds receiving 5 and 100 mg/kg, respectively, displayed significant ($p<0.05$) reductions of 28 and 49% in tubular diameter and increases of 23 and 34% in epithelial height. The epididymal ducts of treated birds presented collapsed and sometimes highly folded lumen, together with an increase in the basement membrane. By contrast, control birds presented wider and regular lumen and a slight basement membrane.

AR expression: At both doses, Roundup caused a major (but unquantified) decrease in AR expression within the Sertoli cell nuclei within the testis. However, the effect did not occur within the epididymal region. The specificity of the AR antibody used was confirmed.

Comment: This study is notable for the low numbers of birds used (especially for hormonal assay); the non-dose related depression of oestradiol concentration; and the lack of an experimental group treated with glyphosate alone, which prevented identification of the formulation constituent(s) causing the reported effects. The observed responses to treatment may have been associated with generalised physiological stress, rather than a specific effect on steroid hormone synthesis.

A4.5 Developmental and reproductive effects of a glyphosate-based herbicide in rats

Dallegrave et al (2003): Groups of 13 – 16 pregnant Wistar rats (90 days old, 200 – 280 g bw, bred at UFRGS, Porto Alegre, Brazil) received Roundup formulation (Lot BS 1096/98, Monsanto Brazil, containing 360 g/L glyphosate and 18% w/v POEA; no other components specified) by oral gavage at 500, 750 or 1000 mg glyphosate/kg bw/d[19] (and *ca* 250, 375 or 500 mg POEA/kg bw/d) (dose volume of 10 mL/kg in distilled water) from GD 6 – 15. Control rats received vehicle alone. Caesarean sections were performed on GD 21, and foetal bodyweight and the numbers of corpora lutea, implantation sites, live and dead foetuses and resorptions were recorded. Foetuses were examined for external malformations and skeletal alterations. However, there was no investigation of their internal organs.

Maternotoxicity: At 1000 mg/kg, there was 50% maternal mortality between GD 7 and 14, but the study authors did not describe any clinical signs or identify the cause of death. No mortality occurred at 0 – 750 mg/kg. There was no treatment-related effect on maternal water intake. The 750 mg/kg group displayed a consistent deficit of *ca* 2.0% in food intake over GD 3 – 21; this is not considered to be treatment-related because it was already present before dosing had commenced. Dams in the 1000 mg/kg group showed a deficit of up to *ca* 4.0% in food intake during the dosing period, maximising on GD 9 but reversing after cessation of treatment. This was accompanied by slight mean bodyweight loss between GD 6 and 9. Subsequent weight gain was similar to the other groups, except for a transient increase over GD 15 – 16. However, there were no statistically significant inter-group differences in food consumption or relative or total gestational bodyweight gain (which was 107, 85, 107 and 102 g at 0, 500, 750 and 1000 mg/kg). Also failing to attain significance was

[19] The doses are believed to have been based on glyphosate acid technical because Dallegrave et al stated that the dosing regimen was chosen by reference to a NOAEL for glyphosate of 1000 mg/kg bw/d for maternal and foetal effects in a developmental toxicity study in rats.

a dose-related trend towards increased relative liver weights (4.57, 4.73, 4.89 and 5.11% in the respective groups). Absolute organ weight data were not presented.

Litter parameters: At Caesarean section, there were 15, 15, 16 and 7 dams and 154, 148, 162 and 75 foetuses available for examination at 0, 500, 750 and 1000 mg/kg. There were no effects on implantation index, resorption rate, mean number of foetuses per dam or mean foetal bodyweight. Gravid uterus weight was not measured. The only remarkable litter parameter was an increase in male:female sex ratio to 1.5:1 at 1000 mg/kg, compared with 1.06:1, 1.01:1 and 0.94:1 in the control, 500 and 750 mg/kg groups. Nevertheless, the finding was not statistically significant (p=0.724, X^2 test) and there is no evidence that it arose from selective mortality of female foetuses *in utero.* Therefore, despite markedly reducing maternal survival at the high dose, the test formulation does not appear to have compromised foetal survival or growth.

Foetal development: There was no treatment-related effect on the incidence of external foetal malformations. However, as shown in the following table, an unequivocal treatment- and dose-related increase in skeletal alterations (all combined) occurred from 500 mg/kg upwards. These mainly involved ossification deficits suggestive of developmental delay but also included abnormalities such as absent ribs and caudal vertebrae, and wavy ribs. The most common individual alterations (incomplete skull ossification and enlarged fontanel) showed a dose-response relationship, but the incidences of some others were significantly ($p<0.05$) elevated at 750 and/or 500 mg/kg but not the high dose. It is not possible to exclude a relationship to treatment in these cases, because (a) no historical control or litter incidence data were presented, (b) the range of doses tested was very narrow, and (c) there were only half as many foetuses at 1000 mg/kg as in the remaining groups (which would reduce the chance of observing abnormalities).

Table 4.6: Percentage incidence of selected skeletal abnormalities in rat foetuses

Region or structure	Abnormality	Glyphosate Dose (mg/kg bw/d)			
		0	**500**	**750**	**1000**
Whole skeleton	All combined	15	33**	42**	57**
Skull, general	Incomplete ossification	10	29*	39*	56*
	Enlarged fontanel	1.9	26*	37*	53*
Interparietal	Bipartite	0.6	19*	4.9*	0.0
Supraoccipital	Bipartite	9.7	20*	1.2	0.0
	Incomplete ossification	3.2	0.0	1.2	13*
Maxilla	Short	0.6	0.7	0.0	1.3
Squama	Incomplete ossification	0.0	0.0	3.1*	2.7*
Caudal vertebrae	Absent	1.9	0.0	7.4*	15*
Ribs	Absent	1.3	2.7	3.1	4.0
	Incomplete ossification	1.9	2.0	5.6	4.0
	Wavy	0.6	2.0	4.9*	0.0
Sternebra	Incomplete ossification	1.9	14.9*	0.0	2.7
	Bipartite	3.9	14.2*	0.6	9.3
Limbs	Incomplete ossification	0.0	0.0	17.9*	1.3
Scapula	Incomplete ossification	0.6	3.4	1.2	4.0
Metacarpal bones	Incomplete ossification	1.3	1.4	0.6	2.7
Femur	Incomplete ossification	3.2	3.4	13*	0.0
Tibia / fibula	Incomplete ossification	2.6	2.7	12*	8.0
Metatarsal bones	Unossified	4.5	1.4	14*	11

Hind phalanges	Unossified	7.1	21*	22*	2.7
Ischium	Incomplete ossification	4.5	2.7	9.3	0.0
Pubis	Incomplete ossification	3.9	2.7	11*	0.0

*p<0.05 **p<0.001 vs control (X^2 test)

Conclusions

The NOEL for maternotoxicity was 750 mg glyphosate/kg bw/d, based on mortality and depression in food intake at the highest dose of 1000 mg/kg bw/d. There was no NOEL for effects on foetal development, due to increased incidences of skeletal abnormalities at and above the lowest dose of 500 mg glyphosate/kg bw/d.

Comment: Williams et al (2012) have criticised reporting deficiencies and anomalies in this paper, and also noted that foetuses were fixed in formalin and trypsin-digested prior to staining and skeletal examination instead of the standard method of alcohol fixation followed by maceration with potassium hydroxide. According to Williams, proteolysis could have digested peptide bonds in the bone matrix, creating areas that appeared to be incompletely ossified. Also deserving comment are the doses of POEA (*ca* 250, 375 and 500 mg/kg bw/d), which far exceed the maternal NOEL and LOEL of 15 and 100 mg/kg bw/d in rats (Holson, 1990). The mid and high doses are also greater than the foetal NOEL of 300 mg/kg bw/d[20].

Dallegrave et al (2007): Groups of 15 Wistar rats (90 days old, 250 – 350 g bw, bred at UFRGS, Porto Alegre, Brazil) received Roundup formulation (Monsanto Brazil, containing 360 g/L glyphosate and 18% w/v POEA; no other components specified) by oral gavage at 50, 150 or 450 mg glyphosate/kg bw/d (dose volume of 10 mL/kg in distilled water) throughout pregnancy and lactation. Control rats received vehicle alone. At delivery, litter size, the number of living and dead pups, birth weight and sex ratio were recorded. Offspring development was monitored by weekly evaluation of bodyweight and daily assessment of developmental landmarks including ear and eye opening, fur emergence, incisor eruption, testis descent, preputial separation and vaginal opening.

From each litter, one rat/sex was killed at puberty (PND 65 for males; first oestrus after PND 65 for females) and a further animal/sex was killed at adulthood (PND 140). Systemic toxicity was determined on the basis of the relative weights of the heart, lungs, liver, spleen, kidneys, adrenals and brain. Reproductive toxicity in males was evaluated as relative weight of the testis, epididymis, seminal vesicle with coagulating gland and prostate, together with spermatid and sperm numbers in the cauda epididymis, sperm morphology, testicular histology and blood testosterone concentration. In females, assessment of reproductive toxicity was limited to the relative weights of the uterus, oviducts and ovaries without histological examination.

Maternotoxicity and litter parameters: There were no maternal deaths or effects on relative bodyweight gain of dams during pregnancy or lactation. There were also no effects on litter parameters at birth, the survival and growth of pups during lactation or attainment of general developmental landmarks.

Female sexual characteristics: Vaginal patency was delayed by two to three days in the treated groups, which was statistically significant (p<0.05, ANOVA-Bonferroni

[20] In Holson (1990), rat dams gavaged with POEA over GD 6 – 15 showed clinical signs and decreased food consumption at 100 mg /kg bw/d, together with mortality and decreased bodyweight gain at 300 mg/kg. However, there were no foetal effects at 300 mg/kg bw/d, the highest dose administered (Williams et al, 2012).

test) vs controls. Latencies of 34.9, 37.6, 36.9 and 36.7 days were recorded at 0, 50, 150 and 450 mg/kg respectively. Nevertheless, the study authors did not consider the finding to be biologically significant because the latency period was "well within" historical control values (these were not cited, however). There was no effect on the weights of the reproductive organs.

Male sexual characteristics: Although there was no effect on attainment of testicular descent, preputial separation was advanced by one day in the 450 mg/kg group (see following table). Despite achieving statistical significance, this was not considered treatment-related because the latency was within the historical control range (not cited). Testis and accessory sex organ weights were not affected by treatment.

However, the numbers and morphology of sperms in the treated groups showed noteworthy displacements from control values, which the study authors considered were biologically significant. As shown in the table below, these comprised:

1. Statistically significant deficits of *ca* 25% in sperm numbers and daily sperm production at adulthood in the 50 and 450 mg/kg groups, although not at 150 mg/kg.
2. A statistically significant doubling in the proportion of abnormal sperm at puberty in the 50 mg/kg, with a non-significant increase at 450 mg/kg but little or no effect at the mid dose. At adulthood, all treated groups displayed a *ca* 1.5-fold elevation in abnormal sperm incidence relative to controls, which did not achieve significance (p=0.066, ANOVA). Furthermore, in the treated groups the proportion of sperm-producing tubules was depressed by *ca* 6 – 11% at puberty and 18 – 29% at adulthood.
3. Dose-related depression in serum testosterone levels, seen at all doses at puberty (significant at 450 mg/kg) but wholly or partially reversing by adulthood.
4. Histological abnormalities within the testis. At puberty, there were growth disorders and degeneration characterised by spermatid vacuolisation and a decrease in elongated spermatids at and above 150 mg/kg. At adulthood there was dose-related, intense tubular degeneration characterised by the absence of tubular lumen (see table).

Based on the above findings, the study authors considered that there was no NOEL for effects on the male reproductive system, and suggested that the test formulation was a probable endocrine disruptor. However, they acknowledged that the study had not elucidated a mechanism of action or identified which component of Roundup was causing the observed effects.

Table 4.7: Reproductive parameters (mean values) in male offspring

Parameter		Maternal glyphosate dose (mg/kg bw/d)			
		0	**50**	**150**	**450**
Age at preputial separation (d)		31.7	31.7	31.5	30.7*
Bodyweight at preputial separation (g)		73.0	68.1	72.2	70.7
Daily sperm production (x 10^6) (n=15)	**PND 140**	20.5	15.3*	19.7	14.7*
Sperm number (x 10^6) (n=15)	**PND 140**	345	251*	369	257*
Abnormal sperm (%) (n=15)	**PND 65**	8.6	16.7*	9.2	11.6
	PND 140	5.4	8.3	8.4	7.7
Tubules with spermatogenesis (%)	**PND 65**	84	77	79	75
(n=5)	**PND 140**	92	74	75	65
Blood testosterone concentration (ng/mL)	**PND 65**	5.2	4.0	3.2	1.5*

Parameter	Maternal glyphosate dose (mg/kg bw/d)			
	0	**50**	**150**	**450**
(n=15) PND 140	3.9	3.4	6.3	3.3
Testis: spermatid vacuolisation & decrease in elongated spermatids (incidence at PND 65)	NS	NS	4/5	4/5
Testis: tubular degeneration (incidence at PND 140)	NS	3/5	4/5	4/5

*p<0.05 vs control, ANOVA – Bonferroni test
NS = Not stated

Comment

Interpretation of the results is hindered by the lack of historical control data, which may have defined effect levels and clarified whether there were genuine treatment-related effects on variables that did not show dose-response relationships. These include daily sperm production, sperm numbers in the cauda epididymis and the proportion of abnormal sperms, which showed the least displacement at 150 mg/kg. The reviewing toxicologist considers that the reporting of histological findings in the testis was insufficiently detailed, as it lacked descriptive detail, severity gradings and control data. The study would also have been strengthened by histological examination of the female reproductive organs.

In an independent assessment of this study, Williams et al (2012) have remarked that:

- In the 450 mg/kg bw/d group, the age at preputial separation was within the physiological range for rats;
- Hastening of puberty would be not be expected, given that the 450 mg/kg group had the lowest mean circulating testosterone level on PND 65;
- The increased percentage of abnormal sperm at 50 mg/kg bw/d may be a random finding, given the lack of effects at higher doses;
- Dallegrave et al's reporting of the testicular histology was deficient and the abnormalities described may be a tissue processing artefact, rather than an effect of treatment;
- Testicular abnormalities have not been reported in offspring in reproduction studies with glyphosate, all of which involved much greater glyphosate exposures.

Conclusions

In the absence of any apparent maternotoxicity, the NOEL in dams was 450 mg glyphosate/kg bw/d. The study did not demonstrate treatment-related effects in female offspring at up to and including the highest dose of 450 mg glyphosate/kg bw/d. The study is considered to be insufficiently reliable enough to demonstrate whether there were treatment-related effects in male offspring.

Romano et al (2010): The test compound in this study was Roundup Transorb (Monsanto Co, St Louis, MO, USA / Monsanto of Brazil Ltda, Sao Paulo, Brazil; containing glyphosate isopropylamine salt 648 g/L equivalent to 480 g/L glyphosate, with 594 g/L of unidentified "inert ingredients"). The formulation was diluted in water to yield a dosage volume of 0.25 mL/100 g bw, and administered PO by gavage to newly weaned male Wistar rats (16 – 18/group) from PND 23 – 53 at 5.0, 50 or 250 mg/kg bw/d. A control group received vehicle alone. The study authors described their test compound as "glyphosate-Roundup Transorb", so it is ambiguous whether they were referring to the active or product. However, given that their choice of doses

was based on a NOEL of 50 mg/kg bw/d *for glyphosate* in another study, it will be assumed that the doses are equivalent to 5.0, 50 or 250 mg active/kg bw/d.

Pups were weighed daily throughout the treatment period and examined to determine the age of puberty (balano-preputial separation) from PND 33 onwards. At termination on PND 53, serum was collected via cardiac puncture for measurement of testosterone, oestradiol and corticosterone concentrations. The testes and adrenal glands were weighed and processed for histological examination. Quantitative morphometry of the seminiferous tubules was then performed to examine for disturbance of spermatogenesis. However, spermatozoa were not examined or quantified.

There were no treatment-related effects on bodyweight throughout the dosing period, including puberty ($p>0.05$). However, attainment of puberty was delayed by *ca* 1.0 and 1.5 days at 50 and 250 mg/kg respectively ($p<0.01$ and <0.001 vs control). As shown in the following table, relative testicular weight increased dose-relatedly by up to *ca* 9%, attaining statistical significance at 250 mg/kg. At this same dose, there was also a significant, 29% increase in relative adrenal weight. Absolute organ and terminal body weights were not provided.

Serum testosterone concentrations were depressed by 30%, 45% and 50% at 5, 50 and 250 mg/kg bw/d. Histologically, this finding was correlated with decreased numbers of germ cells, seen as a dose-related reduction in the height of the seminiferous tubule germinal epithelium and increased diameter of the lumen. Displacements from control were statistically significant at all doses (see table below). By contrast, serum corticosterone and oestradiol concentrations, adrenal morphology and the overall diameter of the seminiferous tubules were not affected.

Table 4.8: Treatment-related effects in rats

Variable examined	**Dose (mg/kg bw/d)**			
	0	**5**	**50**	**250**
Mean testicular weight (mg/100 g bw)	531	539	553	580*
Mean adrenal weight (mg/100 g/bw)	11.3	12.8	12.3	14.6*
Serum testosterone concentration (ng/dL)	155	109**	85***	77***
Seminiferous tubule: Germinal epithelium height (µM)	86	72**	69**	65**
Lumen diameter (µM)	94	117**	114**	130**

*p<0.05 **p<0.001 vs control

Comment

The study was performed before the publication of the EPA OPPTS Test Guideline 890.1500 for investigating pubertal development in male rats[21], but the treatment period (PND 23 – 53) was in line with the Guideline-specified protocol. However, the study was not Guideline-compliant in numerous other aspects of its design and reporting. In particular, there were no bodyweight data except for the mean values at preputial separation. It is therefore impossible to verify independently that inter-group variation in bodyweight and/or bodyweight gain did not influence the timing of puberty, or other parameters. It is also unclear whether the experimenters ensured that litter mates were not allocated to the same experimental group, as required by the Guideline.

[21] Endocrine disruptor screening program Test Guideline OPPTS 890.1500: Pubertal development and thyroid function in intact juvenile/peripubertal male rats. EPA 740-C-09-004, October 2009.

Furthermore, Williams et al (2012) have questioned the reliability of the preputial separation data and morphometric analysis of testis pathology, claiming that the latter was affected by tissue fixation artefacts and confounded by variation in the maturity of seminiferous tubules.

Conclusions

The study is considered to be insufficiently reliable to demonstrate whether there were treatment-related effects in the experimental model used.

Romano et al (2012): Roundup Transorb (see Romano et al, 2010) was administered to pregnant Wistar rats PO by gavage at a dose equivalent to 50 mg glyphosate/kg bw/d from GD 18 to PND 5. The test compound was diluted in water and given at a dose volume of 2.5 mL/kg bw. A control group (size unspecified) received water alone. On PND 4, litters were culled to eight pups/dam and then maintained until weaning at PND 21. Their bodyweight was recorded on PND 21, 30, 40 and 60. Throughout the post-weaning period, male offspring were evaluated for preputial separation, indicating attainment of puberty.

Preference test: On PND 60, subgroups of five male offspring from treated and control dams alternately underwent a sexual preference test, in which they were placed individually on a circular stage with one male and one female stimulus rat, housed in separate cages on opposite sides of the apparatus. The stage was divided into neutral, male and female areas, with the male and female areas divided into seven zones. Stimulus males were gonad-intact and sexually mature, whereas the stimulus females had been ovariectomised and brought into oestrus with oestradiol (50 µg/kg SC at -54 hours) and progesterone (2.0 mg/kg SC at -6 hours). After a five-minute adaptation interval, there was a 20-minute observation period during which the test males' stay times in the two zones nearest the stimulus males and females were recorded. Preference scores were calculated by subtracting the total time spent in the male zones from the time spent in the female zones. Following the preference trial, the test males were not subjected to other experiments.

Mating behaviour: Four males from treated and control dams were scored for the numbers of mounts, attempted mounts, intromissions and ejaculations over a 40-minute interval when placed individually with an oestrus-induced female rat. The time to first ejaculation and ejaculatory intervals were also recorded.

Reproductive tract: On PND 60, the testes, epididymides (caput, corpus and cauda) and seminal vesicles were weighed, sperm counts were performed, and the histology and morphometry of the seminiferous epithelium examined by light microscopy.

Other parameters: Serum concentrations of testosterone and oestradiol were measured by RIA, and FSH and LH concentrations were measured using chemiluminescence immunoassay. Pituitary mRNA and protein levels of β-LH, β-FSH and GH were analysed by real-time PCR (for mRNA) and SDS-PAGE followed by nitrocellulose membrane hybridisation / antibody detection (for proteins).

Results

Maternal observations: No information was provided on the survival, appearance, behaviour or bodyweight of dams during or after the dosing period. It is therefore unknown whether any maternotoxicity occurred.

Growth of offspring and attainment of puberty: The study authors did not present data on bodyweight or pituitary GH levels, but claimed that neither was affected by treatment. In males from Roundup-treated dams, however, age and bodyweight at preputial separation were decreased by about two days (mean of 45 vs 47 days; $p<0.05$) and 30 g (mean of 215 vs 245 g; $p<0.05$).

Preference test: As shown in the table below, male rats from the Roundup-treated dams spent significantly longer in close proximity to female stimulus animals, and had a significantly higher preference score.

Table 4.9: Results of sexual preference test

Parameter	Time (sec)	
	Control	Roundup
Mean total time in male area	431	312
Mean total time in female area	502	625**
Mean partner preference score	71	313**

**p<0.01 vs control (Student's t-test) N = 5/group

Mating behaviour: Based on the interquartile ranges, the study authors claimed a significant increase in mounting, intromission and ejaculatory latency for males from Roundup-treated dams. The remaining parameters did not differ significantly between the groups.

Table 4.10: Results of mating behaviour evaluation

Parameter	Time (min)	
	Control	Roundup
Latency for the first mount^	0.6 – 1.0	5.2 – 7.0*
Latency for the first intromission^	0.6 – 1.0	5.2 – 7.0*
Latency for the first ejaculation^	1.0 – 1.7	5.5 – 7.0*

^Data are interquartile range (25 – 75%) N = 4/group
*p<0.05 vs control (Mann-Whitney U-test)

Reproductive tract: There were no effects on the relative weights of the testes or undrained seminal vesicles on PND 60. However, the relative weight of drained seminal vesicles was 10% higher in the Roundup group, suggesting a lower fluid volume. The corpus and cauda segments of the epididymis were slightly but significantly heavier in the Roundup group than controls. Compared with controls, sperm production was approximately twice as high in rats from Roundup-treated dams (see table below), and sperm reserves in the caput + corpus were increased by 50%. Sperm transit time through the cauda was reduced by *ca* 1/3rd. In the absence of any significant difference in the diameter of the seminiferous tubules, the Roundup group displayed a minor but statistically significant increase in epithelial height and decrease in luminal diameter.

Table 4.11: Findings in the reproductive system of male rats

Parameter		Control	Roundup
Total sperm production (X 10^6/testis)		52	99*
Total sperm production (X 10^6/g testis)		35	71*
Daily sperm production (X 10^6/testis)		8.5	16*
Daily sperm production (X 10^6/g testis)		5.7	12*
Sperm reserve, caput + corpus (X 10^6)		14	21*
Sperm transit time through cauda (days)		6.3	4.0*
Seminiferous	Tubular diameter (µm)	467	451

epithelium	Epithelial height (µm)	92	98*
	Luminal diameter (µm)	257	239*
Seminal vesicle	Weight, undrained (mg/100 g bw)	160	155
	Weight, drained (mg/100 g bw)	100	110*
Epididymis	Weight, corpus (mg/100 g bw)	10	13*
	Weight, cauda (mg/100 g bw)	36	43*

*p<0.05 vs control (Student's t-test) N = 8/group

Other parameters: In males from Roundup-treated dams, serum testosterone and oestradiol concentrations were approximately twice as high as in controls (see following table). Pituitary LH and FSH mRNA levels were very slightly but significantly increased by Roundup treatment. However, although there were concomitant increases of *ca* 70% in pituitary LH protein and serum LH levels, there was no treatment-related effect on FSH levels in the pituitary or serum.

Table 4.12: Hormonal levels in the serum and pituitary

Parameter	Control	Roundup
Serum testosterone conc. (ng/dL) (N = 12)	60	140**
Serum oestradiol conc. (pg/mL) (N = 12)	1.4	2.8**
Pituitary LH mRNA content (AU) (N = 8)	1.00	1.02*
Pituitary LH protein content (AU) (N = 8)	1.1	1.9**
Serum LH conc. (pg/mL) (N = 8)	70	120*
Pituitary FSH mRNA content (AU) (N = 8)	1.00	1.02*

*p<0.05 **p<0.01 vs control (Student's t-test)

Conclusions

The study authors interpreted their findings as indicating that maternal glyphosate exposure during the perinatal period caused hypersecretion of androgens in the male offspring, combined with hastening of puberty, increased gonadal activity and sperm production, greater predilection for the company of female rats and increased libido (the latter notwithstanding the statistically significant increase in the *delay* before copulation). The authors acknowledged that their findings contradicted the depression in serum testosterone level and sperm production and reduced height of the seminiferous epithelium observed by Romano et al (2010) and Dallegrave et al (2007) (see above). However, they attributed the discrepancies in experimental outcome to differences in timing of exposure, which occurred over GD18 to PND 5 in this study but extended through gestation to the end of lactation (PND 21) in Dallegrave et al (2007) and was from PND 23 to 53 in Romano et al (2010).

Comment

Numerous aspects of the design of this study and its findings deserve comment.

- Although the study authors attribute their findings to glyphosate, dams were treated with a commercial formulation containing 594 g/L of unidentified "inert ingredients". Offspring may consequently have been exposed to these formulation adjuvants *in utero* or via maternal milk and it is possible that they influenced the experimental outcome, either directly or by interaction with the active constituent. The study did not control for the presence of adjuvants.

- Since no observations on the dams were presented, it is unknown whether maternotoxicity (including effects on maternal nursing behaviour) occurred. The study authors appear not to have considered the possibility that at least some experimental findings in offspring arose from effects on the mothers.
- The study authors did not state when serum and pituitary hormone parameters were measured.
- Rats that underwent the sexual preference test were not used for other experiments, but no information was provided on whether those undergoing evaluation of mating behaviour were also subjected to hormone assays and/or reproductive tract histology. Either of these end-points could have been affected by sexual activity.
- In a mating evaluation, one would expect relatively large variation in the behaviour of individual males, especially given that the outcome would be partially dependent on the behaviour of the partnering females. However, the group sizes were very small (N = 4). No group mean values were provided; data were reported as interquartile ranges (25 – 75%). In a set of four observations, there would be only one data point per quartile. Therefore, because they were based on so few observations, it is open to question whether the apparent increases in mounting, intromission and ejaculation latency time were biologically significant, even though statistical significance was attained.
- In an extensive critique of this study, DeSesso and Williams (2012) point out that surfactants inhibit the enzyme aromatase, which is responsible for conversion of circulating testosterone to oestradiol. Surfactants, if present in the test formulation, could therefore have disrupted the expression and function of endocrine hormones in the dams and/or offspring.
- The study authors did not identify from which dams/litters the evaluated males had originated. DeSesso and Williams question whether the study was controlled for litter effects, adding that because litter mates are more similar to each other than offspring from separate litters, the observed inter-group differences may be due to animals being derived from the same limited number of litters rather than a true effect of treatment.
- DeSesso and Williams note the lack of evidence that precautions were taken to prevent the sexual preference test being confounded by environmental cues including auditory and visual stimuli, odours and pheromones.
- These authors also observe major differences in the control values for attainment of puberty, serum testosterone and oestradiol concentrations and seminiferous tubule morphometry when comparing Romano's 2010 and 2012 studies. The magnitude of these differences exceeds the size of the treatment-related changes within each study.
- Romano et al (2010; see above) report that preputial separation in controls occurred at means of *ca* 37 days and 146 g bw, compared with 47 days and 245 g bw in their 2012 paper. Mean values from test animals in 2012 (45 days and 215 g bw) lie within this range, and also within the range specified for control Wistar rats in US EPA TG 890.1500 (40 – 46 days and 177 – 241

g bw)[22]. By contrast, mean values from controls in both studies lie *outside* the EPA's Guideline ranges (DeSesso and Williams, 2012).

A4.6 Reproductive effects of glyphosate in male rabbits

Yousef et al (1995): Following an initial six-week observation period, male NZW rabbits (4/group, 8 months old, mean initial bodyweight 2863 g) were given oral doses of glyphosate (from Monsanto, USA) in gelatin capsules for six weeks at 1% or 10% of the LD_{50}. The study authors did not explicitly identify the dosing interval or specify the doses in terms of mg/kg bw. The rabbits were then held without treatment for a further six weeks to study reversibility of effects. The animals were weighed weekly in the morning before access to feed and water. Semen was collected weekly throughout the study using a teaser doe and artificial vagina, with ejaculate volume being recorded after removal of the gel mass. Semen osmolality, fructose concentration and methylene blue reduction time was measured together with sperm concentration and assessment of live, dead and abnormal spermatozoa.

Results

No information was provided on survival of the test and control animals, but for reasons unknown, one rabbit was removed from the control, low and high dose groups during the recovery period. Other than stating that most treated animals showed indications of reduced libido (especially at the high dose), the study authors did not comment on clinical signs. Control mean bodyweight increased by *ca* 2 and 3% respectively during the treatment and recovery periods. By contrast, the low and high dose groups lost weight during treatment, with weight loss being greatest at the low dose (see following table). During recovery, there was little bodyweight change at the low dose, whereas the high dose group showed a bodyweight gain of *ca* 8%.

Table 4.13: Bodyweight (g) of rabbits over the experimental period

Time period	N	Control	GLY 1/100th LD50	GLY 1/10th LD50
Pre-treatment	4	2944	2979	3173*
Treatment	4	3008	2811*	3125
Bw change over treatment^		+64	-168	-48
Recovery	3	3108	2816*	3368*
Bw change over recovery^		+100	+5	+243

^Calculated by evaluator *p<0.05 vs control

Treated rabbits displayed a partially reversible, non-dose related *ca* 25% reduction in semen volume during the treatment period, accompanied by a reversible 3-fold increase in the percentage of dead sperm and partially reversible, dose-related depression in initial semen fructose concentration and prolongation in methylene blue reduction time (see next table). According to the study authors, fructose formation by the accessory glands is dependent on testosterone production by the testis; hence, decreased semen fructose suggested a corresponding decline in testosterone secretion. Yousef et al considered that prolonged MBRT could reflect deficits in nutrition status, viability, activity and oxygen consumption by sperm from treated rabbits.

Interpretation of sperm concentration data is confounded by a progressive doubling in the control group between the pre-treatment and recovery periods. By contrast, sperm concentration in the high dose group remained constant during treatment, but declined

[22] Endocrine disruptor screening program Test Guideline OPPTS 890.1500: Pubertal development and thyroid function in intact juvenile/peripubertal male rats. EPA 740-C-09-004, October 2009.

by *ca* 8% at the low dose. In both treated groups, sperm concentration then rose by *ca* 1.8-fold during recovery. The percentage of abnormal sperm became significantly ($p<0.05$) elevated in the treated groups during the dosing and recovery periods, but again, interpretation is confounded by a two-fold increase in abnormal sperm occurring in controls (mainly) during recovery. The most common types of abnormalities were claimed to be coiled or double tail and tapering or small head. Semen osmolality in treated rabbits changed little during the dosing period, but became statistically significantly lower than in controls. This was caused by increased osmolality in the control group, rather than any effect of treatment.

Table 4.14: Semen characteristics of rabbits. Values are overall means over 6 weeks before, during and after treatment. [n = 4 before and during treatment and n = 3 during recovery]

Parameter	Time period	Control	GLY 1/100th LD50	GLY 1/10th LD50
Semen volume (mL)	P	0.88	0.83	0.88
	T	0.83	0.60*	0.62*
	R	0.82	0.68*	0.73*
Sperm conc. (X 10^6/mL)	P	264	265	262
	T	413	242*	262*
	R	596	473*	467*
Abnormal sperm (%)	P	9.4	9.7	10.3
	T	12.5	21.9*	22.6*
	R	20.4	25.7*	24.1*
Dead sperm (%)	P	6.6	6.4	6.5
	T	8.9	19.5*	21.4*
	R	4.1	6.2*	7.5*
Methylene Blue Reduction Time (min)	P	5.07	5.22	5.07
	T	3.53	6.54*	7.26*
	R	3.48	5.0*	5.29*
Initial fructose conc. (mg/100 mL)	P	337	324	336
	T	359	281*	267*
	R	312	298	297
Semen osmolality (units unstated)	P	248	255	253
	T	283	252*	261*
	R	278	284	278

P = Pre-treatment T = Treatment period R = Recovery *$p<0.05$ vs control

Comment

The study has significant shortcomings in its design and reporting of the experimental methods and results. The dosing interval and administered doses of glyphosate are unknown, and the authors did not explain how the reference LD50 value was derived. Although glyphosate treatment does appear to have caused decreases in ejaculate volume, sperm viability and sperm activity (the latter possibly resulting from depression in semen fructose concentration), the causal mechanism is unidentifiable. It is uncertain whether the results were obtained in the presence of systemic toxicity, as bodyweight loss during treatment was three-fold more severe at the low dose than the high dose. Any effects on semen osmolality, sperm concentration and sperm morphology are uninterpretable due to major, unexplained variation over time within the control group. The small size of the experimental and control groups may have contributed to the experimental outcome.

Furthermore, Williams et al (2000) have observed that:

- The rabbits used in this study were small for their age, bringing into question their health status and reproductive maturity;
- The proper method of semen collection was not used. Multiple ejaculates were not pooled to decrease the inter- and intra animal variability in sperm number and concentration;
- Sperm concentration data from treated and control rabbits were within the normal range in mature NZW rabbits; and
- It is unclear whether control animals were subjected to sham handling and dosing procedures, raising questions of indirect non-treatment related effects given the sensitivity of rabbits to stress.

Based on these deficiencies, the data from this study cannot be used to support any meaningful conclusions.

A4.7 Dermal carcinogenicity of a glyphosate-based herbicide in mice

George et al (2010): Carcinogenicity bioassay: The biological activity of Roundup Original* (a commercial formulation containing 360 g/L glyphosate acid equivalent as the isopropylamine salt, with 15% POEA; no other components identified; manufactured by Monsanto Co., St Louis, MO USA) was tested in a mouse two-stage initiation / promotion model of dermal carcinogenesis. Eight groups of 20 male Swiss mice (12 – 15 g initial bodyweight; from the Indian Institute of Toxicology Research breeding colony) were treated according to the following scheme:

Group	Treatment protocol
1	Nil
2	Roundup*, 25 mg/kg bw, 3X/wk for 32 wk
3	DMBA, 52 µg/mouse, single dose then TPA, 5µg/mouse, 3X/wk for 32 wk
4	Roundup, 25 mg/kg bw, single dose then TPA, 5µg/mouse, 3X/wk for 32 wk
5	Roundup, 25 mg/kg bw, 3X/week for 3 wk then TPA, 5µg/mouse, 3X/wk for 32 wk
6	DMBA, 52 µg/mouse, single dose
7	TPA, 5µg/mouse, 3X/wk for 32 wk
8	DMBA 52 µg/mouse single dose then Roundup, 25 mg/kg bw, 3X/wk for 32 wk

*The study authors include Roundup Original, but not pure glyphosate, in the list of experimental materials. They state that mice were treated with "glyphosate 25 mg/kg bw". It is unclear whether they mean "Roundup at 25 mg/kg bw" [in which case the dose of glyphosate would be 9 mg/kg bw], or "sufficient Roundup to deliver a glyphosate dose of 25 mg/kg bw". I have assumed the former, and use the name "Roundup" to preserve the distinction between the active constituent and commercial formulations bearing this trade name.

DMBA = 7,12-dimethyl benz[a]anthracene
TPA = 12-*O*-tetradecanoyl-phorbol13-acetate

The initiator (DMBA), promoter (TPA) and Roundup formulation were applied to the clipped intact dorsal skin. According to the study authors, "Vehicle for glyphosate, DMBA and TPA were 50% ethanol and acetone, respectively" [sic]. Animals were weighed and examined weekly for the presence of tumours. All mice were sacrificed after 32 weeks of treatment.

Proteomic study: Groups of four male mice (which had not been used for the carcinogenicity bioassay) were treated dermally once with Roundup (50 mg/kg bw), DMBA (104 µg/mouse) or TPA (10 µg/mouse). The study authors did not state whether vehicles were used. A further four untreated animals served as controls. At 24 hours post-treatment, mice were sacrificed and skin samples from the treatment sites were excised, homogenised, lysed, sonicated, centrifuged and pooled for each respective group. Proteins in the supernatants were then separated by two-dimensional gel electrophoresis (2-DE), with the first dimension on immobilised pH gradient strips (pH 3 – 10) and the second dimension on polyacrylamide gel. Analysis was performed in triplicate. Protein expression levels were measured using PDQuest software, and protein spots that varied > two-fold from control were identified by matrix-assisted laser desorption / ionisation time-of-flight and liquid chromatography / mass spectrometry. The identity of some proteins was confirmed by immunoblotting.

Results

Carcinogenicity bioassay: All mice survived until scheduled termination. All 20 positive controls (Group 3 animals treated with DMBA and TPA) developed skin tumours (squamous cell papillomas), with some animals bearing multiple tumours (see Table). Skin tumours also developed on eight / 20 mice receiving DMBA and Roundup. Compared with TPA, Roundup induced the formation of fewer (by 85%), smaller tumours, which first appeared after a more prolonged (by 2.5-fold) treatment period. There was no comment on whether the tumours were preceded or accompanied by dermal irritation or other visible abnormalities at test sites. No dermal tumours were observed on mice from Groups 2, 4, 5, 6 or 7. Therefore, Roundup behaved as a tumour promoter in this experimental model, but not as an initiator or complete carcinogen.

Table 4.15: Tumour formation on the skin of treated and control mice

Group	Treatment	Incidence of TBM^	Days until 1st tumour	% of TBM^	Total no. of tumours	Mean no. tumours / mouse	Mean tumour vol (mm^3)/TBM^
1	None	0 / 20	NA	0	0	0	NA
3	DMBA + TPA	20 / 20*	52	100	156	7.8	96.4
8	DMBA + Roundup	8 / 20*	130	40	23	2.8	26.2

^TBM = Tumour bearing mice NA = Not applicable
*$p<0.05$ vs untreated controls (ANOVA)

Proteomic study: As revealed by 2-DE, single doses of Roundup, TPA or DMBA caused a >two-fold increase or decrease in the expression of 22 proteins. Expression levels of 13 of these proteins were said to be affected similarly by Roundup and TPA, but quantitative data were provided for only nine of these (see Table). DMBA up-regulated four of this sub-set of proteins similarly to Roundup and TPA, but had little or no effect on the expression of superoxide dismutase 1 (see Table). Use of the Western blotting technique confirmed that Roundup and TPA both up-regulated calcyclin and calgranulin-B by *ca* three- and four-fold, respectively, and down-regulated superoxide dismutase by about ten-fold (all $p<0.05$ vs control). Western blotting also demonstrated that DMBA did not influence the expression levels of these particular proteins.

Table 4.16: Expression levels of skin proteins in mice

Protein	Difference from untreated control		
	Roundup	TPA	DMBA
Translation elongation factor eEF1A1	+2.80	+2.79	+2.67
Carbonic anhydrase III	+1.62	+3.72	+2.81
Calcyclin	+2.48*	+2.20*	ND
Annexin II	+2.38	+1.72	ND
Fab fragment of anti-VEGF antibody	+3.64	+3.69	+5.80
Peroxyredoxin-2	+2.73	+2.74	+2.20
Superoxide dismutase 1	-4.97*	-4.56*	+1.16
Stefin A3	+2.29	+1.49	ND
Calgranulin-B (two "spots" corresponding to the same protein)	+9.52*	+7.61*	ND
	+9.34*	+7.43*	ND

*p<0.05 vs control ND = Not detected using 2-DE

Conclusions

The study authors concluded that glyphosate is a tumour promoter in mouse skin which, based on the similarities in protein expression profile, has a mechanism similar to TPA. They noted that several of the proteins whose activity levels were up-regulated have biologically significant roles in cell proliferation[23], while suggesting that down-regulation of superoxide dismutase (which protects cells against reactive oxygen intermediates) could potentiate tumour formation.

Comment

In the reviewing toxicologist's opinion, the carcinogenicity bioassay was not controlled adequately. The test compound was a mixture containing glyphosate, POEA and possibly other adjuvants, and yet no animals were treated with glyphosate, POEA or other formulation constituents in isolation. Therefore, the study could not identify which formulation constituent(s) promoted the growth of tumours in Group 8, show that tumour promotion was caused by any single chemical, or exclude the possibility that promoting activity arose from an interaction between two or more formulation components.

The study reporting would have been strengthened if the authors had commented on whether Roundup Original caused irritation or other effects on the skin where it was applied. This would have been of particular interest because POEA is a severe dermal irritant (Birch, 1977), consistent with the properties of surfactants in general, which interact with and solubilise lipid components of the skin and mucous membranes (Williams et al, 2000). The presence or absence of dermal responses such as inflammation, de-fatting, cell proliferation, scabbing, scarring or fissuring could have assisted in identifying the mechanism(s) by which Roundup promoted the formation of tumours. In this context, it is notable that POEA is not a mutagen (Stegeman and Li, 1990; Williams et al, 2000).

[23] According to the study authors, **Translation eF1A1** is responsible for binding aminoacyl-tRNA to ribosomes during polypeptide synthesis and its increased expression is directly proportional to cellular proliferation, oncogenic transformation, apoptosis and delayed cell senescence; **Carbonic anhydrase III** is involved in the cellular response to oxidative stress; **VEGF** is involved in angiogenesis (a pre-requisite for neoplastic growth); **Stefin A3** plays a role in skin growth and its induction by TPA leads to keratinocyte differentiation and proliferation; **Annexin II** is up-regulated in several human cancers; **Peroxyredoxin-2** is over-expressed in some cancers; and **Calcyclin** and **Calgranulin-B** are implicated in cell cycle progression, differentiation, cancer development and metastasis.

Another point deserving comment is that the proteomic analysis was carried out only at 24 hours after a single application of DMBA, Roundup or TPA. This is fundamentally different from the carcinogenicity bioassay, which involved repeated dosing over 32 weeks after DMBA application. No analysis was performed on skin from test sites during or at the end of the treatment period, on the tumours themselves, or on skin that had been treated with both DMBA *and* Roundup or TPA. The study did not demonstrate that the changes in protein expression observed after one dose of DMBA, TPA or Roundup were sustained throughout the experimental period, were a toxicological endpoint rather than homeostatic regulation, or were causally associated with the eventual development of tumours. Furthermore, the study could not detect changes in the expression of additional proteins after repeated treatment. Consequently, it is uncertain that the promoting activity that the study authors attributed to glyphosate is mechanistically similar to that of TPA.

Overall, this study has shown that Roundup Original is a tumour promoter on mouse skin, its activity is weaker than that of the positive control, TPA, and is dependent on prior induction with the initiator DMBA. The causative agent(s) and its (or their) mode of action remain unidentified. Given that Roundup Original is not a complete carcinogen, it is unlikely to pose a carcinogenic hazard for persons exposed dermally.

A4.8 Epidemiological Study

Eriksson et al (2008): This was a population-based case-control study of exposure to pesticides as a risk factor for non-Hodgkin lymphoma (NHL), consisting of 910 cases and 1016 controls. The subjects were men and women aged 18 – 74 years living in Sweden, diagnosed with NHL between December 1999 and April 2002. All cases were diagnosed and classified histopathalogically according to WHO criteria. Controls were selected from the national population registry.

Exposure assessment was performed by a questionnaire which included work history, exposure to pesticides, organic solvents and several other (unidentified) chemicals. For dose-response analysis of pesticides, information was collected on the number of years, days per year and hours per day of exposure. The questionnaire also included smoking habit, medications, leisure activities and residential proximity to industrial installations, but data on these variables were not included in the review. Supplementary phone interviews were conducted if necessary. All exposures of less than a full day, or occurring during the same calendar year as the diagnosis or one year prior, were disregarded.

Data were analysed by unconditional logistic regression (univariate and multivariate) adjusted for age, sex and year of diagnosis or enrolment. In the univariate analysis, different pesticides were analysed separately, and the unexposed category consisted of subjects who were not exposed to any of the included pesticides. All controls were used in the analyses of NHL subgroups. In the dose-response calculations made for agents with at least 20 exposed subjects, the median number of days of exposure among controls was used as a cut-off. Latency period calculations and multivariate analyses (performed because most pesticide exposures involved more than one chemical) included agents with statistically significantly increased ORs, or with an OR >1.50 and at least 10 exposed subjects.

Results

Univariate analysis adjusted for age, sex and year of diagnosis or enrolment revealed a significant association between NHL and exposure to glyphosate (29 cases and 18 controls; OR = 2.02; 95% CI = 1.10 – 3.71), exposure to glyphosate with a latency of >10 years before diagnosis (unstated no. of cases and controls; OR = 2.26; 95% CI = 1.16 – 4.40) and exposure to glyphosate for >10 days (17 cases and 9 controls; OR = 2.36; 95% CI = 1.04 – 5.37). However, NHL was not associated with exposure to glyphosate with a latency of 1 – 10 years before diagnosis (unstated no. of cases and controls; OR = 1.11; 95% CI = 0.24 – 5.08) or exposure to glyphosate for <10 days (12 cases and 9 controls; OR = 1.69; 95% CI = 0.70 – 4.07). Multivariate analysis adjusting for exposure to other chemicals yielded a low and statistically non-significant risk estimate for glyphosate (OR = 1.51; 95% CI = 0.77 – 2.94).

When the different sub-types of NHL were analysed separately, exposure to glyphosate was associated with a significantly enhanced risk of *small lymphocytic lymphoma / chronic lymphocytic leukaemia* (195 cases; OR = 3.35; 95% CI = 1.42 – 7.89) and *unspecified NHL* (38 cases; OR = 5.63; 95% CI = 1.44 – 22.0). Odds ratios for other types of lymphoma were not statistically significant.

Comment

The same research group have published a previous (Hardell et al, 2002) epidemiology study on the association between pesticide exposure and NHL, in which univariate analysis found a significant association with glyphosate (OR = 3.04; 95% CI = 1.08 – 8.52) based on 8 cases and 8 controls. Noting the small sample size and the broad CI, the Australian DoHA (2005) concluded that strength of association was questionable, and it was equivocal whether glyphosate was indeed a risk factor for NHL.

The current follow-up study improves on its predecessor in several respects, as it was based on a larger population (910 vs 515 cases), had larger sample sizes, included both men and women, and collected exposure data from living individuals only.[24] The follow-up would therefore have increased statistical power and diminished recall bias. Compared with the 2002 study, the risk estimate was lower (OR of 2.02 vs 3.04) but the association between glyphosate exposure and NHL was strengthened, as evidenced by the narrower 95% CI (1.10 – 3.71 vs 1.08 – 8.52). However, the 2008 and 2002 studies failed to demonstrate associations by multivariate analysis, which yielded ORs of only 1.51 and 1.85, with 95% CIs that had lower bounds of less than 1.0 (0.77 – 2.94 and 0.55 – 6.20). Eriksson et al (2008) noted that many glyphosate users had previously been exposed to MCPA, and suggested this as an explanation for why neither chemical showed a significant OR when subjected to multivariate analysis.

At best, the association between glyphosate and NHL in this study is equivocal, remains potentially confounded by established risk factors such as immunosuppression and Epstein-Barr virus (as noted previously by the Australian DoHA, 2005), and could also have been affected by recall, exposure measurement and information bias if NHL cases or their interviewers believed that their disease may be related to pesticides (Mink, unpublished). Mink has also observed that, by excluding 88 potential cases who died before they could be interviewed, the study

[24] In Hardell et al (2002), the next-of-kin provided information for deceased individuals.

population did not represent those cases with more aggressive disease. Furthermore, the dose-response analysis may have been confounded by exposure to other herbicides, and was based on unequal cut-off points for glyphosate (≤10 days or >10 days) and "other" herbicides (≤32 days or >32 days) (Mink, unpublished).

APPENDIX 5: PHARMACOKINETICS OF GLYPHOSATE AND ITS METABOLITE AMPA IN RATS

Anadon et al (2009): Laboratory grade glyphosate (Sigma Chemical Co, St Louis, MO, USA; purity 95%) was administered to male Wistar rats (Charles River Inc, Margate, Kent, UK; bw 200 – 210 g) at 100 mg/kg bw IV (in 0.1 mL glycerol formal) or 400 mg/kg PO (gavage to fasted animals in 0.5 mL corn oil). Groups of 8 rats were killed and exsanguinated at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h post-dosing, and the concentrations of glyphosate and aminomethyl phosphonic acid (AMPA) were measured in plasma by HPLC with fluorescence detection.

Glyphosate, IV administration: Following an initial peak concentration (C_{max}) of 166 µg/mL plasma pharmacokinetics were biphasic, consistent with a two-compartment open model, with rapid distribution and gradual elimination. The volume of distribution at steady state was 2.99 L/kg, suggesting extensive diffusion into the tissues. Clearance was 0.995 L/h/kg. The elimination half-life from plasma was 9.99 h and the area under the concentration vs time curve (AUC) was 100 mg.h/L.

Glyphosate, PO administration: Absorption from the GIT was gradual, with a C_{max} of 4.62 µg/mL occurring in plasma at 5.2 h. Oral bioavailability was poor (23.2%). Clearance was the same as following IV administration and the AUC was similar (at 93.3 mg.h/L), but the elimination half-life from plasma was appreciably more prolonged (14.4 h).

AMPA: The metabolite first appeared in plasma within 0.25 h of PO dosing, and had similar pharmacokinetic behaviour to glyphosate. The C_{max} (0.42 µg/mL) occurred at 2.4 h. An AUC of 6.1 mg.h/L was attained, *ca* 6.5% of glyphosate's AUC in plasma. The elimination half-life of 15.1 h was similar to that of the parent chemical after PO administration.

U.S. Environmental Protection Agency
Office of Pesticide Programs (OPP)

Risks of Glyphosate Use to Federally Threatened California Red-legged Frog

October 2008

Risks of Glyphosate Use to Federally Threatened California Red-legged Frog

(*Rana aurora draytonii*)

Pesticide Effects Determination

Environmental Fate and Effects Division
Office of Pesticide Programs
Washington, D.C. 20460

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1. Executive Summary

The purpose of this assessment is to evaluate potential direct and indirect effects on the California red-legged frog *(Rana aurora draytonii*) (CRLF) arising from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) regulatory actions regarding use of glyphosate and its salts on agricultural and non-agricultural sites. In addition, this assessment evaluates whether these actions can be expected to result in modification of the species' designated critical habitat. This assessment was completed in accordance with the U.S. Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) *Endangered Species Consultation Handbook* (USFWS/NMFS, 1998 and procedures outlined in the U.S. Environmental Protection Agency Overview Document (U.S. EPA, 2004).

The CRLF was listed as a threatened species by USFWS in 1996. The species is endemic to California and Baja California (Mexico) and inhabits both coastal and interior mountain ranges. A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (USFWS, 1996) in California.

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. Both the parent acid and several of its salts are registered as active ingredients and all are considered in this assessment. As of the 1993 Re-registration Eligibility Decision (RED), labeled uses of glyphosate included over 100 terrestrial food crops. In addition, there are many other uses under the categories of terrestrial food, non-food and feed crop; forestry; aquatic food crop and non-food outdoor and industrial; greenhouse food and non-food crop; indoor non-food and outdoor residential. The following uses are considered as part of the federal action evaluated in this assessment: many agricultural crops, non-grass forage/fodder/straw/hay, rights-of-way/fence rows/hedgerows, farm structures/buildings and equipment, pastures, grasses grown for seed and Christmas tree plantations; ornamental shade trees, ground cover, herbaceous plants, non-flowering plants and lawns; nursery stock and turf; commercial, urban and residential outdoor buildings/structures, premises, path/patio, paved areas and recreational areas; rangeland and forestry conifer release, nursery plantings (fir transplant purposes), trees (all or unspecified) and aquatic uses on emergent plants.

Glyphosate is stable towards abiotic hydrolysis and direct photolysis in water. Its major route of transformation identified in laboratory studies and in the field is microbial degradation, where the major metabolite is **a**mino**m**ethyl **p**hosphonic **a**cid (AMPA). Glyphosate is very soluble in water. It has low potential to volatilize from soil or water, as suggested by its low vapor pressure and Henry's Law Constant. Glyphosate adsorbs strongly to soils and sediments. Based on its strong adsorption to soil/sediments alone, leaching to ground water or entering surface water dissolved in runoff would be minimized. However, surface water can be contaminated by transport of suspended soil particulates, followed by desorption from the soil particulates and/or from sediments.

Offsite exposure is also possible via spray drift, colloidal transport, inadvertent direct overspray and wind transport of soil particulates loaded with adsorbed glyphosate residues. Glyphosate is very hydrophilic and is unlikely to bioaccumulate in fish.

Glyphosate is an acid which can be associated with different counter cations to form salts. For comparison purposes in this assessment, each salt is considered in terms of its "glyphosate equivalent," (acid equivalent; ae) as determined by multiplying by the acid equivalence ratio (the ratio of the molecular weight of *N*-(phosphonomethyl)glycine to the molecular weight of the salt). For the assessment of risk to technical glyphosate, both application rates and the toxicity endpoint values are expressed as acid equivalents.

Risks from exposure to glyphosate formulations are also assessed because some of the formulations are more toxic than the technical material. For aquatic organisms, exposures to glyphosate formulations following terrestrial and aquatic applications are considered separately. Terrestrial uses allow for application of formulations that contain a surfactant that is toxic to aquatic organisms (polyethoxylated tallow amines (POEA)), whereas the toxic surfactant is not allowed in formulations designated for aquatic use.

Since CRLFs exist within both aquatic and terrestrial habitats, exposure to glyphosate is assessed separately for the two habitats and for the CRLF and its prey in each habitat. The highest aquatic exposure to both glyphosate and its formulations is expected to result from uses with direct aquatic applications. Estimated environmental concentrations (EECs) for these uses were derived with simple dilution calculations based on the mass of the applied pesticide and the volume of the water body. For glyphosate and its formulations, peak EECs for aquatic uses were 210 µg ae/L and 1840 µg form./L, respectively. These estimates are supplemented with analysis of available California surface water monitoring data from U. S. Geological Survey's National Water Quality Assessment (NAWQA) program and the California Department of Pesticide Regulation (DPR). These data sources included biweekly monitoring for glyphosate at three sites between 2002-2003. Both glyphosate and AMPA, its degradate, were detected at least once at all sites, with maximum reported concentrations of 7.46 µg/L for glyphosate and 1.07 µg/L for AMPA. Both peak concentrations were detected at an agricultural site in Stanislaus County.

To estimate glyphosate exposures to the terrestrial-phase CRLF, and its potential prey resulting from uses involving glyphosate applications, the T-REX model is used for foliar uses. The AgDRIFT model is also used to estimate deposition of glyphosate on terrestrial and aquatic habitats from spray drift. The TerrPlant model is used to estimate glyphosate exposures to terrestrial-phase CRLF habitat, including plants inhabiting semi-aquatic and dry areas, resulting from uses involving foliar glyphosate applications.

The effects determination assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF itself, as well as indirect effects, such as reduction of the prey base or modification of its habitat. Direct effects to the CRLF in the aquatic habitat are based on toxicity information for freshwater fish, which are generally used as a surrogate for aquatic-phase amphibians and on aquatic-phase

amphibians. In the terrestrial habitat, direct effects are based on toxicity information for birds, which are used as a surrogate for terrestrial-phase amphibians. Given that the CRLF's prey items and designated critical habitat requirements in the aquatic habitat are dependant on the availability of freshwater aquatic invertebrates and aquatic plants, toxicity information for these taxonomic groups is also discussed. In the terrestrial habitat, indirect effects due to depletion of prey are assessed by considering effects to terrestrial insects, small terrestrial mammals, and frogs. Indirect effects due to modification of the terrestrial habitat are characterized by available data for terrestrial monocots and dicots.

Acute toxicity data are available for the degradate, AMPA, with freshwater fish, birds and aquatic invertebrates. Since AMPA appears to be less toxic than the parent, this degradate was not considered in exposure estimations.

Risk quotients (RQs) are derived as quantitative estimates of potential high-end risk. Acute and chronic RQs are compared to the U.S. Environmental Protection Agency (referred to as 'the Agency' in subsequent text) levels of concern (LOCs) to identify instances where glyphosate use within the action area has the potential to adversely affect the CRLF and its designated critical habitat via direct toxicity or indirectly based on direct effects to its food supply (i.e., freshwater invertebrates, algae, fish, frogs, terrestrial invertebrates, and mammals) or habitat (i.e., aquatic plants and terrestrial upland and riparian vegetation). When RQs for a particular type of effect are below LOCs, the pesticide is determined to have "no effect" on the subject species. Where RQs exceed LOCs, a potential to cause adverse effects is identified, leading to a conclusion of "may affect." If a determination is made that use of glyphosate within the action area "may affect" the CRLF and its designated critical habitat, additional information is considered to refine the potential for exposure and effects, and the best available information is used to distinguish those actions that "may affect, but are not likely to adversely affect" (NLAA) from those actions that are "likely to adversely affect" (LAA) the CRLF and its critical habitat.

Based on the best available information, the Agency makes a May Affect, and Likely to Adversely Affect determination for the CRLF from the use of glyphosate. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical.

There are no direct effects on the aquatic-phase CRLF for any of the terrestrial or aquatic uses. The terrestrial-phase CRLF eating broadleaf plants, small insects and small herbivorous mammals on a dietary-basis may be at risk to direct effects following chronic exposure to glyphosate at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). In addition, terrestrial phase amphibians may be at risk following acute exposure to one particular formulation (Registration No. 524-424), at application rates of 1.1 lbs formulation/A and above (ornamental lawns and turf and industrial outdoor uses). Indirect effects to the aquatic-phase CRLF, based on reduction in the prey base may occur with aquatic nonvascular plants with aquatic weed management uses at an application rate of 3.75 lb a.e./A. Indirect effects to the

terrestrial-phase CRLF, based on reduction in the prey base may occur with small insects at any registered rate, large insects at an application rate of 7.95 lb a.e./A (forestry uses), terrestrial phase amphibians following chronic exposure at application rates of 7.5 lb a.e./A and above and following acute exposure to one formulation at application rates of 1.1 lbs formulation/A and above and mammals following chronic exposure at application rates of 3.84 lbs a.e./A and above (i.e., many crops, forestry, rights of way and areas with impervious surfaces).

Indirect effects to both the aquatic- and terrestrial-phase CRLF, based on habitat effects may occur with aquatic non-vascular plants following aquatic weed management use and with aquatic emergent plants and terrestrial plants exposed via spray drift with aerial application at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A.

A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat is presented in **Tables 1.1 and 1.2**. Use-specific determinations for direct and indirect effects to the CRLF are provided in **Tables 1.3 and 1.4**. Further information on the results of the effects determination is included as part of the Risk Description in Section 5.2. Given the LAA determination for the CRLF and potential modification of designated critical habitat, a description of the baseline status and cumulative effects for the CRLF is provided in **Attachment 2**.

Table 1.1 Effects Determination Summary for Glyphosate Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA[1]	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		Terrestrial-phase (Juveniles and Adults): The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. Initial area of concern and action area are the entire state of California. Glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the

Table 1.1 Effects Determination Summary for Glyphosate Use and the CRLF		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		freshwater invertebrate population prey base would be very low, and the monitoring data are considerably lower than the modeled concentrations utilized in the risk assessment**.** For non-vascular plants, the LOC for aquatic plants is exceeded for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOC for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		Terrestrial prey items, riparian habitat For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way). The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. Initial area of concern and action area are the entire state of California.

[1] No effect (NE); May affect, but not likely to adversely affect (NLAA); May affect, likely to adversely affect (LAA)

Table 1.2 Effects Determination Summary for Glyphosate Use and CRLF Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
Modification of aquatic-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. For non-vascular plants, the LOC for aquatic plants is exceeded, only for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations. The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low.
Modification of terrestrial-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way).

[1] Habitat Modification or No effect (NE)

Table 1.3 Glyphosate Use-specific Direct Effects Determinations[1] for the CRLF				
Use(s)	**Aquatic Habitat**		**Terrestrial Habitat**	
	Acute	**Chronic**	**Acute**	**Chronic**
Forestry, areas with impervious surfaces and rights of way (application rates of 7.5 lbs a.e./A and above)	NE	NE	NE	LAA
One particular formulation (Reg No. 524-424): industrial sites, rights-of-way, ornamental lawns and turf at 1.1to 5.5 lbs formulation/A.	NA	NA	LAA	NA
All other uses at application rates of 3.85 lb a.e./A and below (all crops, forestry and impervious surfaces at lower rates, rangeland, residential, rights of way at lower rates and turf)	NE	NE	NE	NE
[1] NE = No effect; NLAA = May affect, but not likely to adversely affect; LAA = Likely to adversely affect; NA = data not available for this formulation.				

Table 1.4 Glyphosate Use-specific Indirect Effects Determinations[1] Based on Effects to Prey										
Use(s)	**Algae**	**Aquatic Invertebrates**		**Terrestrial Invertebrates (Acute)**	**Aquatic-phase frogs and fish**		**Terrestrial-phase frogs**		**Small Mammals**	
		Acute	**Chronic**		**Acute**	**Chronic**	**Acute**	**Chronic**	**Acute**	**Chronic**
Forestry, areas with impervious surfaces and rights of way (application rates of 7.5 lb a.e./A and above)	NE	NE	NE	LAA	NE	NE	NE	LAA	NE	LAA
One particular formulation (Reg No. 524-424): industrial sites, rights-of-way, ornamental lawns and turf at 1.1to 5.5 lbs formulation/A.	NA	NA	NA	LAA	NA	NA	LAA	NA	NLAA	NA
Most crops, forestry, rights of way and areas with impervious surfaces at application rates of 3.84 lbs a.e./A and above.	NE	NE	NE	LAA	NE	NE	NE	NE	NE	LAA
Aquatic uses at 3.75 lb a.e./A	LAA	NE	NE	NE	NE	NE	NE	NE	NE	NE

[1] NE = No effect; NLAA = May affect, not likely to adversely affect; LAA = Likely to adversely affect; NA = data not available for this formulation.

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential modification to critical habitat.

2. Problem Formulation

Problem formulation provides a strategic framework for the risk assessment. By identifying the important components of the problem, it focuses the assessment on the most relevant life history stages, habitat components, chemical properties, exposure routes, and endpoints. The structure of this risk assessment is based on guidance contained in U.S. EPA's *Guidance for Ecological Risk Assessment* (U.S. EPA 1998), the Services' *Endangered Species Consultation Handbook* (USFWS/NMFS 1998) and is consistent with procedures and methodology outlined in the Overview Document (U.S. EPA 2004) and reviewed by the U.S. Fish and Wildlife Service and National Marine Fisheries Service (USFWS/NMFS 2004).

2.1 Purpose

The purpose of this endangered species assessment is to evaluate potential direct and indirect effects on individuals of the federally threatened California red-legged frog *(Rana aurora draytonii*) (CRLF) arising from FIFRA regulatory actions regarding use of glyphosate on a large number of agricultural crops, non-grass forage/fodder/straw/hay, rights-of-way/fence rows/hedgerows, farm structures/buildings and equipment, pastures, grasses grown for seed and Christmas tree plantations; ornamental shade trees, ground cover, herbaceous plants, non-flowering plants and lawns; nursery stock and turf; commercial, urban and residential outdoor buildings/structures, premises, path/patio, paved areas and recreational areas; rangeland and forestry conifer release, nursery plantings (fir transplant purposes), trees (all or unspecified) and aquatic uses on emergent plants. In addition, this assessment evaluates whether use on these crops is expected to result in modification of the species' designated critical habitat. This ecological risk assessment has been prepared consistent with a settlement agreement in the case *Center for Biological Diversity (CBD) vs. EPA et al*. (Case No. 02-1580-JSW(JL)) settlement entered in Federal District Court for the Northern District of California on October 20, 2006.

In this assessment, direct and indirect effects to the CRLF and potential modification to its designated critical habitat are evaluated in accordance with the methods described in the Agency's Overview Document (U.S. EPA 2004). Screening level methods include use of standard models such as GENEEC2, PRZM-EXAMS, T-REX, TerrPlant and AgDRIFT, all of which are described at length in the Overview Document. Use of such information is consistent with the methodology described in the Overview Document (U.S. EPA 2004), which specifies that "the assessment process may, on a case-by-case basis, incorporate additional methods, models, and lines of evidence that EPA finds technically appropriate for risk management objectives" (Section V, page 31 of U.S. EPA 2004).

In accordance with the Overview Document, provisions of the Endangered Species Act (ESA), and the Services' *Endangered Species Consultation Handbook,* the assessment of effects associated with registrations of glyphosate is based on an action area. The action area is the area directly or indirectly affected by the federal action, as indicated by the

exceedence of the Agency's Levels of Concern (LOCs). It is acknowledged that the action area for a national-level FIFRA regulatory decision associated with a use of glyphosate may potentially involve numerous areas throughout the United States and its Territories. However, for the purposes of this assessment, attention will be focused on relevant sections of the action area including those geographic areas associated with locations of the CRLF and its designated critical habitat within the state of California. As part of the "effects determination," one of the following three conclusions will be reached regarding the potential use of glyphosate in accordance with current labels:

- "No effect";
- "May affect, but not likely to adversely affect"; or
- "May affect and likely to adversely affect".

Designated critical habitat identifies specific areas that have the physical and biological features, (known as primary constituent elements or PCEs) essential to the conservation of the listed species. The PCEs for CRLFs are aquatic and upland areas where suitable breeding and non-breeding aquatic habitat is located, interspersed with upland foraging and dispersal habitat.

If the results of initial screening-level assessment methods show no direct or indirect effects (no LOC exceedances) upon individual CRLFs or upon the PCEs of the species' designated critical habitat, a "no effect" determination is made for use of glyphosate as it relates to this species and its designated critical habitat. If, however, potential direct or indirect effects to individual CRLFs are anticipated or effects may impact the PCEs of the CRLF's designated critical habitat, a preliminary "may affect" determination is made for the FIFRA regulatory action regarding glyphosate.

If a determination is made that use of glyphosate within the action area(s) associated with the CRLF "may affect" this species or its designated critical habitat, additional information is considered to refine the potential for exposure and for effects to the CRLF and other taxonomic groups upon which these species depend (e.g., aquatic and terrestrial vertebrates and invertebrates, aquatic plants, riparian vegetation, etc.). Additional information, including spatial analysis (to determine the geographical proximity of CRLF habitat and glyphosate use sites) and further evaluation of the potential impact of glyphosate on the PCEs is also used to determine whether modification of designated critical habitat may occur. Based on the refined information, the Agency uses the best available information to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that "may affect and are likely to adversely affect" the CRLF or the PCEs of its designated critical habitat. This information is presented as part of the Risk Characterization in Section 5 of this document.

The Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because glyphosate is expected to directly impact living organisms within the action area (defined in Section 2.7), critical habitat analysis for glyphosate is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked

to biologically mediated processes (i.e., the biological resource requirements for the listed species associated with the critical habitat or important physical aspects of the habitat that may be reasonably influenced through biological processes). Activities that may modify critical habitat are those that alter the PCEs and appreciably diminish the value of the habitat. Evaluation of actions related to use of glyphosate that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. Actions that may affect the CRLF's designated critical habitat have been identified by the Services and are discussed further in Section 2.6.

2.2 Scope

Glyphosate is an herbicide approved for use on crops grown in California as well as for many non-agricultural and residential sites. These include the following categories: terrestrial non-food, food and feed crop; forestry; aquatic food crop and non-food outdoor and industrial; greenhouse food and non-food crop; indoor non-food and outdoor residential. Registered uses of glyphosate on crops that are not grown in California, including soybeans, will not be considered in this assessment. In addition to the parent acid, several salts of glyphosate can be used as active ingredients. All of these species are included in this assessment and will be referred to collectively as "glyphosate" throughout this document.

The end result of the EPA pesticide registration process (*i.e.*, the FIFRA regulatory action) is an approved product label. The label is a legal document that stipulates how and where a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type (*e.g.*, liquid or granular), acceptable methods of application, approved use sites, and any restrictions on how applications may be conducted. In addition, the labels usually specify application rates and frequency of application. Thus, the use or potential use of glyphosate in accordance with the approved product labels for California is "the action" relevant to this ecological risk assessment.

Although current registrations of glyphosate allow for use nationwide, this ecological risk assessment and effects determination addresses currently registered uses of glyphosate in portions of the action area that are reasonably assumed to be biologically relevant to the CRLF and its designated critical habitat. Further discussion of the action area for the CRLF and its critical habitat is provided in Section 2.7.

The primary degradate of glyphosate is AMPA, which can be formed through photolysis or through metabolism in both aerobic and anaerobic conditions. Acute ecotoxicity studies with freshwater fish and invertebrates and birds indicate that AMPA is not more toxic than the parent, glyphosate. Therefore, the degradate was not included in the assessment.

The Agency does not routinely include in its risk assessments an evaluation of mixtures of active ingredients, either those mixtures of multiple active ingredients in product formulations or those in the applicator's tank. In the case of the product formulations of active ingredients (that is, a registered product containing more than one active

ingredient), each active ingredient is subject to an individual risk assessment for regulatory decision regarding the active ingredient on a particular use site. If effects data are available for a formulated product containing more than one active ingredient, they may be used qualitatively or quantitatively in accordance with the Agency's Overview Document and the Services' Evaluation Memorandum (U.S., EPA 2004; USFWS/NMFS 2004).

Glyphosate has registered products that contain multiple active ingredients (**Table 2.1**). Analysis of the available acute oral mammalian toxicity data for multiple active ingredient products relative to the single active ingredient is provided in **Appendix A**. The results of this analysis show that an assessment based on the toxicity of the single active ingredient of glyphosate is appropriate. There are no currently registered product LD_{50} values, with associated 95% Confidence Intervals (CIs) available for mixtures containing glyphosate. As discussed in USEPA (2000), a quantitative component-based evaluation of mixture toxicity requires data of appropriate quality for each component of a mixture. In this mixture evaluation, an LD_{50} with associated 95% CI is needed for the formulated product. The same quality of data is also required for each component of the mixture. Given that the formulated products for mixtures containing glyphosate do not have LD_{50} data available, it is not possible to undertake a quantitative or qualitative analysis for potential interactive effects.

In some products, glyphosate is formulated with surfactants which have been shown to increase the toxicity of the parent compound. Therefore, risk from formulations containing surfactants is considered in this assessment as well as from glyphosate alone. Products containing surfactants will be referred to as "formulations" throughout this document and those containing only glyphosate are referred to as "glyphosate". Products containing the surfactant POEA are off-labeled for aquatic uses in California, so these products will only be assessed for terrestrial uses. This document only assesses a surfactant when it is included as part of the formulated product; it does not assess surfactant that may be included in the tank mix.

Table 2.1. Multiple Active-Ingredient Formulations for Glyphosate									
		Percent (%) Active Ingredient							
REG_NR	PROD_NAME	2,4-D	Dicamba	Diquat dibromide	Glyphosate	Imazethapyr	Oxyfluorfen	S-Metolachlor	Sulfuric acid, monourea adduct
00010001179	TOUCHDOWN DIQUAT HOME AND GARDEN CONCENTRATE			0.73	13.4				
00010001180	TOUCHDOWN DIQUAT HOME AND GARDEN READY TO USE			0.06	0.81				
00010001185	SEQUENCE HERBICIDE				21.8			29	
00010001186	TOUCHDOWN 008		0.6		43.5				
00023902694	ORTHO SEASON-LONG GRASS & WEED KILLER			0.1	8		1.5		
00024100404	STANDOUT HERBICIDE				21.9	2.7			
00035200675	ETK-2301 HERBICIDE				9.6				
00968800211	CHEMSICO HERBICIDE CONCENTRATE DT			1.9	14.6				
00968800213	CHEMSICO HERBICIDE RTU DT			0.1	0.81				
07136800030	NUFARM GLYKAMBA BROADSPECTRUM HERBICIDE		4.1		23.3				
07136800035	RECOIL BROAD SPECTRUM HERBICIDE	11.38			23.03				

2.3 Previous Assessments

The ecological risks associated with use of glyphosate as an herbicide have been assessed several times since 1974 when it was first registered for use in the United States. Findings from relevant ecological risk assessments are briefly summarized below.

- Glyphosate was assessed for the Reregistration Eligibility Decision in 1993. The Agency concluded that direct risks to birds, mammals, invertebrates and fish would be minimal. Under certain conditions, aquatic plants were expected to be at risk from glyphosate use. Additional data were needed for non-target terrestrial plants, including incident data and vegetative vigor testing on non-target terrestrial plants. The assessment stated that many endangered plants may be at risk from use of glyphosate with the registered use patterns. In addition, it was determined that the Houston Toad may be at risk from use of glyphosate on alfalfa.

- In 2003, the USDA Forest Service had a risk assessment conducted for glyphosate uses in Forest Service vegetation management programs (USDA, 2003). For forestry uses, all commercial formulations of glyphosate contained the isopropylamine salt of glyphosate (IPA). Application rates ranged from 0.5 lbs a.e./A to 7 lbs a.e./A with the most typical at 2 lb a.e./A. The USDA assessment did not conduct a separate assessment for amphibians. The document concluded that the amphibian data indicated that glyphosate is no more toxic to amphibians than it is to fish. The USDA risk assessment also used a "relative potency" method to estimate the chronic NOAEC for fish in more sensitive species. This appears to be similar to the Agency's acute to chronic ratio estimations. The NOAEC from a less sensitive fish study was divided by 10 to provide a NOAEC for a more sensitive fish. A similar approach was used for an estimation of a chronic NOAEC for glyphosate formulations on freshwater fish and invertebrates. Finally, as a note, some of the endpoints utilized in the USDA risk assessment were not the same endpoints as used in the Agency risk assessments. For example, the chronic mammal endpoint is also used as the acute endpoint for mammals (175 mg/kg from the developmental study in rabbits).

  Based on the available data, the USDA concluded that the risks were minimal to mammals, birds, fish, invertebrates and aquatic plants. Risks to fish following application of the more toxic formulations were not considered to be high; however, the assessment did state that at an application rate of 7 lb a.e./A, the acute exposures slightly exceeded the acute LC_{50} for a more tolerant freshwater fish and exceeded it by a factor of 2 for the less tolerant fish. These values were estimated from a worst-case scenario where there was a severe rainfall of about 7 inches over a 24-hour period in an area where runoff is favored. For terrestrial plants, the assessment concluded that for relatively tolerant plants, when a low-boom spray is utilized as the method of application, there is no indication that glyphosate would result in damage from spray drift at distances from the application site of 25 feet or greater. For more sensitive plants, the distance increased to approximately 100 feet. The

applications requiring the use of backpack-directed spray, the distances would be less. No risks to terrestrial plants from runoff were expected.

- In 2004, the Agency assessed glyphosate's potential to affect 11 federally listed Pacific salmonids. That assessment determined that use of glyphosate "may affect, but is not likely to adversely affect" the species based on acute toxicity to fish for uses with application rates above 5 lb ai/A. For uses with application rates below 5 lb ai/A, the Agency determined glyphosate would have no effect on the 11 subject species.

- In 2006, the Agency assessed glyphosate for a new use on bentgrass (0.74 lb a.i./A) and for new uses on Indian mulberry (noni), dry peas, lentils, garbanzo, safflower and sunflower with the highest proposed ground application rate of 3.73 lbs ae/A. For all proposed new uses, the Agency concluded that there was minimal risk of direct acute effect to terrestrial animals (birds and mammals) and aquatic animals (fish, amphibians, and invertebrates) and minimal risk to terrestrial plants (both non-target and endangered plant species), aquatic non-vascular (algae and diatoms) and vascular (duckweed) plants from offtarget spray drift and runoff from ground-based application technology. In addition, there were no chronic risks to animals.

2.4 Stressor Source and Distribution

Glyphosate [*N*-(phosphonomethyl)glycine] is an acid, and it can also be associated with different counter cations to form salts. Several salts of glyphosate are currently marketed, as well as the acid, and are considered as the active ingredient in end-use products. The parent acid is the chemical species that exhibits herbicidal activity and so is the actual chemical stressor considered in this ecological risk assessment regardless of the salt, unless otherwise specified. In order to have comparable results, each salt is considered in terms of its glyphosate equivalent, (acid equivalent; ae), determined by multiplying the application rate by the acid equivalence ratio, defined as the ratio of the molecular weight of *N*-(phosphonomethyl)glycine to the molecular weight of the salt. Table 2.2 shows the salts of glyphosate that may be used as the source of the actual herbicide-active chemical species. Products that no longer have active registrations are included as well for reference purposes. For the purpose of this assessment, the acid and all salt species are referred to collectively as "glyphosate" throughout this document.

Table 2.2. Identification of Glyphosate and its Salts			
Counter Cation	**PC Code**	**CAS No.**	**Acid Equivalence Ratio**
Glyphosate acid (no counter cation)	417300	1071-83-6	1
Isopropyl amine	103601	38641-94-0	0.74
Monoammonium	103604	114370-14-8	0.94
Diammonium	103607	40465-66-5	0.83
N-methylmethanamine	103608	34494-07-7	0.79
Potassium	103613	39600-42-5; 70901-20-1	0.81
Sesquisodium	103603	70393-85-0	Inactive Registration
Ethanolamine	103605	--	Inactive Registration
Trimethyl sulfonium	128501	81591-81-3	Inactive Registration

Surfactants

In some end use products, the active ingredient is formulated with a surfactant to improve efficacy. Studies show that these formulated products can be more toxic than the active ingredient alone and so in this assessment, formulated products are considered independently of those containing only the active ingredient.

Surfactants (**surf**ace **act**ing **a**ge**nt**") are wetting agents that lower the surface tension of a liquid, allowing easier spreading, and lower the interfacial tension between two liquids. Usually they are organic chemicals that contain a hydrophobic group ("tail") and a hydrophilic group ("head") in the same molecule. For the most part, surfactants are mixtures of the same class with different length of the carbon chain. Usually, the mixture indicates the carbon-chain range in the surfactant (e.g., C10- C14 fraction).

Pesticides of high solubility in water, such as glyphosate, do not "wet" (cover) properly the waxy (hydrophobic) surfaces of plants. To attain proper coverage of plant surfaces and distribution of the herbicide, surfactants are added into the formulation of the pesticide. Proper coverage arises from hydrophobic interactions between the surfactant tail (usually long carbon chains) and the waxy surfaces of plants. Therefore, the ecological effects of the pesticide-surfactant combination may differ from that of the single pesticide or the single surfactant. Glyphosate labels also recommend using a nonionic surfactant in the tank mix to further enhance the "wettability" of glyphosate.

One class of surfactants used in glyphosate formulations are the polyethoxylated tallow amines (POEA). Use of POEA containing products is not allowed for aquatic uses in California. However, other formulations may contain a different class of surfactant. The

nature of the surfactant included in the formulation is considered to be Confidential Business Information (CBI) and is not included on product labels.

2.4.1 Physical and Chemical Properties

The physical and chemical properties of glyphosate are shown in Table 2.3. Based on these physical and chemical properties alone, glyphosate has low potential to volatilize from soils (vapor pressure) or from water (Henry's Law Constant). It is also unlikely to bioaccumulate in fish given the low value of the Log *n*-octanol/water partition coefficient. **Appendix B** provides the structure and further chemical/molecular information on glyphosate. The molecular structure characteristics of glyphosate are important as they help understanding its mode of action at a molecular level as well as the binding of glyphosate to soil/sediment particulates**.**

Table 2.3 Physical and Chemical Properties of Glyphosate

Physical/Chemical Property	**Value**
Molecular Formula	$C_3H_8NO_5P$
Molecular Weight	170.8 g/mole
Melting Point	210-212° C (tech.) 215-219° C (pure)
Solubility in water, 25° C	12,000 mg L^{-1}
Vapor Pressure, Pa	1.3 x 10^{-7} (25° C)
Henry's Law Constant, Pa $\cdot m^3 \cdot mol^{-1}$	2.1 x 10^{-9}
Log K_{ow}	< -3
Dissociation Constants	pKa_1 = 0.8 pKa_2 = 2.35 pKa_3 = 5.84 pKa_4 = 10.48

2.4.2 Environmental Fate Properties

Table 2.4 summarizes the environmental fate behavior of glyphosate in different media. The environmental fate data shown in this Table are taken from required studies submitted in support of registration of glyphosate. These studies are conducted in a limited number of test systems (e.g., soils, water-sediments). These data are specific only for these systems. They may vary for other systems and may not be the same under actual use conditions.

The major route of transformation of glyphosate identified in laboratory studies is microbial degradation. In soils incubated under aerobic conditions, the half-life of glyphosate ranges from 1.8 to 5.4 days and in aerobic water-sediment systems is 7 days. However, anaerobic conditions limit the metabolism of glyphosate (half-life 8 to 199 days in anaerobic water-sediment systems). In laboratory studies, glyphosate was not observed to break down by abiotic processes in water, such as hydrolysis and direct aquatic photolysis, but soil photolysis occurred with a half-life of 6.6 days. In the field,

dissipation half-lives were measured to be 2.4 to 160 days (n=6). Glyphosate dissipation appeared to correlate with climate, being more persistent in cold than in warm climates. Along with significant mineralization to carbon dioxide, the major metabolite of glyphosate is **a**mino **m**ethyl **p**hosphonic **a**cid (AMPA).

No data are available about the environmental fate behavior of glyphosate formulations.

Table 2.4. Summary of Glyphosate Environmental Fate Behavior

Transformation

Study	Value	Major Degradates[1], Comments	MRID #	Study Status
Abiotic Hydrolysis Half-life	Stable (at 25° C for at least 30 days)	None	00108192; 44320642	161-1 Satisfied
Direct Aqueous Photolysis	Stable (for at least 30 days)	None	41689101; 44320643	161-2 Satisfied
Soil Photolysis Half-life	Stable (for at least 30 days)	Degradation in dark control was equal to that in irradiated samples	44320645.	161-3 Satisfied
Aerobic Soil Metabolism Half-life	1.8 and 5.4 days (sandy loam) 2.6 days (silt loam)	AMPA (max 29% at 40 d) CO_2 (≥70% after 1 year)	42372501; 44320645	162-1 Satisfied
Anaerobic Aquatic Metabolism Half-life	208 days (Water- silty clay loam sediment system)	AMPA (max 25% at 15 d) CO_2 (≥ 35% after 1 year) Initial degradation was rapid but slowed considerably. Non-linear modeling predicts DT_{50} = 8.1 day and DT_{90} > 1 yr	41723701; 42372502	162-3 Satisfied
Aerobic Aquatic Metabolism Half-life	14.1 days (Water- silty clay loam sediment)	AMPA (19-25% at 7-30 d) CO_2 (≥ 23% after 30 d)	41723601; 42372503	162-4 Satisfied

Mobility

Study	Value						MRID #	Study Status
Batch Equilibrium (mL/g)	*Soil*	*Avg K_d*	*Avg K_{oc}*	*K_F*	*1/n*	*K_{Foc}*	44320646	163-1 Satisfied
	sand	170	58,000	64	0.75	22,000		
	sandy loam	18	3,100	9.4	0.72	1,600		
	sandy loam	230	13,000	90	0.76	5,000		
	silty clay loam	680	33,000	470	0.93	21,000		
	silty clay loam	1,000	47,000	700	0.94	33,000		

Field Dissipation					
Study	**Value**			**MRID #**	**Study Status**
Terrestrial Field Dissipation Half-life	Glyph. 1.7 d 7.3 d 8.3 d 13 d 17 d 25 d 114 d 142 d	AMPA 131 d 119 d 958 d 896 d 142 d 302 d 240 d no data	(TX) (OH) (GA) (CA) (AZ) (MN) (NY) (IA)	Bare ground studies. Glyphosate and AMPA were found predominantly in the 0 to 6 inch layers	42607501; 42765001	
Aquatic Field Dissipation	7.5 days			In a farm pond in Missouri. At 3 sites (OR, GA, MI), half-lives could not be calculated due to recharging events.	40881601	
	Water: Dissipated rapidly immediately after treatment. Sediment: Glyphosate remained in pond sediments at ≥ 1 ppm at 1 year post treatment.			In ponds in Michigan and Oregon and a stream in Georgia Accumulation was higher in the pond than in the stream sediments	41552801.	
Forestry Dissipation	Foliage: < 1 day Ecosystem: Glyphosate: 100 d AMPA: 118 d			3.75 lb ae/A, aerial application	41552801.	

[1] Major degradates are defined as those which reach >10% of the applied.

2.4.3 Environmental Transport Mechanisms

The available field and laboratory data indicate that both glyphosate and AMPA adsorb strongly to soil. Soil partitioning coefficients (K_d) measured in batch equilibrium studies ranged from 18 to 1000 mL/g, with corresponding organic carbon partitioning coefficients (K_{oc}) of 3100 to 58000 mL/g_{oc}. The coefficient of variation for K_{oc} is less than the coefficient of variation for K_d, indicating that pesticide binding to the organic matter fraction of the soil explains some of the variability among the adsorption coefficients, and that K_{oc} is therefore the appropriate parameter to use in determining the soil mobility of the compound. Based on measured K_{oc} values, glyphosate is classified as slightly mobile to hardly mobile according to the FAO classification scheme and would not be expected to leach to groundwater or to move to surface water at high levels through dissolved runoff. However, glyphosate does have the potential to contaminate surface water from erosion via spray drift or transport of residues adsorbed to soil particles suspended in runoff, and transport of glyphosate with colloidal matter has been recognized as well.

The potential for volatilization from soil and water is expected to be low due to the low vapor pressure and low Henry's Law constant. Several studies conducted in use locations outside of California demonstrate that both glyphosate and AMPA can be found in

rainwater near use locations. In most cases, these detections were found during the spraying season in the vicinity of local use areas and can be attributed to spray drift rather than to volatilization or long range transport (Baker et al., 2006; Quaghebeur et al., 2004). The highest concentrations were found in urban locations. At one site in Belgium that was 5 m from a spraying location in an urban parking lot, glyphosate was detected in rainwater for several months following an application (Quaghebeur et al., 2004). Deposition was measured to be 205 µg a.i./m^2 at one week after spraying and 0.829 µg/m^2 two months after spraying. These data suggest that volatilization of glyphosate from hard surfaces is possible despite its low vapor pressure, but detections at 5 m were low and so unlikely to have spread far or to have had an impact on exposure.

2.4.4 Mechanism of Action

Glyphosate is a foliar, non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. Glyphosate is a potent and specific inhibitor of the enzyme 5-enolpyruvylshikimate 3-phosphate (ESPS) synthase. This enzyme is the sixth enzyme on the shikimate pathway and it is essential for the biosynthesis of aromatic amino acids and other aromatic compounds in algae, higher plants, bacteria and fungi. Inhibition of this enzyme leads to plant cell death. The shikimate pathway is absent in mammals.

2.4.5 Use Characterization

2.4.5.1 Labeled Use Pattern

Analysis of labeled use information is the critical first step in evaluating the federal action. The current labels for glyphosate represent the FIFRA regulatory action; therefore, labeled use and application rates specified on the label form the basis of this assessment. The assessment of use information is critical to the development of the action area and selection of appropriate modeling scenarios and inputs.

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective, systemic herbicide widely used to control weeds in agricultural crops and non-agricultural sites. **Table 2.5** presents a listing of all registered uses for crops grown in California, grouped into categories. In addition to terrestrial food (agricultural crop) uses, this assessment also considers non-agricultural uses such as rights of way, nurseries, Christmas tree plantations, and around buildings and paved areas, as detailed below. Glyphosate also has aquatic uses which allow direct application to water bodies for control of emergent plants.

Table 2.5. Glyphosate Uses Assessed for the CRLF	
Group Name	**Uses represented**
Aquatic uses on emergent plants	
Avocado	
Blueberry; Passion Fruit (Granadilla)	
Citrus	Grapefruit; Lemon; Orange; Tangelos; Tangerine; Kumquat
Cole crops	Broccoli; Cabbage; Cauliflower; Horseradish; Mustard
Corn	Corn- (Field-, Pop-, Sweet- [silage]); Millet – Proso (Broomcorn); Sunflower
Cotton	
Eggplant; Okra; Tomatillo; Tomato	
Fodder	Alfalfa; Clover; Non-grass forage/Fodder/Straw/Hay
Forestry	Christmas Tree Plantations; Conifer release; Forest Nursery Plantings (fir transplant purposes); Forest trees (all or unspecified)
Fruit	Apple; Apricot; Cherry; Fig; Nectarine; Peach; Pear; Pomegranate; Prune
Garlic; Leek	
Grains/Cereal	Barley; Oats; Rye; Safflower; Sorghum (including silage); Triticale; Wheat
Grapes	
Leafy Vegetables	Brussels Sprouts; Chicory; Endive (Escarole);Lettuce; Parsley
Melons	Melons (Cantaloupe, Honeydew, Mango, Musk Melons, Watermelons, Winter Melons [Casaba/Crenshaw/Honeydew/ Persian]), Pumpkins
Non-Crop Uses	Agricultural/Farm structures/Buildings and Equipment; Commercial Storages/Warehouses Premises; Household/Domestic Dwellings Outdoor Premises; Industrial Areas; Non-agricultural Outdoor Buildings/Structures; Path/Patios; Paved Areas (Private roads/Sidewalks); Urban Areas
Nuts	Almond; Pecan; Pistachio;Walnuts (English/Black)
Olive	
Onions	
Ornamentals	Ornamental and/or shade trees, groundcover, herbaceous plants, non-flowering plants, Nursery stock

Table 2.5. Glyphosate Uses Assessed for the CRLF	
Group Name	**Uses represented**
Residential	Ornamental lawns and turf; Recreational areas
Rangeland	Bermudagrass; Pastures; Rangeland
Rights of way	Agricultural rights-of-way/Fence Rows/Hedgerows
Root crops	Potato White/Irish; Rutabaga; Sweet Potato; Turnip (greens); Turnip (root)
Row crops	Artichoke; Artichoke- Jerusalem; Asparagus; Beans; Beets; Carrots (including tops); Celery; Pepper; Peas- Dried Type; Peas
Strawberry	
Sugar beet (including tops), Parsnip	
Turf	Ornamental sod farm (turf), Grasses grown for seed

Table 2.6 presents application rates and methods for the groups of uses considered in this assessment. The reported application rates represent the maximum application rate used in any crop/use site within each group. The information was extracted from existing product labels. When available, the number and frequency of applications were taken from the label. In some cases, the number of applications had to be estimated based on maximum seasonal application rates and maximum single application rates. For these uses, application intervals were assumed to be 14 days. All of the glyphosate application rates are in units of lb acid equivalents (ae)/A, regardless of the source of glyphosate in the end-use product.

Unlike for the active ingredient, labels only provide formulation application rates in terms of volume applied rather than in terms of mass applied, as is required for estimating exposure concentrations. For this assessment, application rates for formulations were back-calculated based on application rates for glyphosate and the fraction of active ingredient in the formulation. To calculate an application rate for the formulated product, the seasonal application rate of glyphosate acid was converted from acid equivalents to active ingredient, and this rate was then divided by the fraction of active ingredient in the formulated product, according to the following equation:

$$\textit{Seasonal application rate}\ (\text{lb formulated product/A}) = \frac{[\textit{Seasonal application rate}\ (\text{lb ae/A}) \div \textit{acid equivalence ratio}]}{[\textit{fraction of a.i. in formulated product}]}$$

The formulation rates have only been calculated for seasonal applications, and not separated out for single maximum application rates. Additionally, application methods corresponding to formulation application rates have not been extracted from the label. In

order to be conservative, when quantitative estimations are necessary, calculations are based on the assumption of aerial application.

The uses considered in this risk assessment represent all currently registered uses according to a review of all current labels. Historical uses, mis-reported uses, and misuse that may have been listed in the California PUR data are not considered part of the federal action and, therefore, are not considered in this assessment.

Table 2.6 Maximum Application Rates Assessed for Glyphosate and Glyphosate Formulations

GROUP NAME	GLYPHOSATE		GLYPHOSATE FORMULATIONS
	Application Method	**Max. Single App. Rate * Apps/season** [1] (lb ae/A)	**Max. Seasonal App. Rate** [2] (lb formulation/A)
Aquatic uses on emergent plants	N/A	3.75 * 1	32.9
Avocado	Ground	3.75 * 2	28.2
Blueberry; Passion Fruit (Granadilla)	Aerial	3.85 (*1st app*), 2.3 (*2nd app*) [3]	8.7
Citrus	Ground	3.85; 2.3	28.2
Cole crops	Aerial	3.85; 2.3	23.9
Corn	Aerial	0.75 * 8	25.7
Cotton	Ground	3.75; 2.25	14.1
Eggplant; Okra; Tomatillo; Tomato	Aerial	3.75; 2.35	8.7
Fodder	Ground	3.75 * 2	20.1
Forestry	Aerial	7.95 * 1	32.1
Fruit	Ground	3.84 * 1	26.7
Garlic; Leek	Ground	3.75; 2.25	8.5
Grains/Cereal	Ground	3.75; 2.25	25.7
Grapes	Ground	3.84 * 2	11.5
Leafy Vegetables	Aerial	3.85; 2.3	21.3
Melons	Aerial	3.85; 2.3	8.7
Non-Crop Uses	Ground	7.95 * 1	34.0
Nuts	Ground	3.84 * 2	11.5
Olive	Ground	3.84 * 2	11.5

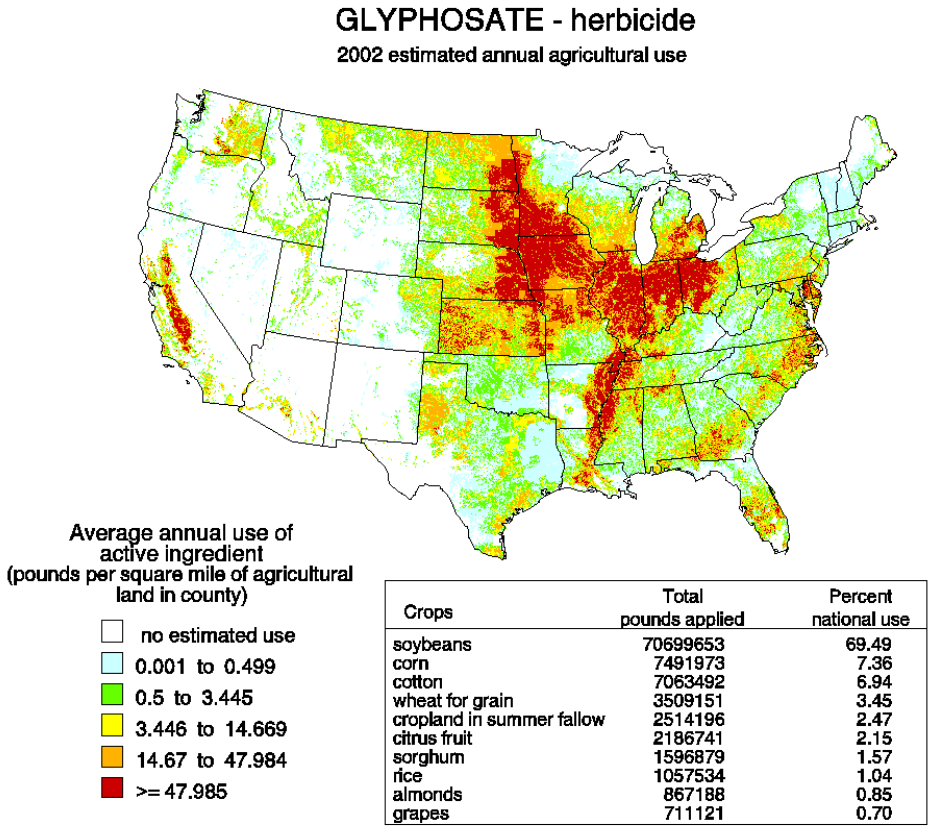
Table 2.6 Maximum Application Rates Assessed for Glyphosate and Glyphosate Formulations			
GROUP NAME	**GLYPHOSATE**		**GLYPHOSATE FORMULATIONS**
	Application Method	**Max. Single App. Rate * Apps/season** [1] (lb ae/A)	**Max. Seasonal App. Rate** [2] (lb formulation/A)
Onions	Aerial	3.85; 2.3	20.1
Ornamentals	Aerial	3.75 * 2	34.0
Residential	Ground	3.75 * 2	34.0
Rangeland	Aerial	3.75 * 2	34.0
Rights of way	Aerial	7.5 * 1	34.0
Root crops	Aerial	3.85; 2.3	25.7
Row crops	Ground	3.75; 2.25	25.7
Strawberry	Ground	3.75; 2.25	15.1
Sugar beet (including tops), Parsnip	Aerial	3.75; 2.35	18.4
Turf	Aerial	3.75 * 2	34.0

[1] Application intervals are 14 days.
[2] Application rates in lb formulation/A were calculated based on labeled application rates in lbs ae/A, fraction a.i. in the product, and the appropriate acid equivalent ratio for the salt in the active ingredient, as described above.
[3] Throughout table, when two application rates are listed consecutively, they represent different maximum application rates for the first and second single applications, with two applications allowed per season.

2.4.5.2 Use Statistics

As shown in **Figure 2.1**, glyphosate is used on agricultural crops across the country, with the highest usage concentrated in the Upper Midwest and Mississippi River basin. The use of glyphosate on soybeans represents about 70% of the national agricultural use. This map was downloaded from a U.S. Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) website.[1]

[1] http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=02&map=m1099



Crops	Total pounds applied	Percent national use
soybeans	70699653	69.49
corn	7491973	7.36
cotton	7063492	6.94
wheat for grain	3509151	3.45
cropland in summer fallow	2514196	2.47
citrus fruit	2186741	2.15
sorghum	1596879	1.57
rice	1057534	1.04
almonds	867188	0.85
grapes	711121	0.70

Figure 2.1 Glyphosate Use in Total Pounds per Square Mile

The Agency's Biological and Economic Analysis Division (BEAD) provides an analysis of both national- and county-level usage information (Kaul and Jones, 2006) using state-level usage data obtained from USDA-NASS[2], Doane (www.doane.com; the full dataset is not provided due to its proprietary nature) and the CDPR PUR database[3]. California State law requires that every pesticide application be reported to the state and made available to the public. Therefore, CDPR PUR is considered the most comprehensive source of pesticide usage data for the state and includes both agricultural and non-agricultural sites. It does not include home and garden use, industrial and institutional use, or any other uses by non-professional applicators. The usage data reported for glyphosate by county in this California-specific assessment were generated using CDPR PUR data.

Eight years (1999-2006) of usage data from CDPR PUR were obtained for every glyphosate application made on every use site at the field level. Usage data are available

[2] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.

[3] The California Department of Pesticide Regulation's Pesticide Use Reporting database provides a census of pesticide applications in the state. See http://www.cdpr.ca.gov/docs/pur/purmain.htm.

for glyphosate and several salts, including glyphosate-diammonium salt, glyphosate-isopropylamine salt, glyphosate-monoammonium salt, glyphosate-potassium salt, and glyphosate-trimesium. Total annual pounds applied and total annual area treated are calculated at the county level by site and pesticide active ingredient. Pesticide usage was also aggregated across all observations for eight years for each chemical-county-unit treated combination. Because pesticide applications are made in different area units, the units of area treated are provided where available. Years in which there is no reported use in a county are included as zeros in the calculation of the eight-year averages for pounds and area treated. Averages reflect years without use.

Between 1999 and 2006, glyphosate was reportedly used in all 58 counties in California. According to available information, the total amount of glyphosate active ingredients applied in California increased from about 4.4 million pounds (a.i.) in 1999 to about 7.8 million pounds (a.i.) in 2006 (CDPR PUR) (**Table 2.7**). The counties with the highest and lowest average total pounds from 1999-2006 were Fresno (56,868.9 lb a.i./year) and Alpine (6.2 lb a.i./year), respectively (**Table 2.8**). Glyphosate has a number of residential and industrial uses that are not represented in these data.

Table 2.7. Total Amount of Glyphosate Active Ingredients (lbs a.i.) Applied in California from 1999-2006 (Source: CDPR PUR)								
Active Ingredient	**Total Pounds Applied in California**							
	1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
GLYPHOSATE	30	843	55,486	157,014	116,168	113,383	307,172	523,482
GLYPHOSATE, DIAMMONIUM SALT	0	0	46	59,865	127,636	150,813	141,093	101,340
GLYPHOSATE, ISOPROPYLAMINE SALT	4,300,644	4,639,986	4,406,668	5,027,361	5,618,418	5,803,284	4,590,548	4,781,541
GLYPHOSATE, MONOAMMONIUM SALT	28,298	5,608	1,211	1,173	199,208	151,703	81,283	86,388
GLYPHOSATE, POTASSIUM SALT	0	0	0	0	79	95,034	1,861,410	2,247,232
GLYPHOSATE-TRIMESIUM	91,772	194,849	146,562	146,941	58,913	48,520	25,502	13,384
TOTAL	**4,420,744**	**4,841,286**	**4,609,973**	**5,392,354**	**6,120,422**	**6,362,738**	**7,007,008**	**7,753,367**

Table 2.8. Summary of County-Level Glyphosate Usage Information For California From 1999 to 2006 (Source: CDPR PUR)					
County	**AVG Annual Pounds Applied**	**AVG Application Rate**	**95 Percentile Application Rate**	**99 Percentile Application Rate**	**AVG MAX Application Rate**
ALAMEDA	5145.5	1.3	3.2	4.8	10.9
ALPINE	6.2	2.9	2.9	2.9	2.9
AMADOR	774.2	1.1	2.3	5.3	6.4
BUTTE	10777.8	0.9	1.7	2.8	21.4
CALAVERAS	766.3	1.7	4.5	5.1	9.0
COLUSA	6626.9	0.9	1.8	2.8	8.2
CONTRA COSTA	5930.4	1.0	2.1	3.2	7.7
DEL NORTE	229.9	1.2	1.5	1.7	1.8
EL DORADO	1319.5	1.0	2.2	10.5	21.2
FRESNO	56868.9	0.9	2.0	4.4	37.1
GLENN	9324.1	1.3	2.4	2.8	6.5
HUMBOLDT	566.1	0.5	1.2	2.1	4.8
IMPERIAL	15930.4	1.4	2.1	3.0	13.0
INYO	202.0	1.0	2.4	2.4	2.4
KERN	37356.2	1.2	2.2	3.3	57.5
KINGS	15437.9	0.9	1.7	2.3	26.3
LAKE	1288.9	3.8	7.2	7.6	12.9
LASSEN	505.6	2.4	3.3	4.0	5.3
LOS ANGELES	16188.7	2.5	5.3	6.8	26.1
MADERA	22289.5	1.0	2.2	4.5	15.2
MARIN	525.7	1.5	3.0	4.5	7.0
MARIPOSA	763.7	1.9	5.4	6.1	30.0
MENDOCINO	1741.1	1.2	2.6	3.5	7.0
MERCED	22682.8	2.1	5.2	8.0	25.4
MODOC	575.8	1.0	2.0	3.0	5.0
MONO	152.5	1.5	4.0	4.0	4.0
MONTEREY	7519.7	1.8	2.8	3.8	17.9
NAPA	3511.6	1.3	2.7	4.1	18.0
NEVADA	728.9	0.7	1.6	3.1	9.7
ORANGE	6976.5	2.2	5.7	6.9	9.6
PLACER	1198.4	1.9	5.6	7.9	11.2
PLUMAS	313.3	1.4	2.2	2.8	3.9
RIVERSIDE	11039.4	3.3	12.3	28.7	36.2
SACRAMENTO	7904.4	1.3	4.0	4.9	10.5
SAN BENITO	1435.3	4.1	6.0	8.5	13.0
SAN BERNARDINO	3419.1	1.4	2.8	3.9	8.7
SAN DIEGO	6801.2	0.6	1.8	4.0	26.3
SAN FRANCISCO	591.2	N/A	N/A	N/A	N/A
SAN JOAQUIN	16478.4	0.8	1.9	4.3	29.2

Table 2.8. Summary of County-Level Glyphosate Usage Information For California From 1999 to 2006 (Source: CDPR PUR)					
County	**AVG Annual Pounds Applied**	**AVG Application Rate**	**95 Percentile Application Rate**	**99 Percentile Application Rate**	**AVG MAX Application Rate**
SAN LUIS OBISPO	4267.4	2.2	4.6	8.9	15.8
SAN MATEO	1223.4	3.2	8.3	11.9	23.4
SANTA BARBARA	5757.9	2.1	8.7	12.3	19.8
SANTA CLARA	8142.1	1.0	2.1	4.6	13.8
SANTA CRUZ	621.1	3.2	9.2	15.3	19.0
SHASTA	2085.4	1.5	3.0	3.9	8.4
SIERRA	188.3	1.6	4.4	6.7	6.7
SISKIYOU	860.1	0.9	2.2	3.5	7.3
SOLANO	4389.3	0.8	2.1	3.2	10.6
SONOMA	5286.9	1.0	2.3	2.9	9.3
STANISLAUS	16380.9	2.4	4.1	5.5	18.3
SUTTER	5268.7	1.2	2.0	3.3	7.8
TEHAMA	3271.7	2.8	7.3	7.5	11.5
TRINITY	1097.2	0.7	2.0	2.8	10.3
TULARE	37981.1	1.7	7.9	10.1	25.4
TUOLUMNE	2714.2	2.1	2.8	3.2	7.3
VENTURA	9031.7	1.6	3.2	8.1	37.3
YOLO	6917.5	0.7	1.4	2.1	6.3
YUBA	1668.7	1.1	2.1	2.8	5.4

Table 2.9 summarizes the five highest uses for each active ingredient in California in 2006. The highest use was a non-agricultural use in Santa Clara county; about 460,000 pounds of glyphosate isopropylamine was used for landscape maintenance. For agricultural crops in California, glyphosate was most heavily used on oranges, with about 182,000 pounds of glyphosate isopropylamine used in Tulare county. The next highest usage in an agricultural setting was on tree nuts (almonds, pistachios), cotton, corn, nectarines, and peaches.

Table 2.9. Top 5 Uses For Glyphosate and Its Salts in California in 2006.				
Active Ingredient	**County**	**Site Name**	**Total Pounds 2006**	**Total Area 2006 (acres)**
GLYPHOSATE	KERN	ALMOND	92,655	114,828
	FRESNO	ALMOND	51,378	45,125
	KERN	PISTACHIO	34,452	46,226
	MERCED	ALMOND	32,093	32,964
	KINGS	RIGHTS OF WAY	26,884	N/A
		Total	**237,462**	
GLYPHOSATE, DIAMMONIUM SALT	KERN	ALMOND	31,775	58,739
	COLUSA	ALMOND	9,893	16,950
	FRESNO	ALMOND	9,550	11,035
	MERCED	ALMOND	5,149	3,667
	COLUSA	TOMATO, PROCESSING	4,204	5,536
		Total	**60,570**	
GLYPHOSATE, ISOPROPYLAMINE SALT	SANTA CLARA	LANDSCAPE MAINTENANCE	460,113	N/A
	LOS ANGELES	LANDSCAPE MAINTENANCE	141,647	N/A
	LOS ANGELES	RIGHTS OF WAY	135,505	N/A
	TULARE	ORANGE	114,639	117,980
	IMPERIAL	RIGHTS OF WAY	105,572	N/A
		Total	**957,477**	
GLYPHOSATE, MONOAMMONIUM SALT	LOS ANGELES	LANDSCAPE MAINTENANCE	9,882	N/A
	LOS ANGELES	RIGHTS OF WAY	6,328	N/A
	SAN JOAQUIN	RIGHTS OF WAY	5,168	N/A
	SANTA CLARA	RIGHTS OF WAY	4,719	N/A
	SANTA CLARA	LANDSCAPE MAINTENANCE	4,419	N/A
		Total	**30,517**	
GLYPHOSATE, POTASSIUM SALT	KERN	ALMOND	181,668	164,038
	FRESNO	ALMOND	95,304	70,535
	FRESNO	COTTON	73,668	68,945
	KINGS	COTTON	58,394	62,818
	TULARE	CORN (FORAGE - FODDER)	65,124	57,999
		Total	**474,159**	
GLYPHOSATE-TRIMESIUM	FRESNO	NECTARINE	2,179	923
	SAN JOAQUIN	SOIL FUMIGATION/PREPLANT	2,067	1,933
	GLENN	ALMOND	2,052	817
	FRESNO	PEACH	1,003	368
	SUTTER	UNCULTIVATED AG	849	881
		Total	**8,150**	

2.5 Assessed Species

The CRLF was federally listed as a threatened species by USFWS effective June 24, 1996 (USFWS 1996). It is one of two subspecies of the red-legged frog and is the largest native frog in the western United States (USFWS 2002). A brief summary of information regarding CRLF distribution, reproduction, diet, and habitat requirements is provided in Sections 2.5.1 through 2.5.4, respectively. Further information on the status, distribution, and life history of and specific threats to the CRLF is provided in **Attachment 1**.

Final critical habitat for the CRLF was designated by USFWS on April 13, 2006 (USFWS 2006; 71 FR 19244-19346). Further information on designated critical habitat for the CRLF is provided in Section 2.6.

2.5.1 Distribution

The CRLF is endemic to California and Baja California (Mexico) and historically inhabited 46 counties in California including the Central Valley and both coastal and interior mountain ranges (USFWS 1996). Its range has been reduced by about 70%, and the species currently resides in 22 counties in California (USFWS 1996). The species has an elevational range of near sea level to 1,500 meters (5,200 feet) (Jennings and Hayes 1994); however, nearly all of the known CRLF populations have been documented below 1,050 meters (3,500 feet) (USFWS 2002).

Populations currently exist along the northern California coast, northern Transverse Ranges (USFWS 2002), foothills of the Sierra Nevada (5-6 populations), and in southern California south of Santa Barbara (two populations) (Fellers 2005a). Relatively larger numbers of CRLFs are located between Marin and Santa Barbara Counties (Jennings and Hayes 1994). A total of 243 streams or drainages are believed to be currently occupied by the species, with the greatest numbers in Monterey, San Luis Obispo, and Santa Barbara counties (USFWS 1996). Occupied drainages or watersheds include all bodies of water that support CRLFs (i.e., streams, creeks, tributaries, associated natural and artificial ponds, and adjacent drainages), and habitats through which CRLFs can move (i.e., riparian vegetation, uplands) (USFWS 2002).

The distribution of CRLFs within California is addressed in this assessment using four categories of location including recovery units, core areas, designated critical habitat, and known occurrences of the CRLF reported in the California Natural Diversity Database (CNDDB) that are not included within core areas and/or designated critical habitat (see **Figure 2.2**). Recovery units, core areas, and other known occurrences of the CRLF from the CNDDB are described in further detail in this section, and designated critical habitat is addressed in Section 2.6. Recovery units are large areas defined at the watershed level that have similar conservation needs and management strategies. The recovery unit is primarily an administrative designation, and land area within the recovery unit boundary is not exclusively CRLF habitat. Core areas are smaller areas within the recovery units that comprise portions of the species' historic and current range and have been determined by USFWS to be important in the preservation of the species. Designated

critical habitat is generally contained within the core areas, although a number of critical habitat units are outside the boundaries of core areas, but within the boundaries of the recovery units. Additional information on CRLF occurrences from the CNDDB is used to cover the current range of the species not included in core areas and/or designated critical habitat, but within the recovery units.

Recovery Units

Eight recovery units have been established by USFWS for the CRLF. These areas are considered essential to the recovery of the species, and the status of the CRLF "may be considered within the smaller scale of the recovery units, as opposed to the statewide range" (USFWS 2002). Recovery units reflect areas with similar conservation needs and population statuses, and therefore, similar recovery goals. The eight units described for the CRLF are delineated by watershed boundaries defined by US Geological Survey (USGS) hydrologic units and are limited to the elevational maximum for the species of 1,500 m above sea level. The eight recovery units for the CRLF are listed in **Table 2.10** and shown in **Figure 2.2**.

Core Areas

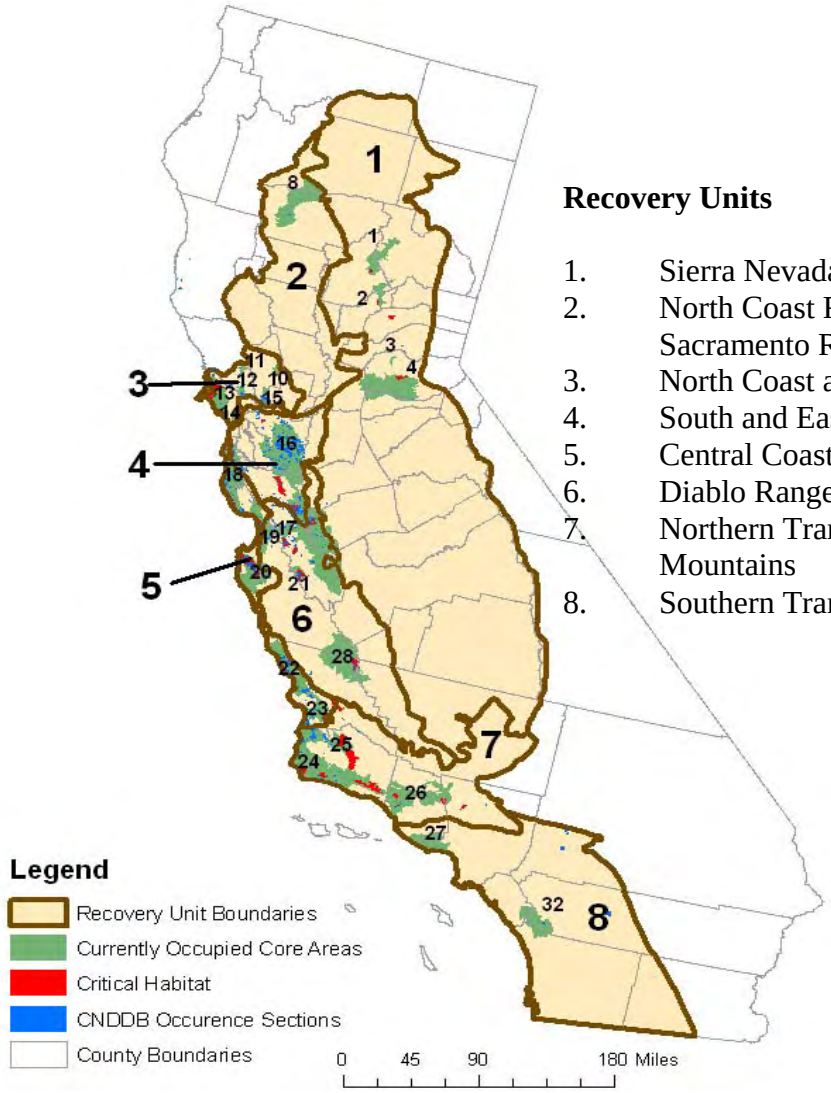
USFWS has designated 35 core areas across the eight recovery units to focus their recovery efforts for the CRLF (see **Figure 2.2**). **Table 2.10** summarizes the geographical relationship among recovery units, core areas, and designated critical habitat. The core areas, which are distributed throughout portions of the historic and current range of the species, represent areas that allow for long-term viability of existing populations and reestablishment of populations within their historic range. These areas were selected because they: 1) contain existing viable populations; or 2) they contribute to the connectivity of other habitat areas (USFWS 2002). Core area protection and enhancement are vital for maintenance and expansion of the CRLF's distribution and population throughout its range.

For purposes of this assessment, designated critical habitat, currently occupied (post-1985) core areas, and additional known occurrences of the CRLF from the CNDDB are considered. Historically occupied sections of the core areas are not evaluated as part of this assessment because the USFWS Recovery Plan (USFWS 2002) indicates that CRLFs are extirpated from these areas. A summary of currently and historically occupied core areas is provided in **Table 2.10** (currently occupied core areas are bolded). While core areas are considered essential for recovery of the CRLF, core areas are not federally-designated critical habitat, although designated critical habitat is generally contained within these core recovery areas. It should be noted, however, that several critical habitat units are located outside of the core areas, but within the recovery units. The focus of this assessment is currently occupied core areas, designated critical habitat, and other known CNDDB CRLF occurrences within the recovery units. Federally-designated critical habitat for the CRLF is further explained in Section 2.6.

Table 2.10 California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat				
Recovery Unit [1] (Figure 2.a)	**Core Areas [2,7] (Figure 2.a)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
Sierra Nevada Foothills and Central Valley (1) (eastern boundary is the 1,500m elevation line)	**Cottonwood Creek (partial) (8)**	--	✓	
	Feather River (1)	BUT-1A-B	✓	
	Yuba River-S. Fork Feather River (2)	YUB-1	✓	
	--	NEV-1[6]		
	Traverse Creek/Middle Fork American River/Rubicon (3)	--	✓	
	Consumnes River (4)	ELD-1	✓	
	S. Fork Calaveras River (5)	--		✓
	Tuolumne River (6)	--		✓
	Piney Creek (7)	--		✓
	East San Francisco Bay (partial)(16)	--	✓	
North Coast Range Foothills and Western Sacramento River Valley (2)	**Cottonwood Creek (8)**	--	✓	
	Putah Creek-Cache Creek (9)	--		✓
	Jameson Canyon – Lower Napa Valley (partial) (15)	--	✓	
	Belvedere Lagoon (partial) (14)	--	✓	
	Pt. Reyes Peninsula (partial) (13)	--	✓	
North Coast and North San Francisco Bay (3)	Putah Creek-Cache Creek (partial) (9)	--		✓
	Lake Berryessa Tributaries (10)	NAP-1	✓	
	Upper Sonoma Creek (11)	--	✓	
	Petaluma Creek-Sonoma Creek (12)	--	✓	
	Pt. Reyes Peninsula (13)	MRN-1, MRN-2	✓	
	Belvedere Lagoon (14)	--	✓	
	Jameson Canyon-Lower Napa River (15)	SOL-1	✓	
South and East San Francisco Bay (4)	--	CCS-1A[6]		
	East San Francisco Bay (partial) (16)	ALA-1A, ALA-1B, STC-1B	✓	
	--	STC-1A[6]		
	South San Francisco Bay (partial) (18)	SNM-1A	✓	
Central Coast (5)	**South San Francisco Bay (partial) (18)**	SNM-1A, SNM-2C, SCZ-1	✓	
	Watsonville Slough- Elkhorn Slough (partial) (19)	SCZ-2 [5]	✓	
	Carmel River-Santa Lucia (20)	MNT-2	✓	

Table 2.10 California Red-legged Frog Recovery Units with Overlapping Core Areas and Designated Critical Habitat				
Recovery Unit [1] (Figure 2.a)	**Core Areas [2,7] (Figure 2.a)**	**Critical Habitat Units [3]**	**Currently Occupied (post-1985) [4]**	**Historically Occupied [4]**
	Estero Bay (22)	--	✓	
	--	SLO-8[6]		
	Arroyo Grande Creek (23)	--	✓	
	Santa Maria River-Santa Ynez River (24)	--	✓	
Diablo Range and Salinas Valley (6)	**East San Francisco Bay (partial) (16)**	MER-1A-B, STC-1B	✓	
	--	SNB-1[6], SNB-2[6]		
	Santa Clara Valley (17)	--	✓	
	Watsonville Slough- Elkhorn Slough (partial)(19)	MNT-1	✓	
	Carmel River-Santa Lucia (partial)(20)	--	✓	
	Gablan Range (21)	SNB-3	✓	
	Estrella River (28)	SLO-1A-B	✓	
Northern Transverse Ranges and Tehachapi Mountains (7)	--	SLO-8[6]		
	Santa Maria River-Santa Ynez River (24)	STB-4, STB-5, STB-7	✓	
	Sisquoc River (25)	STB-1, STB-3	✓	
	Ventura River-Santa Clara River (26)	VEN-1, VEN-2, VEN-3	✓	
	--	LOS-1[6]		
Southern Transverse and Peninsular Ranges (8)	**Santa Monica Bay-Ventura Coastal Streams (27)**	--	✓	
	San Gabriel Mountain (29)	--		✓
	Forks of the Mojave (30)	--		✓
	Santa Ana Mountain (31)	--		✓
	Santa Rosa Plateau (32)	--	✓	
	San Luis Rey (33)	--		✓
	Sweetwater (34)	--		✓
	Laguna Mountain (35)	--		✓

[1] Recovery units designated by the USFWS (USFWS 2000, pg 49).
[2] Core areas designated by the USFWS (USFWS 2000, pg 51).
[3] Critical habitat units designated by the USFWS on April 13, 2006 (USFWS 2006, 71 FR 19244-19346).
[4] Currently occupied (post-1985) and historically occupied core areas as designated by the USFWS (USFWS 2002, pg 54).
[5] Critical habitat unit where identified threats specifically included pesticides or agricultural runoff (USFWS 2002).
[6] Critical habitat units that are outside of core areas, but within recovery units.
[7] Currently occupied core areas that are included in this effects determination are bolded.



Recovery Units

1. Sierra Nevada Foothills and Central Valley
2. North Coast Range Foothills and Western Sacramento River Valley
3. North Coast and North San Francisco Bay
4. South and East San Francisco Bay
5. Central Coast
6. Diablo Range and Salinas Valley
7. Northern Transverse Ranges and Tehachapi Mountains
8. Southern Transverse and Peninsular Ranges

Figure 2.2 Recovery Unit, Core Area, Critical Habitat, and Occurrence Designations for CRLF

Core Areas

1. Feather River
2. Yuba River- S. Fork Feather River
3. Traverse Creek/ Middle Fork/ American R. Rubicon
4. Cosumnes River
5. South Fork Calaveras River*
6. Tuolumne River*
7. Piney Creek*
8. Cottonwood Creek
9. Putah Creek – Cache Creek*
10. Lake Berryessa Tributaries
11. Upper Sonoma Creek
12. Petaluma Creek – Sonoma Creek
13. Pt. Reyes Peninsula
14. Belvedere Lagoon
15. Jameson Canyon – Lower Napa River
16. East San Francisco Bay
17. Santa Clara Valley
18. South San Francisco Bay
19. Watsonville Slough-Elkhorn Slough
20. Carmel River – Santa Lucia
21. Gablan Range
22. Estero Bay
23. Arroyo Grange River
24. Santa Maria River – Santa Ynez River
25. Sisquoc River
26. Ventura River – Santa Clara River
27. Santa Monica Bay – Venura Coastal Streams
28. Estrella River
29. San Gabriel Mountain*
30. Forks of the Mojave*
31. Santa Ana Mountain*
32. Santa Rosa Plateau
33. San Luis Ray*
34. Sweetwater*
35. Laguna Mountain*

* Core areas that were historically occupied by the California red-legged frog are not included in the map

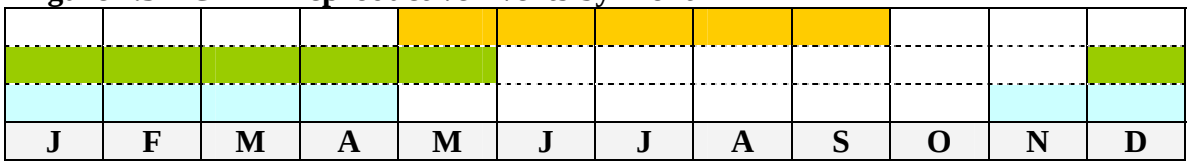
Other Known Occurrences from the CNDBB

The CNDDB provides location and natural history information on species found in California. The CNDDB serves as a repository for historical and current species location sightings. Information regarding known occurrences of CRLFs outside of the currently occupied core areas and designated critical habitat is considered in defining the current range of the CRLF. See: http://www.dfg.ca.gov/bdb/html/cnddb_info.html for additional information on the CNDDB.

2.5.2 Reproduction

CRLFs breed primarily in ponds; however, they may also breed in quiescent streams, marshes, and lagoons (Fellers 2005a). According to the Recovery Plan (USFWS 2002), CRLFs breed from November through late April. Peaks in spawning activity vary geographically; Fellers (2005b) reports peak spawning as early as January in parts of coastal central California. Eggs are fertilized as they are being laid. Egg masses are typically attached to emergent vegetation, such as bulrushes (*Scirpus* spp.) and cattails (*Typha* spp.) or roots and twigs, and float on or near the surface of the water (Hayes and Miyamoto 1984). Egg masses contain approximately 2000 to 6000 eggs ranging in size between 2 and 2.8 mm (Jennings and Hayes 1994). Embryos hatch 10 to 14 days after fertilization (Fellers 2005a) depending on water temperature. Egg predation is reported to be infrequent and most mortality is associated with the larval stage (particularly through predation by fish); however, predation on eggs by newts has also been reported (Rathburn 1998). Tadpoles require 11 to 28 weeks to metamorphose into juveniles (terrestrial-phase), typically between May and September (Jennings and Hayes 1994, USFWS 2002); tadpoles have been observed to over-winter (delay metamorphosis until the following year) (Fellers 2005b, USFWS 2002). Males reach sexual maturity at 2 years, and females reach sexual maturity at 3 years of age; adults have been reported to live 8 to 10 years (USFWS 2002). **Figure 2.3** depicts CRLF annual reproductive timing.

Figure 2.3 – CRLF Reproductive Events by Month



Light Blue = **Breeding/Egg Masses**
Green = **Tadpoles (except those that over-winter)**
Orange = **Young Juveniles**
Adults and juveniles can be present all year

2.5.3 Diet

Although the diet of CRLF aquatic-phase larvae (tadpoles) has not been studied specifically, it is assumed that their diet is similar to that of other frog species, with the aquatic phase feeding exclusively in water and consuming diatoms, algae, and detritus

(USFWS 2002). Tadpoles filter and entrap suspended algae (Seale and Beckvar, 1980) via mouthparts designed for effective grazing of periphyton (Wassersug, 1984, Kupferberg *et al.*; 1994; Kupferberg, 1997; Altig and McDiarmid, 1999).

Juvenile and adult CRLFs forage in aquatic and terrestrial habitats, and their diet differs greatly from that of larvae. The main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic and terrestrial invertebrates found along the shoreline and on the water surface. Hayes and Tennant (1985) report, based on a study examining the gut content of 35 juvenile and adult CRLFs, that the species feeds on as many as 42 different invertebrate taxa, including Arachnida, Amphipoda, Isopoda, Insecta, and Mollusca. The most commonly observed prey species were larval alderflies (*Sialis* cf. *californica*), pillbugs (*Armadilliadrium vulgare*), and water striders (*Gerris* sp). The preferred prey species, however, was the sowbug (Hayes and Tennant, 1985). This study suggests that CRLFs forage primarily above water, although the authors note other data reporting that adults also feed under water, are cannibalistic, and consume fish. For larger CRLFs, over 50% of the prey mass may consists of vertebrates such as mice, frogs, and fish, although aquatic and terrestrial invertebrates were the most numerous food items (Hayes and Tennant 1985). For adults, feeding activity takes place primarily at night; for juveniles feeding occurs during the day and at night (Hayes and Tennant 1985).

2.5.4 Habitat

CRLFs require aquatic habitat for breeding, but also use other habitat types including riparian and upland areas throughout their life cycle. CRLF use of their environment varies; they may complete their entire life cycle in a particular habitat or they may utilize multiple habitat types. Overall, populations are most likely to exist where multiple breeding areas are embedded within varying habitats used for dispersal (USFWS 2002). Generally, CRLFs utilize habitat with perennial or near-perennial water (Jennings et al. 1997). Dense vegetation close to water, shading, and water of moderate depth are habitat features that appear especially important for CRLF (Hayes and Jennings 1988). Breeding sites include streams, deep pools, backwaters within streams and creeks, ponds, marshes, sag ponds (land depressions between fault zones that have filled with water), dune ponds, and lagoons. Breeding adults have been found near deep (0.7 m) still or slow moving water surrounded by dense vegetation (USFWS 2002); however, the largest number of tadpoles have been found in shallower pools (0.26 – 0.5 m) (Reis, 1999). Data indicate that CRLFs do not frequently inhabit vernal pools, as conditions in these habitats generally are not suitable (Hayes and Jennings 1988).

CRLFs also frequently breed in artificial impoundments such as stock ponds, although additional research is needed to identify habitat requirements within artificial ponds (USFWS 2002). Adult CRLFs use dense, shrubby, or emergent vegetation closely associated with deep-water pools bordered with cattails and dense stands of overhanging vegetation (http://www.fws.gov/endangered/features/rl_frog/rlfrog.html#where).

In general, dispersal and habitat use depends on climatic conditions, habitat suitability, and life stage. Adults rely on riparian vegetation for resting, feeding, and dispersal. The

foraging quality of the riparian habitat depends on moisture, composition of the plant community, and presence of pools and backwater aquatic areas for breeding. CRLFs can be found living within streams at distances up to 3 km (2 miles) from their breeding site and have been found up to 30 m (100 feet) from water in dense riparian vegetation for up to 77 days (USFWS 2002).

During dry periods, the CRLF is rarely found far from water, although it will sometimes disperse from its breeding habitat to forage and seek other suitable habitat under downed trees or logs, industrial debris, and agricultural features (USFWS 2002). According to Jennings and Hayes (1994), CRLFs also use small mammal burrows and moist leaf litter as habitat. In addition, CRLFs may also use large cracks in the bottom of dried ponds as refugia; these cracks may provide moisture for individuals avoiding predation and solar exposure (Alvarez 2000).

2.6 Designated Critical Habitat

In a final rule published on April 13, 2006, 34 separate units of critical habitat were designated for the CRLF by USFWS (USFWS 2006; FR 51 19244-19346). A summary of the 34 critical habitat units relative to USFWS-designated recovery units and core areas (previously discussed in Section 2.5.1) is provided in Table 2.10.

'Critical habitat' is defined in the ESA as the geographic area occupied by the species at the time of the listing where the physical and biological features necessary for the conservation of the species exist, and there is a need for special management to protect the listed species. It may also include areas outside the occupied area at the time of listing if such areas are 'essential to the conservation of the species.' All designated critical habitat for the CRLF was occupied at the time of listing. Critical habitat receives protection under Section 7 of the ESA through prohibition against destruction or adverse modification with regard to actions carried out, funded, or authorized by a federal agency. Section 7 requires consultation on federal actions that are likely to result in the destruction or adverse modification of critical habitat.

To be included in a critical habitat designation, the habitat must be 'essential to the conservation of the species.' Critical habitat designations identify, to the extent known using the best scientific and commercial data available, habitat areas that provide essential life cycle needs of the species or areas that contain certain primary constituent elements (PCEs) (as defined in 50 CFR 414.12(b)). PCEs include, but are not limited to, space for individual and population growth and for normal behavior; food, water, air, light, minerals, or other nutritional or physiological requirements; cover or shelter; sites for breeding, reproduction, rearing (or development) of offspring; and habitats that are protected from disturbance or are representative of the historic geographical and ecological distributions of a species. The designated critical habitat areas for the CRLF are considered to have the following PCEs that justify critical habitat designation:

- Breeding aquatic habitat;
- Non-breeding aquatic habitat;

- Upland habitat; and
- Dispersal habitat.

Further description of these habitat types is provided in **Attachment 1**.

Occupied habitat may be included in the critical habitat only if essential features within the habitat may require special management or protection. Therefore, USFWS does not include areas where existing management is sufficient to conserve the species. Critical habitat is designated outside the geographic area presently occupied by the species only when a designation limited to its present range would be inadequate to ensure the conservation of the species. For the CRLF, all designated critical habitat units contain all four of the PCEs, and were occupied by the CRLF at the time of FR listing notice in April 2006. The FR notice designating critical habitat for the CRLF includes a special rule exempting routine ranching activities associated with livestock ranching from incidental take prohibitions. The purpose of this exemption is to promote the conservation of rangelands, which could be beneficial to the CRLF, and to reduce the rate of conversion to other land uses that are incompatible with CRLF conservation. Please see **Attachment 1** for a full explanation on this special rule.

USFWS has established adverse modification standards for designated critical habitat (USFWS 2006). Activities that may destroy or adversely modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the species. Evaluation of actions related to use of glyphosate that may alter the PCEs of the CRLF's critical habitat form the basis of the critical habitat impact analysis. According to USFWS (2006), activities that may affect critical habitat and therefore result in adverse effects to the CRLF include, but are not limited to the following:

(1) Significant alteration of water chemistry or temperature to levels beyond the tolerances of the CRLF that result in direct or cumulative adverse effects to individuals and their life-cycles.
(2) Significant increase in sediment deposition within the stream channel or pond or disturbance of upland foraging and dispersal habitat that could result in elimination or reduction of habitat necessary for the growth and reproduction of the CRLF by increasing the sediment deposition to levels that would adversely affect their ability to complete their life cycles.
(3) Significant alteration of channel/pond morphology or geometry that may lead to changes to the hydrologic functioning of the stream or pond and alter the timing, duration, water flows, and levels that would degrade or eliminate the CRLF and/or its habitat. Such an effect could also lead to increased sedimentation and degradation in water quality to levels that are beyond the CRLF's tolerances.
(4) Elimination of upland foraging and/or aestivating habitat or dispersal habitat.
(5) Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
(6) Alteration or elimination of the CRLF's food sources or prey base (also evaluated as indirect effects to the CRLF).

As previously noted in Section 2.1, the Agency believes that the analysis of direct and indirect effects to listed species provides the basis for an analysis of potential effects on the designated critical habitat. Because glyphosate is expected to directly impact living organisms within the action area, critical habitat analysis for glyphosate is limited in a practical sense to those PCEs of critical habitat that are biological or that can be reasonably linked to biologically mediated processes.

2.7 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the federal action and not merely the immediate area involved in the action (50 CFR 402.02). It is recognized that the overall action area for the national registration of glyphosate is likely to encompass considerable portions of the United States based on the large array of agricultural uses. However, the scope of this assessment limits consideration of the overall action area to those portions that may be applicable to the protection of the CRLF and its designated critical habitat within the state of California. The Agency's approach to defining the action area under the provisions of the Overview Document (USEPA 2004) considers the results of the risk assessment process to establish boundaries for that action area with the understanding that exposures below the Agency's defined Levels of Concern (LOCs) constitute a no-effect threshold. For the purposes of this assessment, attention will be focused on the footprint of the action (i.e., the area where pesticide application occurs), plus all areas where offsite transport (i.e., spray drift, downstream dilution, etc.) may result in potential exposure within the state of California that exceeds the Agency's LOCs.

Deriving the geographical extent of this portion of the action area is based on consideration of the types of effects that glyphosate may be expected to have on the environment, the exposure levels to glyphosate that are associated with those effects, and the best available information concerning the use of glyphosate and its fate and transport within the state of California. Specific measures of ecological effect for the CRLF that define the action area include any direct and indirect toxic effect to the CRLF and any potential modification of its critical habitat, including reduction in survival, growth, and fecundity as well as the full suite of sublethal effects available in the effects literature. Therefore, the action area extends to a point where environmental exposures are below any measured lethal or sublethal effect threshold for any biological entity at the whole organism, organ, tissue, and cellular level of organization. In situations where it is not possible to determine the threshold for an observed effect, the action area is not spatially limited and is assumed to be the entire state of California.

The definition of action area requires a stepwise approach that begins with an understanding of the federal action. The federal action is defined by the currently labeled uses for glyphosate. An analysis of labeled uses and review of available product labels was completed. Several of the currently labeled uses are classified as special local needs (SLNs) or are restricted to specific states and are consequently excluded from this assessment. In addition, a distinction has been made between food use crops and those that are non-food/non-agricultural uses. For those uses relevant to the CRLF, the analysis

indicates that, for glyphosate, the following agricultural uses are considered as part of the federal action evaluated in this assessment:

- alfalfa, clover, non-grass forage/fodder/straw/hay, almond, pecan, pistachio, walnuts (english/black), avocado, grapefruit, lemon, orange, tangelos, tangerine, kumquat, broccoli, cabbage, cauliflower, horseradish, mustard, corn- field, corn-pop, corn- sweet corn, (silage), corn (unspecified), millet – proso (broomcorn), sunflower, cotton, cotton (unspecified), apple, apricot, cherry, fig, nectarine, peach, pear, pomegranate, prune, garlic, leek, grapes, brussels sprouts, chicory, endive (escarole), lettuce, parsley, melons, melons- cantaloupe, melons-honeydew, melons- mango, melons- musk, melons- water, melons- winter, casaba/crenshaw/honeydew/persian), pumpkins, olive, onions, potato white/irish, rutabaga sweet potato, turnip (greens), turnip (root), artichoke, artichoke-Jerusalem, asparagus, beans, beets, carrots (including tops), celery, pepper, peas-dried type, peas, strawberry, sugar beet, sugar beet (including tops), parsnip, eggplant, okra, tomatillo, tomato, barley, oats, rye, safflower, sorghum, sorghum (silage), sorghum (unspecified), triticale, wheat, blueberry and passion fruit (granadilla).

In addition, the following non-food and non-agricultural uses are considered:

- Christmas tree plantations, conifer release, forest nursery plantings (fir transplant purposes), forest trees (all or unspecified), emergent aquatic plants, agricultural/farm structures/buildings and equipment, commercial storages/warehouses premises, household/domestic dwellings outdoor premises, industrial areas, non-agricultural outdoor buildings/structures, path/patios, paved areas (private roads/sidewalks), urban areas, ornamental and/or shade trees, ground cover, herbaceous plants, non-flowering plants, nursery stock, bermudagrass, pastures, rangeland, ornamental lawns and turf, recreational areas, agricultural rights-of-way/fence rows/hedgerows, ornamental sod farm (turf), grasses grown for seed and aquatic weed control.

Following a determination of the assessed uses, an evaluation of the potential "footprint" of glyphosate use patterns (i.e., the area where pesticide application occurs) is determined. This "footprint" represents the initial area of concern, based on an analysis of available land cover data for the state of California. The initial area of concern is defined as all land cover types and the stream reaches within the land cover areas that represent the labeled uses described above. Based on glyphosate use patterns, the entire state of California is considered to be the initial area of concern.

Once the initial area of concern is defined, the next step is to define the potential boundaries of the action area by determining the extent of offsite transport via spray drift and runoff where exposure of one or more taxonomic groups to the pesticide exceeds the listed species LOCs.

As previously discussed, the action area is defined by the most sensitive measure of direct and indirect ecological toxic effects including reduction in survival, growth, reproduction, and the entire suite of sublethal effects from valid, peer-reviewed studies.

Due to the lack of a defined no effect concentration in a subchronic freshwater fish study from the open literature (Jiraungkoorskul et. al., 2003), the spatial extent of the action area (i.e., the boundary where exposures and potential effects are less than the Agency's LOC) for glyphosate cannot be determined. Therefore, it is assumed that the action area encompasses the entire state of California, regardless of the spatial extent (i.e., initial area of concern or footprint) of the pesticide use(s).

2.8 Assessment Endpoints and Measures of Ecological Effect

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[4] Selection of the assessment endpoints is based on valued entities (*e.g.,* CRLF, organisms important in the life cycle of the CRLF, and the PCEs of its designated critical habitat), the ecosystems potentially at risk (*e.g.,* waterbodies, riparian vegetation, and upland and dispersal habitats), the migration pathways of glyphosate (*e.g.,* runoff, spray drift, etc.), and the routes by which ecological receptors are exposed to glyphosate *(e.g.,* direct contact, *etc.).*

2.8.1. Assessment Endpoints for the CRLF

Assessment endpoints for the CRLF include direct toxic effects on the survival, reproduction, and growth of the CRLF, as well as indirect effects, such as reduction of the prey base or modification of its habitat. In addition, potential modification of critical habitat is assessed by evaluating potential effects to PCEs, which are components of the habitat areas that provide essential life cycle needs of the CRLF. Each assessment endpoint requires one or more "measures of ecological effect," defined as changes in the attributes of an assessment endpoint or changes in a surrogate entity or attribute in response to exposure to a pesticide. Specific measures of ecological effect are generally evaluated based on acute and chronic toxicity information from registrant-submitted guideline tests that are performed on a limited number of organisms. Additional ecological effects data from the open literature are also considered. It should be noted that assessment endpoints are limited to direct and indirect effects associated with survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According to the Overview Document (USEPA 2004), the Agency relies on acute and chronic effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

A complete discussion of all the toxicity data available for this risk assessment, including resulting measures of ecological effect selected for each taxonomic group of concern, is included in Section 4 of this document. A summary of the assessment endpoints and

[4] From U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.

measures of ecological effect selected to characterize potential assessed direct and indirect CRLF risks associated with exposure to glyphosate is provided in **Table 2.11**.

Table 2.11 Assessment Endpoints and Measures of Ecological Effects	
Assessment Endpoint	**Measures of Ecological Effects[5]**
Aquatic-Phase CRLF (Eggs, larvae, juveniles, and adults)[a]	
Direct Effects	
1. Survival, growth, and reproduction of CRLF	1a. Amphibian acute LC_{50} (ECOTOX) or most sensitive fish acute LC_{50} (guideline or ECOTOX) if no suitable amphibian data are available: bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L. Formulations: fish acute LC_{50} terrestrial uses: 3.17 mg/L and aquatic uses: 824 mg/L 1b. Amphibian chronic NOAEC (ECOTOX) or most sensitive fish chronic NOAEC (guideline or ECOTOX): chronic study with leopard frog NOAEC/LOAEC: 1.8/>1.8 mg a.e./L. Formulations: chronic study with leopard frog LOAEC: 1.9 mg formulation/L terrestrial uses; Study not available for aquatic uses. 1c. Amphibian early-life stage data (ECOTOX) or most sensitive fish early-life stage NOAEC (guideline or ECOTOX): study not available
Indirect Effects and Critical Habitat Effects	
2. Survival, growth, and reproduction of CRLF individuals via indirect effects on aquatic prey food supply (*i.e.*, fish, freshwater invertebrates, non-vascular plants)	2a. Most sensitive fish, aquatic invertebrate, and aquatic plant EC_{50} or LC_{50} (guideline or ECOTOX): bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L; water flea 48-hr EC_{50}: 53.2 mg a.e./L; green algae 96-hr EC_{50}: 12.1 mg a.e./L. For formulations: freshwater fish terrestrial uses 3.17 mg/L and aquatic uses: 824 mg/L; freshwater invertebrates terrestrial uses 3 mg/L and aquatic uses 164.3 mg/L; non-vascular plants: EC_{50}: 0.39 mg/L (terrestrial and aquatic uses) 2b. Most sensitive aquatic invertebrate and fish chronic NOAEC (guideline or ECOTOX): Life cycle study with fathead minnow NOAEC/LOAEC: 25.7/>25.7 mg a.e./L; water flea chronic NOAEC: 49.9 mg a.e./L. For formulations: studies not available.
3. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, food supply, and/or primary productivity (*i.e.*, aquatic plant community)	3a. Vascular plant acute EC_{50} (duckweed guideline test or ECOTOX vascular plant): duckweed growth inhibition EC_{50}: 11.9 mg a.e./L. For formulations, terrestrial uses 2 mg/L and aquatic uses 25 mg/L. 3b. Non-vascular plant acute EC_{50} (freshwater algae or diatom, or ECOTOX non-vascular): green algae 96-hr EC_{50}: 12.1 mg a.e./L. For formulations, 0.39 mg/L for terrestrial and aquatic uses.
4. Survival, growth, and reproduction of CRLF	4a. Distribution of EC_{25} values for monocots

[5] All registrant-submitted and open literature toxicity data reviewed for this assessment are included in Appendix J.

Table 2.11 Assessment Endpoints and Measures of Ecological Effects	
Assessment Endpoint	**Measures of Ecological Effects[5]**
individuals via effects to riparian vegetation	(seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 to >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A 4b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: > 4 to > 5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A
Terrestrial-Phase CRLF (Juveniles and adults)	
Direct Effects	
5. Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	5a. Most sensitive bird[b] or terrestrial-phase amphibian acute LC_{50} or LD_{50} (guideline or ECOTOX): bobwhite acute LD_{50}: > 3196.3 mg a.e./kg bw; bobwhite subacute dietary LC_{50}: > 4971.2 ppm a.e. 5b. Most sensitive bird[b] or terrestrial-phase amphibian chronic NOAEC (guideline or ECOTOX): bobwhite quail reproduction NOAEC 830 ppm a.e.
Indirect Effects and Critical Habitat Effects	
6. Survival, growth, and reproduction of CRLF individuals via effects on terrestrial prey (*i.e.*,terrestrial invertebrates, small mammals , and frogs)	6a. Most sensitive terrestrial invertebrate and vertebrate acute EC_{50} or LC_{50} (guideline or ECOTOX)[c]: honey bee acute contact LD_{50} > 100 µg a.i./bee; rat LD_{50}: >4800 mg a.e./kg; bobwhite acute LD_{50}: > 3196.3 mg a.e./kg bw; bobwhite subacute dietary LC_{50}: > 4971.2 ppm a.e. 6b. Most sensitive terrestrial invertebrate and vertebrate chronic NOAEC (guideline or ECOTOX): No chronic terrestrial invertebrate study available; bobwhite quail reproduction NOAEC 830 ppm a.e.; rat reproduction study NOAEL: 500 mg a.e./kg bw/day, NOAEC: 10000 ppm
7. Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.,* riparian and upland vegetation)	7a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A 4b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A

[a] Adult frogs are no longer in the "aquatic phase" of the amphibian life cycle; however, submerged adult frogs are considered "aquatic" for the purposes of this assessment because exposure pathways in the water are considerably different that exposure pathways on land.
[b] Birds are used as surrogates for terrestrial phase amphibians.

2.8.2 Assessment Endpoints for Designated Critical Habitat

As previously discussed, designated critical habitat is assessed to evaluate actions related to the use of glyphosate that may alter the PCEs of the CRLF's critical habitat. PCEs for the CRLF were previously described in Section 2.6. Actions that may modify critical habitat are those that alter the PCEs and jeopardize the continued existence of the CRLF. Therefore, these actions are identified as assessment endpoints. It should be noted that

evaluation of PCEs as assessment endpoints is limited to those of a biological nature (i.e., the biological resource requirements for the listed species associated with the critical habitat) and those for which glyphosate effects data are available.

Adverse modification to the critical habitat of the CRLF includes, but is not limited to, the following, as specified by USFWS (2006):

1. Alteration of water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs.
2. Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.
3. Significant increase in sediment deposition within the stream channel or pond or disturbance of upland foraging and dispersal habitat.
4. Significant alteration of channel/pond morphology or geometry.
5. Elimination of upland foraging and/or aestivating habitat, as well as dispersal habitat.
6. Introduction, spread, or augmentation of non-native aquatic species in stream segments or ponds used by the CRLF.
7. Alteration or elimination of the CRLF's food sources or prey base.

Measures of such possible effects by labeled use of glyphosate on critical habitat of the CRLF are described in **Table 2.12**. Some components of these PCEs are associated with physical abiotic features (e.g., presence and/or depth of a water body, or distance between two sites), which are not expected to be measurably altered by use of pesticides. Assessment endpoints used for the analysis of designated critical habitat are based on the adverse modification standard established by USFWS (2006).

Table 2.12 Summary of Assessment Endpoints and Measures of Ecological Effect for Primary Constituent Elements of Designated Critical Habitat	
Assessment Endpoint	**Measures of Ecological Effect**
Aquatic-Phase CRLF PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)	
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	a. Most sensitive aquatic plant EC_{50} (guideline or ECOTOX): duckweed growth inhibition EC_{50}: 11.9 mg a.e./L. For formulations, 0.39 mg/L (freshwater diatom) for both terrestrial and aquatic uses. b. Distribution of EC_{25} values for terrestrial monocots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A c. Distribution of EC_{25} values for terrestrial dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	a. Most sensitive EC_{50} values for aquatic plants (guideline or ECOTOX): duckweed growth inhibition EC_{50}: 11.9 mg a.e./L. For formulations: freshwater diatom 96-hr EC_{50}: 0.39 mg/L b. Distribution of EC_{25} values for terrestrial monocots (seedling emergence or vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A c. Distribution of EC_{25} values for terrestrial dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	a. Most sensitive EC_{50} or LC_{50} values for fish or aquatic-phase amphibians and aquatic invertebrates (guideline or ECOTOX): bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L; water flea 48-hr EC_{50}: 53.2 mg a.e./L. For formulations: freshwater fish: terrestrial uses 3.17 mg/L and aquatic uses: 824 mg/L; freshwater invertebrates terrestrial uses 3 mg/L and aquatic uses 164.3 mg/L b. Most sensitive NOAEC values for fish or aquatic-phase amphibians and aquatic invertebrates (guideline or ECOTOX): chronic study with leopard frog NOAEC/LOAEC: 1.8/>1.8 mg a.e./L.; water flea chronic NOAEC: 49.9 mg a.e./L. For formulations: chronic study with leopard frog LOAEC: 1.9 mg formulation/L terrestrial uses; studies not available for aquatic uses or for aquatic invertebrates.
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae)	a. Most sensitive aquatic plant EC_{50} (guideline or ECOTOX): green algae EC_{50}: 12.1 mg a.e./L. For formulations: freshwater diatom 96-hr EC_{50}: 0.39 mg/L

Terrestrial-Phase CRLF PCEs (Upland Habitat and Dispersal Habitat)	
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	a. Distribution of EC_{25} values for monocots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - > 5 lbs a.e/A; EC_{25} vegetative vigor: 0.16 – 0.98 lbs a.e./A b. Distribution of EC_{25} values for dicots (seedling emergence, vegetative vigor, or ECOTOX): EC_{25} seedling emergence: >4 - >5 lbs a.e/A; EC_{25} vegetative vigor: 0.074 – 0.89 lbs a.e./A c. Most sensitive food source acute EC_{50}/LC_{50} and NOAEC values for terrestrial vertebrates (mammals) and invertebrates, birds or terrestrial-phase amphibians, and freshwater fish: rat LD_{50}: >4800 mg a.e./kg; honey bee acute contact LD_{50} > 100 µg a.i./bee; bobwhite acute LD_{50}: > 3196.3 mg a.e./kg bw; bobwhite subacute dietary LC_{50}: > 4971.2 ppm a.e. and bluegill sunfish 96-hr LC_{50}: 43 mg a.e./L. Chronic NOAEC: rat reproduction study NOAEL: 500 mg a.e./kg bw/day, NOAEC: 10000 ppm; no chronic terrestrial invertebrate study available; bobwhite quail reproduction NOAEC 830 ppm a.e.and life cycle study with fathead minnow NOAEC/LOAEC: 25.7/>25.7 mg a.e./L.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	

[a] Physico-chemical water quality parameters such as salinity, pH, and hardness are not evaluated because these processes are not biologically mediated and, therefore, are not relevant to the endpoints included in this assessment.

2.9 Conceptual Model

2.9.1 Risk Hypotheses

Risk hypotheses are specific assumptions about potential adverse effects (*i.e.*,changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (U.S. EPA, 1998). For this assessment, the risk is stressor-linked, where the stressor is the release of glyphosate to the environment. The following risk hypotheses are presumed for this endangered species assessment:

The labeled use of glyphosate within the action area may:

- directly affect the CRLF by causing mortality or by adversely affecting growth or fecundity;
- indirectly affect the CRLF by reducing or changing the composition of food supply;
- indirectly affect the CRLF or modify designated critical habitat by reducing or changing the composition of the aquatic plant community in the ponds and streams comprising the species' current range and designated critical habitat, thus affecting primary productivity and/or cover;
- indirectly affect the CRLF or modify designated critical habitat by reducing or changing the composition of the terrestrial plant community (i.e., riparian habitat)

required to maintain acceptable water quality and habitat in the ponds and streams comprising the species' current range and designated critical habitat;

- modify the designated critical habitat of the CRLF by reducing or changing breeding and non-breeding aquatic habitat (via modification of water quality parameters, habitat morphology, and/or sedimentation);
- modify the designated critical habitat of the CRLF by reducing the food supply required for normal growth and viability of juvenile and adult CRLFs;
- modify the designated critical habitat of the CRLF by reducing or changing upland habitat within 200 ft of the edge of the riparian vegetation necessary for shelter, foraging, and predator avoidance.
- modify the designated critical habitat of the CRLF by reducing or changing dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.
- modify the designated critical habitat of the CRLF by altering chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs.

2.9.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the glyphosate release mechanisms, biological receptor types, and effects endpoints of potential concern. The conceptual models for aquatic and terrestrial phases of the CRLF are shown in **Figures 2.4 and 2.5**, respectively, and the conceptual models for the aquatic and terrestrial PCE components of critical habitat are shown in **Figures 2.6 and 2.7**, respectively. Exposure routes shown in dashed lines are not quantitatively considered because the contribution of those potential exposure routes to potential risks to the CRLF and modification to designated critical habitat is expected to be negligible.

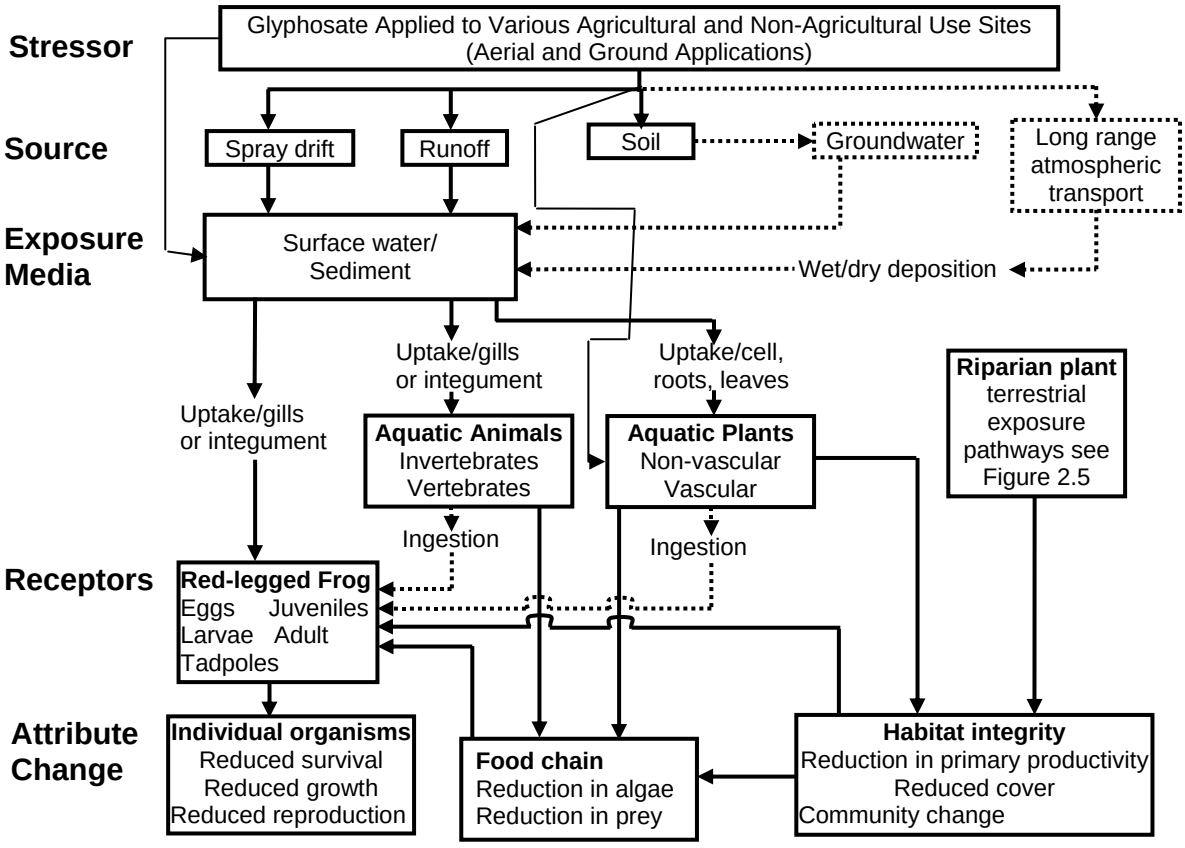


Figure 2.4 Conceptual Model for Aquatic-Phase of the CRLF

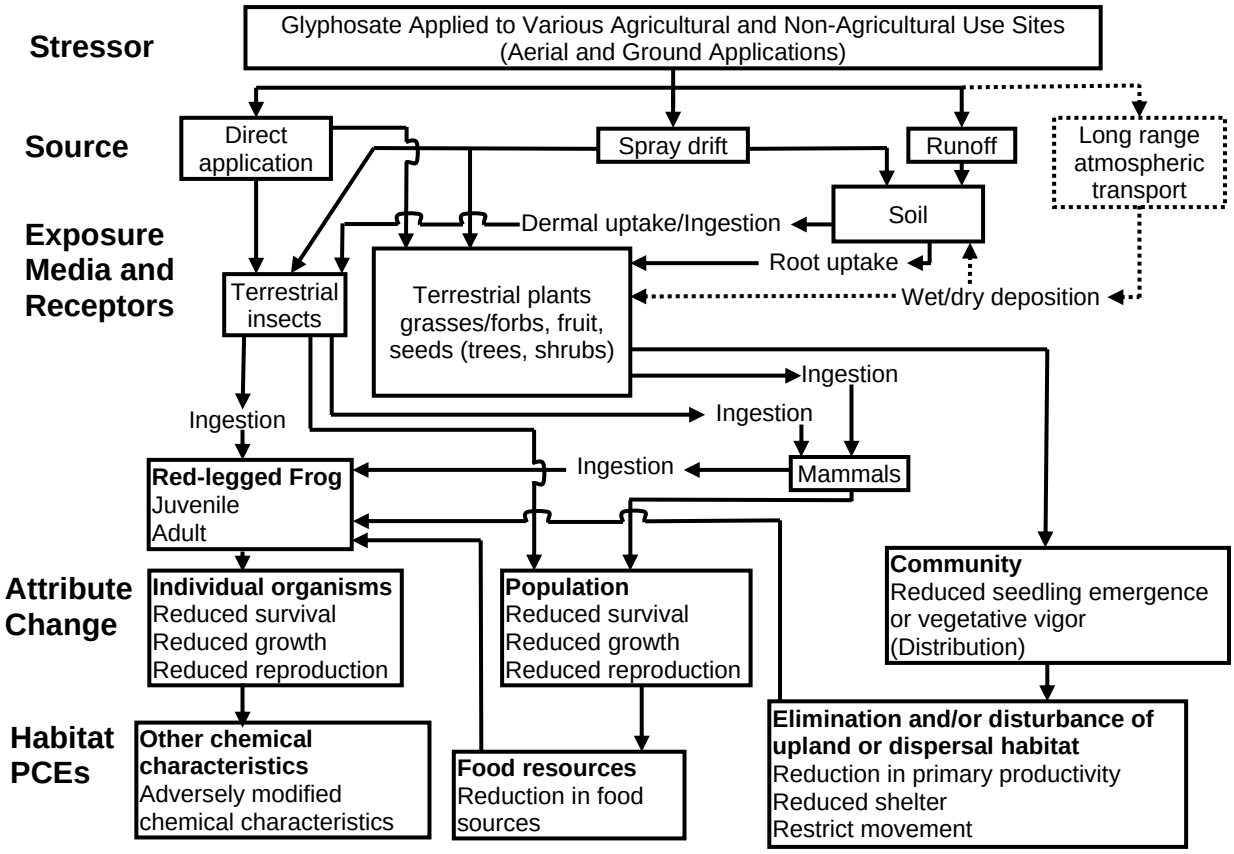


Figure 2.5 Conceptual Model for Terrestrial-Phase of the CRLF

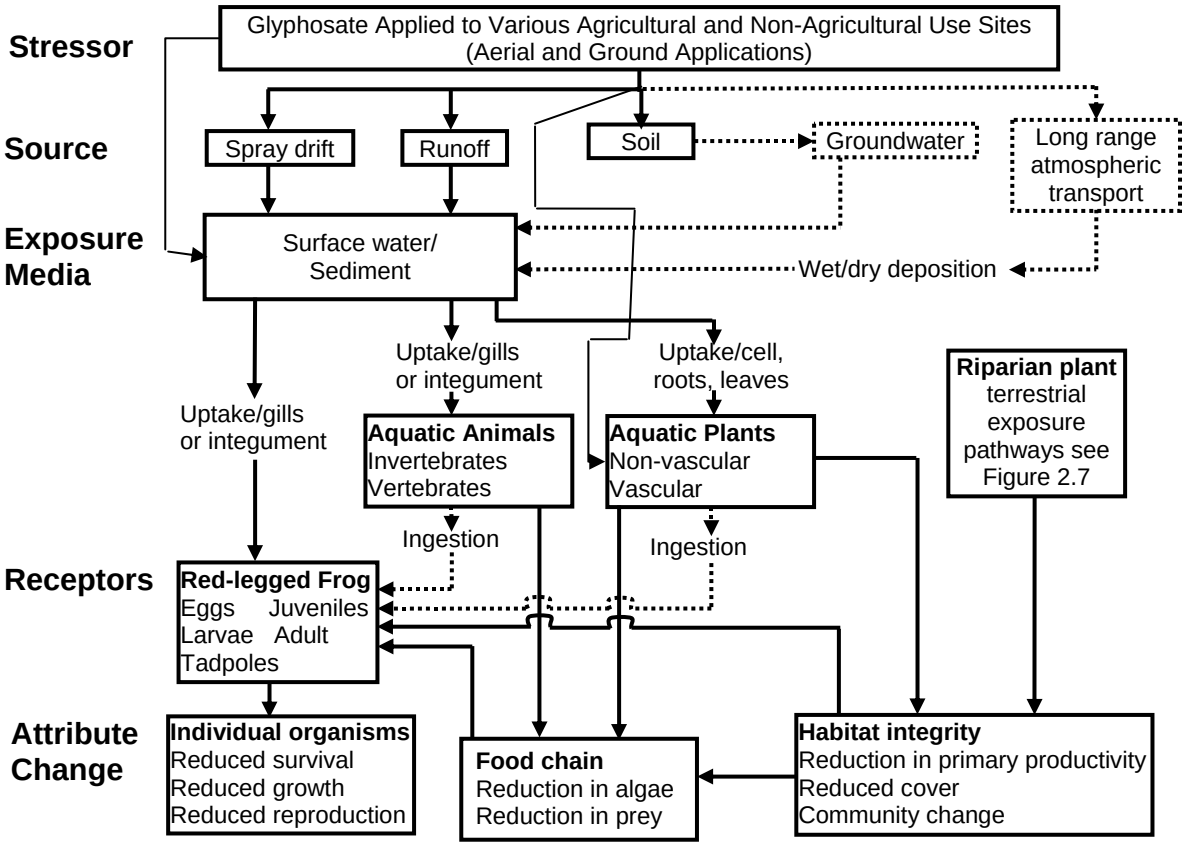


Figure 2.6 Conceptual Model for Pesticide Effects on Aquatic Component of CRLF Critical Habitat

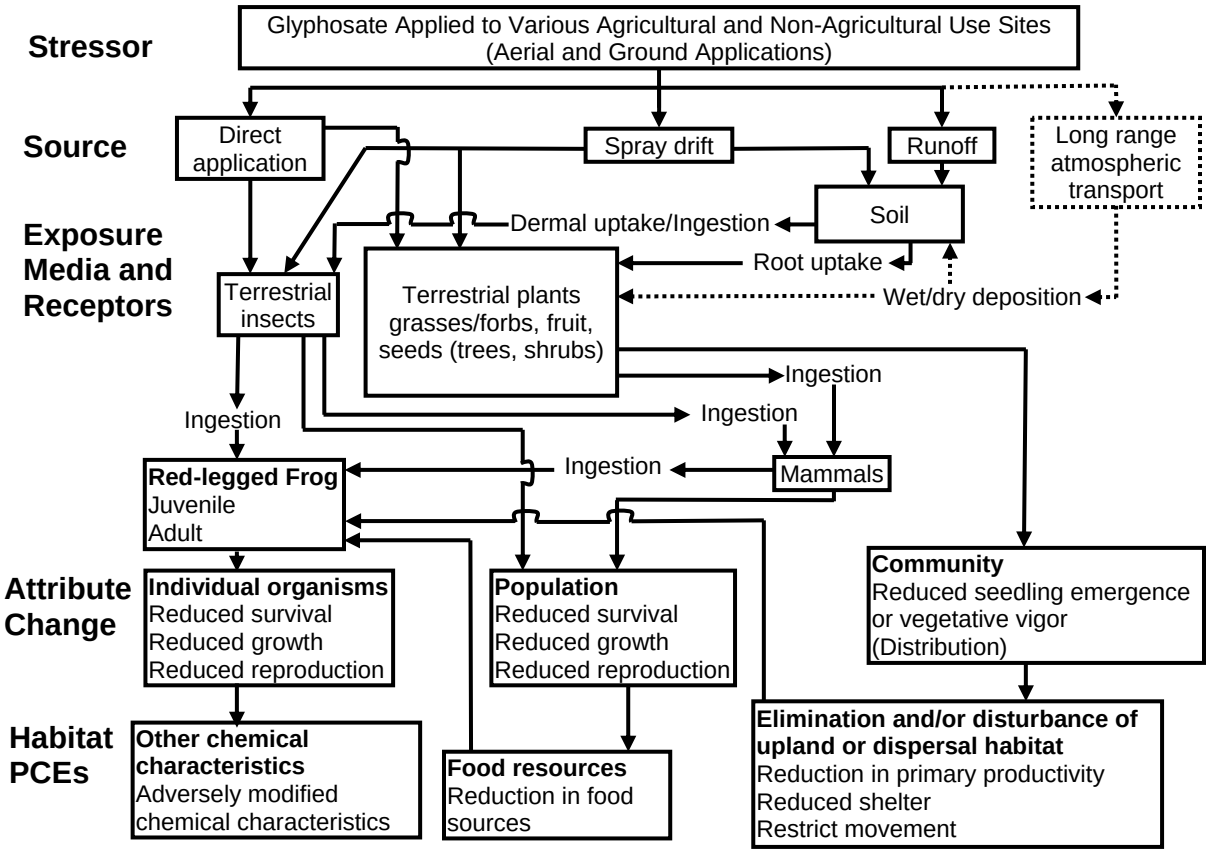


Figure 2.7 Conceptual Model for Pesticide Effects on Terrestrial Component of CRLF Critical Habitat

2.10 Analysis Plan

In order to address the risk hypothesis, the potential for direct and indirect effects to the CRLF, its prey, and its habitat is estimated. In the following sections, the use, environmental fate, and ecological effects of glyphosate are characterized and integrated to assess the risks. This is accomplished using a risk quotient (ratio of exposure concentration to effects concentration) approach. Although risk is often defined as the likelihood and magnitude of adverse ecological effects, the RQ-based approach does not provide a quantitative estimate of likelihood and/or magnitude of an adverse effect. However, as outlined in the Overview Document (U.S. EPA, 2004), the likelihood of effects to individual organisms from particular uses of glyphosate is estimated using the probit dose-response slope and either the LOC (discussed below) or actual calculated RQ value.

2.10.1 Measures to Evaluate the Risk Hypothesis and Conceptual Model

2.10.1.1 Measures of Exposure

The physical/chemical properties and environmental fate data for glyphosate, along with available monitoring data, indicate that runoff and spray drift are the principal potential transport mechanisms of glyphosate to the aquatic and terrestrial habitats of the CRLF. Based on its low vapor pressure and Henry's Law constant, long range atmospheric transport is not expected to be an important transport mechanism. In this assessment, transport of glyphosate through runoff and spray drift is considered in deriving quantitative estimates of glyphosate exposure to CRLF, its prey and its habitats.

Measures of exposure are based on aquatic and terrestrial models that predict estimated environmental concentrations (EECs) of glyphosate using maximum labeled application rates and methods of application. For aquatic exposure, a Tier I approach is used unless there are LOC exceedances. The model used to predict aquatic exposure from terrestrial applications of glyphosate is the screening model GENEEC2. The model used to predict aquatic exposure from terrestrial applications of glyphosate formulations and from aquatic applications of both glyphosate and glyphosate formulations, is a simple dilution calculation based on the standard pond scenario. The model used to predict terrestrial EECs on food items is T-REX. The model used to derive EECs relevant to terrestrial and wetland plants is TerrPlant. These models are parameterized using relevant reviewed registrant-submitted environmental fate data in support of glyphosate registration.

Exposure estimates for the aquatic-phase CRLF and for fish and aquatic invertebrates (serving as potential prey) in water bodies exposed to spray drift or runoff from terrestrial applications of glyphosate are derived using the Tier I simulation model GENEEC2 (Version 2.0; August 1, 2001). GENEEC2 uses a standard pond scenario, which assumes application of the active ingredient to a 10-hectare agricultural field that drains into an adjacent 1-hectare water body, 2 meters deep (20,000 m^3 volume) with no outlet. GENEEC2 considers adsorption of the pesticide to soil or sediment, incorporation of the pesticide at application, direct deposition of spray drift into the water body, and degradation of the pesticide in soil before runoff and within the water body. It is a single event model, meaning that it assumes one single large rainfall/runoff event occurs and removes a large quantity of pesticide at one time from the field to a pond.

Aquatic exposure resulting from terrestrial applications of glyphosate formulations or from aquatic applications of either glyphosate or glyphosate formulations is estimated using a simple dilution calculation based on the standard pond scenario and assuming that the entire applied mass is dispersed evenly in the standard water body. For terrestrial applications of glyphosate formulations, the calculation uses default spray drift parameters to estimate applied mass, and for aquatic applications, the application rate defines the mass applied.

Exposure estimates for the terrestrial-phase CRLF and terrestrial invertebrates and mammals (serving as potential prey) assumed to be in the target area or in an area exposed to spray drift are derived using the T-REX model (version 1.3.1, 12/07/2006). This model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of actual field residue data. The upper limit values from the nomograph represented the 95th percentile of residue values from actual field measurements (Hoerger and Kenega, 1972). For modeling purposes, direct exposures of the CRLF to glyphosate through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey (small mammals) are assessed using the small mammal (15 g) which consumes short grass. The small bird (20g) consuming small insects and the small mammal (15g) consuming short grass are used because these categories represent the largest RQs of the size and dietary categories in T-REX that are appropriate surrogates for the CRLF and one of its prey items. Estimated exposures of terrestrial insects to glyphosate are bound by using the dietary based EECs for small insects and large insects.

EECs for terrestrial plants inhabiting dry and wetland areas are derived using TerrPlant (version 1.2.2, 12/26/2006). This model uses estimates of pesticides in runoff and in spray drift to calculate EECs. EECs are based upon solubility, application rate and minimum incorporation depth.

The spray drift model, AgDRIFT is used to assess exposures of terrestrial phase CRLF and its prey to glyphosate deposited on terrestrial habitats by spray drift. In addition to the buffered area from the spray drift analysis, the downstream extent of glyphosate that exceeds the LOC for the effects determination is also considered.

2.10.1.2 Measures of Effect

Data identified in Section 2.8 are used as measures of effect for direct and indirect effects to the CRLF. Data were obtained from registrant submitted studies or from literature studies identified by the ECOTOXicology database (ECOTOX), a source for locating single chemical toxicity data for aquatic life, terrestrial plants, and wildlife. The database was searched in order to provide more ecological effects data and in an attempt to bridge existing data gaps. ECOTOX was created and is maintained by the USEPA, Office of Research and Development, and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division.

The assessment of risk for direct effects to the terrestrial-phase CRLF makes the assumption that toxicity of glyphosate to birds is similar to or less than the toxicity to the terrestrial-phase CRLF. The same assumption is made for fish and aquatic-phase CRLF. Algae, aquatic invertebrates, fish, and amphibians represent potential prey of the CRLF in the aquatic habitat. Terrestrial invertebrates, small mammals, and terrestrial-phase amphibians represent potential prey of the CRLF in the terrestrial habitat. Aquatic, semi-aquatic, and terrestrial plants represent habitat of CRLF.

The acute measures of effect used for animals in this screening level assessment are the LD_{50}, LC_{50} and EC_{50}. LD stands for "Lethal Dose", and LD_{50} is the amount of a material, given all at once, that is estimated to cause the death of 50% of the test organisms. LC stands for "Lethal Concentration" and LC_{50} is the concentration of a chemical that is estimated to kill 50% of the test organisms. EC stands for "Effective Concentration" and the EC_{50} is the concentration of a chemical that is estimated to produce a specific effect in 50% of the test organisms. Endpoints for chronic measures of exposure for listed and non-listed animals are the NOAEL/NOAEC and NOEC. NOAEL stands for "No Observed-Adverse-Effect-Level" and refers to the highest tested dose of a substance that has been reported to have no harmful (adverse) effects on test organisms. The NOAEC (*i.e.*, "No-Observed-Adverse-Effect-Concentration") is the highest test concentration at which none of the observed effects were statistically different from the control. The NOEC is the No-Observed-Effects-Concentration. For non-listed plants, only acute exposures are assessed (*i.e.*, EC_{25} for terrestrial plants and EC_{50} for aquatic plants).

It is important to note that the measures of effect for direct and indirect effects to the CRLF and its designated critical habitat are associated with impacts to survival, growth, and fecundity, and do not include the full suite of sublethal effects used to define the action area. According the Overview Document (USEPA 2004), the Agency relies on effects endpoints that are either direct measures of impairment of survival, growth, or fecundity or endpoints for which there is a scientifically robust, peer reviewed relationship that can quantify the impact of the measured effect endpoint on the assessment endpoints of survival, growth, and fecundity.

2.10.1.3 Integration of Exposure and Effects

Risk characterization is the integration of exposure and ecological effects characterization to determine the potential ecological risk from agricultural and non-agricultural uses of glyphosate likelihood of direct and indirect effects to CRLF in aquatic and terrestrial habitats. The exposure and toxicity effects data are integrated in order to evaluate the risks of adverse ecological effects on non-target species. For the assessment of glyphosate risks, the RQ method is used to compare exposure and measured toxicity values. EECs are divided by acute and chronic toxicity values. The resulting RQs are then compared to the Agency's LOCs (USEPA, 2004) (see **Appendix C**).

For this endangered species assessment, listed species LOCs are used for comparing RQ values for acute and chronic exposures of glyphosate directly to the CRLF. If estimated glyphosate exposure to the CRLF resulting from a particular use is sufficient to exceed the listed species LOC, then the effects determination for that use is "may affect". When considering indirect effects to the CRLF due to effects to animal prey (aquatic and terrestrial invertebrates, fish, frogs, and mice), the listed species LOCs are also used. If estimated glyphosate exposure to CRLF prey resulting from a particular use is sufficient to exceed the listed species LOC, then the effects determination for that use is a "may affect." If the RQ being considered also exceeds the non-listed species acute risk LOC, then the effects determination is a LAA. If the acute RQ is between the listed species LOC and the non-listed acute risk species LOC, then further lines of evidence (*i.e.*

probability of individual effects, species sensitivity distributions) are considered in distinguishing between a determination of NLAA and a LAA. When considering indirect effects to the CRLF due to effects to algae as dietary items or plants as habitat, the non-listed species LOC for plants is used because the CRLF does not have an obligate relationship with any particular aquatic and/or terrestrial plant. If the RQ being considered for a particular use exceeds the non-listed species LOC for plants, the effects determination is "may affect". Further information on LOCs is provided in **Appendix C**.

2.10.2 Data Gaps

The environmental fate and ecological effects databases for glyphosate are complete for the CRLF assessment. All fate and ecological effects study requirements have been satisified and there are no data gaps.

3. Exposure Assessment

Glyphosate is formulated as a liquid concentrate that can be applied through ground or aerial application. Risks from ground boom and aerial applications are considered in this assessment because they are expected to result in the highest off-target levels of glyphosate due to generally higher spray drift levels. Ground boom and aerial modes of application tend to use lower volumes of application applied in finer sprays than applications coincident with sprayers and spreaders and thus have a higher potential for off-target movement via spray drift.

3.1 Label Application Rates and Intervals

Glyphosate labels may be categorized into two types: labels for manufacturing uses (including technical glyphosate and its formulated products) and end-use products. While technical products, which contain glyphosate of high purity, are not used directly in the environment, they are used to make formulated products, which can be applied in specific areas to control weeds. The formulated product labels legally limit glyphosate's potential use to only those sites that are specified on the labels.

Currently registered agricultural uses of glyphosate relevant to CRLF critical habitat in California include, among others, use on row crops, cotton, nuts, melons, citrus, grapes, berries and other fruit, corn, wheat, and potatoes as well as use on turf, ornamentals, and forest trees. There are many non-agricultural uses of glyphosate as well, including application to rights of way and around buildings, structures, and paved areas. Additionally, for some uses, glyphosate is labeled for direct aquatic application. The uses being assessed, both for glyphosate and its formulations, were summarized previously in **Table 2.6**.

3.2 Aquatic Exposure Assessment

3.2.1 Modeling Approach

Aquatic EECs of glyphosate and glyphosate formulations are derived using a Tier I screening level approach. There are a variety of types of uses, including terrestrial and aquatic applications, either as glyphosate or as glyphosate formulations. Each type of use has different fate and exposure issues and so requires different methods to determine EECs, as described below. For all types of uses, only the highest labeled application rates are considered. If estimates using Tier I modeling and high application rates do not exceed LOCs, then further refinement is not required.

For all uses, exposure estimates are generated using the standard pond scenario and are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and first-order streams. As a group, there are factors that make these water bodies more or less vulnerable than the standard surrogate pond. Static water bodies that have larger ratios of drainage area to water body volume would be expected to have higher peak EECs than the standard pond. These water bodies will be either shallower or have large drainage areas (or both). Shallow water bodies tend to have limited additional storage capacity, and thus, tend to overflow and carry pesticide in the discharge whereas the standard pond has no discharge. As watershed size increases beyond 10 hectares, at some point, it becomes unlikely that the entire watershed is planted to a single crop, which is all treated with the pesticide. Headwater streams can also have peak concentrations higher than the standard pond, but they tend to persist for only short periods of time and are then carried downstream.

3.2.2 Modeling Calculations

3.2.2.1 Direct Aquatic Applications

The highest potential aquatic exposure for glyphosate results from uses which allow application directly to a water body. For both glyphosate and its formulations, peak aquatic exposure from these direct aquatic applications was estimated by calculating simple dilution in the standard pond, which has a volume of 20,000 m^3 and a surface area of 1 ha. In this calculation, an aquatic EEC is determined by dividing the mass of glyphosate applied to the pond by the volume of the pond, representing the peak exposure in a well-mixed water body.

$$\text{EEC } (kg/L) = \frac{[\text{Seasonal application rate } (lb/A) * 1.12\ (kg\ ha^{-1}/lb\ A^{-1}) * 1\ ha/pond]}{[20{,}000{,}000\ L/pond]}$$

Chronic EECs are not estimated because the simple dilution calculation does not account for chemical and environmental fate processes that affect longer term exposure, such as abiotic and biotic degradation, volatilization, and partitioning to sediment. For the same reason, this calculation cannot account for multiple applications and so, in order to be

conservative, it is assumed that the maximum seasonal application rate has been applied in a single application. Further refinement of estimates of chronic exposure or of exposure from single applications is not required unless there are LOC exceedances.

As listed in Table 2.6, the maximum seasonal application rates for aquatic uses are 3.75 lb ae/A for glyphosate and 32.9 lb formulation/A for formulations.

3.2.2.2 Terrestrial Applications

Although direct aquatic applications are expected to lead to the highest exposure concentrations, surface water exposures for terrestrial uses of glyphosate and its formulations have also been calculated, for characterization purposes.

For terrestrial appliations of glyphosate, EECs are quantitatively estimated using the Tier I simulation model GENEEC2 (Version 2.0; August 1, 2001), based on the standard pond scenario. The modeled application site is not crop-specific and represents a generic vulnerable site where high concentration levels are expected due to the occurrence of environmental conditions, including weather and soils, known to favor transport to and persistence in surface water. A summary of the GENEEC2 model inputs used in assessing aquatic exposure from terrestrial applications of glyphosate are provided in **Table 3.1**.

Table 3.1. GENEEC2 Inputs for Aquatic EECs from Terrestrial Applications of Glyphosate

Input Parameter	Value	Comment	Source
Application Rate and Method	7.95 lb ae/A; Aerial spray	For forestry, the use with the maximum labeled applicatiom rate.	Product labels
Application Details	Fine to medium droplet size Not wetted in No buffer		EFED Defaults
K_{oc}	3100 mL/g_{oc}	Lowest non-sand value from five soils	MRID 44320646
Solubility in Water	12,000 mg/L		Product Chemistry
Aerobic Soil Metabolism Half-life	5.4 days	90% upper confidence bound on the mean	MRIDs 42372501, 44320645
Hydrolysis at pH 7	0 days	Stable to hydrolysis	MRID 00108192, 44320642
Aquatic Photolyis	0 days	Stable to photolysis	MRID 41689101; 44320643
Aerobic Aquatic Metabolism	21 days	Single value x 3	MRID 41723601

For terrestrial application of formulations, partitioning and degradation properties for each formulation component in runoff suggest that the final proportion of the residues of these components in the receiving surface waters would not represent what was introduced and what was tested in an aquatic organism toxicity study using the formulated product. For this reason, spray drift is assumed to be the only route of aquatic exposure to the formulation as introduced. The mass of mesotrione from terrestrial applications expected to reach the water body through drift was estimated based on the default assumption of 5% drift for aerial spray. The simple dilution method, described in Section 3.2.2.1, is then applied to determine a peak aquatic EEC. Chronic EECs cannot be estimated because there are no fate data available for formulated products to allow for simulation of dissipation processes. For terrestrially applied formulation, the maximum seasonal application rate is 34.0 lb formulation/A, shared by a variety of non-crop uses, including rights-of-way, rangeland, ornamental, non-agricultural, and residential uses.

3.2.3 Results

EECs for terrestrial and aquatic applications of both glyphosate and its formulations are presented in **Table 3.2**. These EECs are based on the maximum labeled use from each category. Glyphosate EECs represent ug ae/L and formulation EECs represent ug formulation/L. GENEEC2 model outputs and simple dilution calculations are included in **Appendix D**.

Table 3.2. Aquatic EECs for Glyphosate and its Formulations

Use Type	Exposure Routes	Model	Peak	21-Day Avg EEC	60-Day Avg EEC
GLYPHOSATE (ug ae/L)					
Terrestrial	Runoff, spray drift	GENEEC2	87.2	69.0	45.8
Aquatic	Direct application	Simple Dilution	210	NA	NA
FORMULATIONS (ug formulation/L)					
Terrestrial	Spray drift	Simple Dilution	95.2	NA	NA
Aquatic	Direct Application	Simple Dilution	1840	NA	NA

3.2.4 Existing Monitoring Data

A critical step in the process of characterizing EECs is comparing the modeled estimates with available surface water monitoring data. Monitoring of glyphosate and/or AMPA (major biotransformation product) is not extensive, mostly because of the lack of appropriate analytical chemistry methods to identify/quantify glyphosate and AMPA prior to 2001, when a method was developed by the USGS with a method reporting limit of 0.1 µg/L for both species.

Included in this assessment are California-specific glyphosate and AMPA monitoring data for both surface and groundwater from the USGS NAWQA program (http://water.usgs.gov/nawqa). Several open literature studies monitoring glyphosate at

sites outside of California are discussed here as well because they are targeted to specific use sites and so provide insight into potential off-site transport of glyphosate. The California Department of Pesticide Regulation (CDPR) surface water monitoring database (http://www.cdpr.ca.gov/docs/emon/surfwtr/surfdes.htm) does not include glyphosate or AMPA as analytes and so will not be discussed further.

3.2.4.1 USGS NAWQA Surface Water Data

In California, the NAWQA database includes monitoring for glyphosate and AMPA in surface water at three locations, although this monitoring does not target specific chemicals or uses. At each location, 16 to 19 samples were collected between October 2002 and September 2003, generally every two to four weeks. Results are reported in **Table 3.3**. At a mixed use site in Merced County, glyphosate and AMPA were detected above the reporting limit of 0.1 µg/L at one sampling event (8/07/2003), at levels of 0.18 µg/L and 0.22 µg/L, respectively. At a mixed use site in San Joaquin county, glyphosate was detected four times over the sampling period (0.13 to 0.24 µg/L) but AMPA was detected in every sample (0.12 µg/L to 0.56 µg/L). The glyphosate detections showed no temporal pattern. AMPA showed peaks on 3/11/2003 (0.36 µg/L) and on 8/06/2003 (0.56 µg/L). At the only agricultural site, in Stanislaus county, glyphosate was detected in all but one of the samples and AMPA was detected in all samples. At this site, glyphosate detections were low (≤ 0.2 µg/L) until a peak concentration of 7.5 µg/L was reached on 3/12/03. Concentrations steadily decreased for 6 weeks and then remained ≤1.2 µg/L throughout the rest of the sampling period. AMPA levels were lower, with a maximum detected value of 1.1 µg/L reached on 7/24/03.

Table 3.3. NAWQA Surface Water Sampling Results in California

Site Location	Use Type	# of samples	Glyphosate		AMPA	
			# Detects	Range (µg/L)	# Detects	Range (µg/L)
Merced	Mixed	19	1	0.18	1	0.22
San Joaquin	Mixed	16	4	0.13 – 0.24	16	0.12 – 0.56
Stanislaus	Agriculture	16	15	0.10 – 7.46	16	0.23 – 1.07

3.2.4.2 USGS NAWQA Groundwater Data

In California, the NAWQA program monitored for glyphosate and AMPA in groundwater at 48 wells in 7 counties, although this monitoring does not target specific chemicals or uses. Neither compound was detected, although some sampling had reporting limits higher than 0.1 µg/L (0.15 µg/L for glyphosate and 0.31 µg/L for AMPA). This sampling included 30 sites in primarily agricultural areas in Fresno, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare Counties and 18 urban sites in Sacramento County.

3.2.4.3 Additional Studies

A USGS study sampled for glyphosate and AMPA in overland flow and in surface water in the Leary Weber Ditch Basin, Hancock County, Indiana (Baker et al., 2006). The 2.5

mi^2 study basin is primarily agricultural (87%), farmed with corn and soybeans, and flow in the ditch is dominated by tile-drain contributions. Overland flow and surface water samples were collected during two storm events occurring one to two weeks following pesticide application. Glyphosate and AMPA were detected in all overland flow samples (n=12). In the first storm event, glyphosate concentrations in overland flow were approximately 300 to 500 ppb and in the second event, concentrations were approximately 30 to 60 ppb. The median concentration of AMPA in all runoff samples was ~30 ppb. In surface water in the Leary Weber Ditch, glyphosate and AMPA were detected in 13 and 15 of 19 samples, respectively. The maximum glyphosate concentration was ~7 ppb and the median concentration was ~0.2 ppb. The maximum AMPA concentration was ~1 ppb and the median was slightly above the detection limit of 0.1 ppb. (Concentrations were only reported in charts, not numerically, so exact values are not available.)

3.2.4.4 Atmospheric Monitoring Data

Available studies monitoring atmospheric transport in the Central Valley and Sierra Nevada do not include glyphosate as an analyte (http://www.cdpr.ca.gov/docs/empm/pubs/tac/tacstdys.htm; http://www.nature.nps.gov/air/Studies/air_toxics/wacap.cfm). Some monitoring of glyphosate in rainwater has been conducted, but has found only local effects attributed to spray drift, as discussed in Section 2.4.2.

3.2.5 Spray Drift Buffer Analysis

In order to determine terrestrial and aquatic habitats of concern due to glyphosate exposures through spray drift, it is necessary to estimate the distance that spray applications can drift from the treated area and still be present at concentrations that exceed levels of concern. An analysis of spray drift distances was completed using AgDrift Tiers 1 and 3.

Based on glyphosate use patterns, the entire state of California is considered to be the initial area of concern. As stated previously, due to the lack of a defined no effect concentration in a subchronic freshwater fish study from the open literature (Jiraungkoorskul et. al., 2003), the spatial extent of the action area for glyphosate cannot be determined. Therefore, it is assumed that the action area also encompasses the entire state of California. Therefore, buffers can be estimated for a specific use; however, for aggregate uses, the widest buffer for both terrestrial and aquatic uses would be applied and would effectively be the entire state.

The spray drift buffer analysis is presented in the Risk Description, Section 5.2.3.2 under Terrestrial Plants.

3.2.6 Downstream Dilution Analysis

As stated above, for glyphosate, both the initial area of concern and the action area are considered to be the entire state of California. Due to the fact that the glyphosate labels allow for aquatic uses in multiple types of water bodies, multiple applications within a specific watershed may occur within the same time frame. As a result, there is potentially no input of "glyphosate clean" water to dilute existing concentrations of glyphosate downstream because it could be applied in the downstream waterbodies as well. Therefore, no credible watershed dilution can be done. For that reason, a downstream dilution analysis was not conducted.

3.3 Terrestrial Animal Exposure Assessment

T-REX (Version 1.3.1) is used to calculate dietary and dose-based EECs of glyphosate for the CRLF and its potential prey (*e.g.* small mammals and terrestrial insects) inhabiting terrestrial areas. EECs used to represent the CRLF are also used to represent exposure values for frogs serving as potential prey of CRLF adults. T-REX simulates a 1-year time period. For this assessment, spray applications of glyphosate are considered as discussed below.

Terrestrial EECs for foliar formulations of glyphosate were derived for the uses summarized in Table 3.7. A magnitude of residue study for alfalfa (MRID 45646001) provided sufficient data to generate a foliar dissipation half-life for glyphosate. Two half-lifes were generated, 4 and 7 days. The 7 day value was selected as a conservative estimate for use in T-REX. Use-specific input values, including number of applications, application rate and application interval are provided in **Table 3.4**. An example output from T-REX is available in **Appendix E**.

Table 3.4. Input Parameters for Foliar Applications Used to Derive Terrestrial EECs for Glyphosate with T-REX

Use Scenario (Application method)	Application rate (lbs ae/A)	Number of Applications
Forestry and areas with impervious surfaces (aerial)	7.95	1
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf (ground)	3.75	2
Almond, fruit, grape and olive (ground)	3.84	2
Citrus, cole crop, lettuce, melon, onion, potato and wine grape (ground)	3.85 1st application 2.3 2nd application	2
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat (ground)	3.75 1st application 2.25 2nd application	2
Corn (aerial) and wheat (ground)	0.75	8
Rangeland (ground)	1.54	5
Rangeland (aerial)	0.387	20
Right of way (aerial)	7.5	1
Right of way (ground)	3.69	2

T-REX is also used to calculate EECs for terrestrial insects exposed to glyphosate Dietary-based EECs calculated by T-REX for small and large insects (units of a.i./g) are used to bound an estimate of exposure to bees. Available acute contact toxicity data for bees exposed to glyphosate (in units of µg a.i./bee), are converted to µg a.i./g (of bee) by multiplying by 1 bee/0.128 g. The EECs are later compared to the adjusted acute contact toxicity data for bees in order to derive RQs.

For modeling purposes, exposures of the CRLF to glyphosate through contaminated food are estimated using the EECs for the small bird (20 g) which consumes small insects. Dietary-based and dose-based exposures of potential prey are assessed using the small mammal (15 g) which consumes short grass. Upper-bound Kenega nomogram values reported by T-REX for these two organism types are used for derivation of EECs for the CRLF and its potential prey (**Table 3.5**). Only the values for chronic exposure are provided because the acute avian oral and dietary and mammalian oral studies showed no mortalities at the highest dose/concentration tested. Dietary-based EECs for small and large insects reported by T-REX as well as the resulting adjusted EECs are available in **Table 3.6**. An example output from T-REX v. 1.3.1 is available in **Appendix E**.

Table 3.5 Upper-bound Kenega Nomogram EECs for Dietary- and Dose-based Exposures of the CRLF and its Prey to Glyphosate

Use	EECs for CRLF		EECs for Prey (small mammals)	
	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)	Dietary-based EEC (ppm)	Dose-based EEC (mg/kg-bw)
Forestry (aerial) and areas with impervious surfaces 7.95 lbs a.e./A	1073.25	Not applicable	1908.00	1819.13
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf 3.75 lbs/A	632.81	Not applicable	1125.00	1072.6
Almond, fruit, grape and olive 3.84 lb/A	648.00	Not applicable	1152.00	1098.34
Citrus, cole crop, lettuce, melon, onion, potato and wine grape 3.85 first application, 2.3 second application lb/A	388.13 - 649.69	Not applicable	690.00 - 1155.00	657.86 - 1101.2
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat 3.75 first application, 2.25 second application lb/A	379.69 – 889.92	Not applicable	675.00 – 1582.07	643.56 - 1508.38
Corn and wheat 0.75 lb/A	135.00	Not applicable	240.00	228.82
Rangeland 1.54 lb/A	276.93	Not applicable	492.32	469.39
Rangeland 0.387 lb/A	104.49	Not applicable	185.76	177.11
Right of way 7.5 lb/A	1012.50	Not applicable	1800.00	1716.16
Right of way 3.69 lb/A	622.69	Not applicable	1107.00	1055.44

Table 3.6. EECs (ppm) for Indirect Effects to the Terrestrial-Phase CRLF via Effects to Terrestrial Invertebrate Prey Items		
Use	**Small Insect**	**Large Insect**
Forestry (aerial) and areas with impervious surfaces 7.95 lbs a.e./A	1073.25	119.25
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf 3.75 lbs/A	632.81	70.31
Almond, fruit, grape and olive 3.84 lb/A	648.00	72.00
Citrus, cole crop, lettuce, melon, onion, potato and wine grape 3.85 first application, 2.3 second application lb/A	388.13 - 649.69	43.13 – 72.19
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat 3.75 first application, 2.25 second application lb/A	379.69 – 889.92	42.19 – 98.88
Corn and wheat 0.75 lb/A	135.00	15.00
Rangeland 1.54 lb/A	276.93	30.77
Rangeland 0.387 lb/A	104.49	11.61
Right of way 7.5 lb/A	1012.50	112.5
Right of way 3.69 lb/A	622.69	69.19

3.4 Terrestrial Plant Exposure Assessment

TerrPlant (Version 1.1.2) is used to calculate EECs for non-target plant species inhabiting dry and semi-aquatic areas. Parameter values for application rate, drift assumption and incorporation depth are based upon the use and related application method (**Table 3.7**). A runoff value of 0.05 is utilized based on glyphosate's solubility, which is classified by TerrPlant as >100 mg/L. For aerial and ground application methods, drift is assumed to be 5% and 1%, respectively. EECs relevant to terrestrial plants consider pesticide concentrations in drift and in runoff. These EECs are listed by use in **Table 3.7**. An example output from TerrPlant v.1.2.2 is available in **Appendix F.**

Table 3.7 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to Glyphosate via Runoff and Drift						
Use	**Application rate (lbs a.i./A)**	**Application method**	**Drift Value (%)**	**Spray drift EEC (lbs a.i./A)**	**Dry area EEC (lbs a.i./A)**	**Semi-aquatic area EEC (lbs a.i./A)**
Alfalfa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	3.75	Foliar - Ground	1	0.0375	0.225	1.913
Almond, fruit, grape, olive	3.84	Foliar -Ground	1	0.0384	0.230	1.96
Citrus	3.85	Foliar -Ground	1	0.0385	0.231	1.964
Cole crop, lettuce, melon, onion, potato, wine grape	3.85	Foliar -Aerial	5	0.1925	0.385	2.118

Table 3.7 TerrPlant Inputs and Resulting EECs for Plants Inhabiting Dry and Semi-aquatic Areas Exposed to Glyphosate via Runoff and Drift						
Use	**Application rate (lbs a.i./A)**	**Application method**	**Drift Value (%)**	**Spray drift EEC (lbs a.i./A)**	**Dry area EEC (lbs a.i./A)**	**Semi-aquatic area EEC (lbs a.i./A)**
Corn	0.75	Foliar -Aerial	5	0.0375	0.075	0.4125
Forestry	7.95	Foliar -Aerial	5	0.3975	0.795	4.3725
Impervious	7.95	Foliar -Ground	1	0.0795	0.477	4.0545
Nursery, rangeland, sugar beet, tomato, turf	3.75	Foliar -Aerial	5	0.1875	0.375	2.0625
Rangeland	1.54	Foliar -Ground	1	0.0154	0.0924	0.7854
Rangeland	0.387	Foliar -Aerial	5	0.01935	0.0387	0.21285
Rights of way	7.5	Foliar -Aerial	5	0.375	0.75	4.125
Rights of way	3.69	Foliar -Ground	1	0.0369	0.2214	1.8819
Wheat	0.75	Foliar -Ground	1	0.0075	0.045	0.3825

4. Effects Assessment

This assessment evaluates the potential for glyphosate to directly or indirectly affect the CRLF or modify its designated critical habitat. As previously discussed in Section 2.7, assessment endpoints for the CRLF effects determination include direct toxic effects on the survival, reproduction, and growth of CRLF, as well as indirect effects, such as reduction of the prey base or modification of its habitat. In addition, potential modification of critical habitat is assessed by evaluating effects to the PCEs, which are components of the critical habitat areas that provide essential life cycle needs of the CRLF. Direct effects to the aquatic-phase of the CRLF are based on toxicity information for freshwater fish, while terrestrial-phase effects are based on avian toxicity data, given that birds are generally used as a surrogate for terrestrial-phase amphibians. Because the frog's prey items and habitat requirements are dependent on the availability of freshwater fish and invertebrates, small mammals, terrestrial invertebrates, and aquatic and terrestrial plants, toxicity information for these taxa are also discussed. Acute (short-term) and chronic (long-term) toxicity information is characterized based on registrant-submitted studies and a comprehensive review of the open literature on glyphosate and its salts.

As described in the Agency's Overview Document (U.S. EPA, 2004), the most sensitive endpoint for each taxon is used for risk estimation. For this assessment, evaluated taxa include aquatic-phase amphibians, freshwater fish, freshwater invertebrates, aquatic plants, birds (surrogate for terrestrial-phase amphibians), mammals, terrestrial invertebrates, and terrestrial plants.

Toxicity endpoints are established based on data generated from guideline studies submitted by the registrant, and from open literature studies that meet the criteria for inclusion into the ECOTOX database maintained by EPA/Office of Research and Development (ORD) (U.S. EPA, 2004). Open literature data presented in this assessment were obtained from an ECOTOX search on 12/21/2007. In order to be included in the ECOTOX database, papers must meet the following minimum criteria:

(1) the toxic effects are related to single chemical exposure;
(2) the toxic effects are on an aquatic or terrestrial plant or animal species;
(3) there is a biological effect on live, whole organisms;
(4) a concurrent environmental chemical concentration/dose or application rate is reported; and
(5) there is an explicit duration of exposure.

Data that pass the ECOTOX screen are evaluated along with the registrant-submitted data, and may be incorporated qualitatively or quantitatively into this endangered species assessment. In general, effects data in the open literature that are more conservative than the registrant-submitted data are considered. The degree to which open literature data are quantitatively or qualitatively characterized for the effects determination is dependent on whether the information is relevant to the assessment endpoints (*i.e.*, maintenance of CRLF survival, reproduction, and growth) identified in Section 2.8. For example, endpoints such as behavior modifications are likely to be qualitatively evaluated, because quantitative relationships between modifications and reduction in species survival, reproduction, and/or growth are not available. Although the effects determination relies on endpoints that are relevant to the assessment endpoints of survival, growth, or reproduction, it is important to note that the full suite of sublethal endpoints potentially available in the effects literature (regardless of their significance to the assessment endpoints) are considered to define the action area for glyphosate.

Citations of all the open literature studies are attached in **Appendix G**. This includes all studies that were not considered as part of this assessment because they were either rejected by the ECOTOX screen or accepted by ECOTOX but not used (*e.g.*, the endpoint is less sensitive). **Appendix G** also includes a rationale for rejection of those studies that did not pass the ECOTOX screen and those that were not evaluated as part of this endangered species risk assessment. A detailed spreadsheet of the available ECOTOX open literature data, including the full suite of lethal and sublethal endpoints is presented in **Appendix H**.

In addition to registrant-submitted and open literature toxicity information, other sources of information, including use of the acute probit dose response relationship to establish the probability of an individual effect and reviews of the Ecological Incident Information System (EIIS), are conducted to further refine the characterization of potential ecological effects associated with exposure to glyphosate. A summary of the available aquatic and terrestrial ecotoxicity information, use of the probit dose response relationship, and the incident information for glyphosate are provided in Sections 4.1 through 4.4, respectively.

A large number of toxicity studies on glyphosate and/or its formulated products, especially acute toxicity studies have either been submitted to the Agency or are available in the open literature. The vast majority of these studies are on glyphosate formulations with mammals and aquatic species. Due to the proprietary nature of the surfactants and other inerts in the formulated products, the submitted studies with the associated data

evaluation records (DERs) and the studies from the open literature did not usually report any details on the formulations tested other than a generic trade name, such as Roundup or Rodeo and the percent active ingredient. Often, the active ingredient was not identified in the submitted study report or the DER as to whether or not it was glyphosate or one of its salts that was tested. This was also true of the open literature. Most results were not expressed in terms of glyphosate acid equivalents. Therefore, the ecotoxicity data on formulations are presented in terms of the trade name, active ingredient tested (if available) and the percent active ingredient. Where available, the name of the surfactant present in the formulated product is noted. If the active ingredient and percent active ingredient are reported, then the results from the studies are expressed in terms of acid equivalents. In some cases, a best guess was made as to the active ingredient tested based on what is known to be in trade name products. Toxicity endpoint values for the isopropylamine salt of glyphosate (IPA) were converted to acid equivalents by multiplying by 0.74, the ratio of the molecular weight of glyphosate to the IPA salt. The trisodium diglyphosate (sesquisodium salt) toxicity endpoints values were converted to acid equivalents by multiplying by 0.42 and the glyphosate ammonium salt values were converted to acid equivalents by multiplying by 0.77.

Appendix I includes a summary of the mammalian data utilized for the most current assessment of human health risk for glyphosate. These data are used for determination of the action area and potential sublethal effects.

Acute toxicity data are available for fish, aquatic invertebrates and birds with the degradate, aminomethyl phosphonic acid (AMPA). AMPA appears to be less acutely toxic than the parent to freshwater fish and invertebrates and birds. Tables of these studies are provided after the data on the technical material and formulations with the appropriate taxonomic group.

Summary tables of all the available ecotoxicity information for the glyphosate formulated products and degradate are presented in **Appendix J**, incorporated along with the ecotoxicity studies conducted with the technical material glyphosate and/or its salts.

Toxicity data on mixtures were obtained from both the studies submitted to the Agency and from those found in the open literature from ECOTOX. The glyphosate team was unable to obtain copies of all the open literature studies on mixtures. Therefore, the bibliographic references for these studies are included in **Appendix A.** One submitted study was available for a mixture of glyphosate and oxyfluorfen tested on green algae (MRID 45906008). This study is summarized in **Table 4.23**. Many acute mammalian studies were conducted with mixtures of glyphosate and other active ingredients. These are also discussed in **Appendix A**.

4.1 Toxicity of Glyphosate to Aquatic Organisms

Tables 4.1 and **4.2** summarize the most sensitive aquatic toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature, as

previously discussed. A brief tabular summary of submitted and any open literature data considered relevant to this ecological risk assessment for the CRLF is presented below.

The available toxicity data on technical glyphosate and/or its isopropylamine salt (IPA) with aquatic-phase amphibians indicate that glyphosate is less toxic to the selected amphibian species tested than to the selected freshwater fish species tested. In order to protect the wider range of aquatic-phase amphibians (including the CRLF) which may be more sensitive than those amphibians that were tested, the more conservative endpoints from freshwater fish were selected for assessment of risk. Endpoints from the amphibian studies, presented along with the uncertainties associated with these studies, were used as conservative estimates if the endpoints could conceivably be lower than those selected from the fish studies. These endpoints are summarized in the following tables.

Table 4.1 Freshwater Aquatic Toxicity Profile for Glyphosate and/or Its Salts

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID # /Date	Comment
Acute Direct Toxicity to Aquatic-Phase CRLF	Bluegill sunfish (*Lepomis macrochirus*)	96-hr. LC_{50}: 43 mg a.e./L*	44320630/1995	Acceptable
Chronic Direct Toxicity to Aquatic-Phase CRLF	Fathead minnow (*Pimephales promelas*) Leopard Frog (*Rana pipiens*)	NOAEC: 25.7 mg a.e./L (highest concentration tested) NOAEC: 1.8 mg a.e./L (highest concentration tested)	00108171/1975 46650501/2004	Acceptable. Frog study endpoint was used in assessment as a conservative estimate. Supplemental
Indirect Toxicity to Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (i.e. prey items)	Midge (*Chironomus plumosus*)	48-hr LC_{50}: 53.2 mg a.e./L	00162296/1979	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Chronic Toxicity to Freshwater Invertebrates (i.e. prey items)	Water flea (*Daphnia magna*)	NOAEC: 49.9 mg a.e./L	00124763/1982	Acceptable. LOAEC: 95.7 mg a.e./L based on reduced reproductive capacity.
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants	Green algae (*Selenastrum capricornutum*)	4-day EC50: 12.1 mg a.e./L	40236901/1987	Acceptable
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	Duckweed (*Lemna gibba*)	14-day EC50: 11.9 mg a.e./L	44320638/1996	Acceptable

*a.e. = expressed in terms of acid equivalents for glyphosate

Some glyphosate formulations have been found to be more toxic to aquatic organisms than technical glyphosate. Therefore, endpoints for assessment of risk to glyphosate formulations were selected. In California, one of the more toxic surfactants is not allowed to be applied directly to aquatic sites (polyoxy ethylene fatty amine or POEA). Therefore, for aquatic organisms, separate endpoints were selected for terrestrial uses where the POEA surfactant is allowed and for aquatic uses where this surfactant is not allowed. For aquatic animals, significant differences in toxicities between the formulations containing POEA and those that do not contain the surfactant are observed. For assessment of risk, exposure to the formulations is expressed in terms of EEC of the formulation rather than to the glyphosate acid equivalent. For consistency of units, the toxicity endpoints are also expressed in terms of concentration of formulation rather than the glyphosate acid equivalent.

For terrestrial uses, the most conservative endpoints from all the active formulations were selected. For aquatic uses, endpoints needed to be selected from studies on formulations that do not contain the POEA surfactant. Since it was not always possible to tell which formulations tested did not have the POEA surfactant, whenever possible, endpoints were selected from studies conducted with formulations that are currently labeled for aquatic use. This was not possible for the aquatic plant studies. The studies on aquatic plants were conducted with a product with the same basic name that has two separate labels, one for terrestrial uses and one for aquatic uses. It could not be determined from the aquatic plant studies whether or not they were conducted with the formulation for terrestrial uses or with the formulation for aquatic uses. The two formulations are different in terms of the inerts; however, the formulation for terrestrial uses does not have the POEA surfactant in it. Therefore, as a conservative estimate, the studies on this formulation were utilized for the assessment of risk to aquatic plants following exposure to formulations.

Table 4.2 Freshwater Aquatic Toxicity Profile for Glyphosate Formulations

Assessment Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID # /Date	Comment
Acute Direct Toxicity to Aquatic-Phase CRLF				Both studies supplemental
Terrestrial Applications	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50}: 3.17 ppm formulation	40098001/1986	Roundup: 30% a.i.
Aquatic Applications	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50}: 824 ppm formulation	45374001/1999	Glyphosate (360 g/L SL) 27% a.i.
Chronic Direct Toxicity to Aquatic-Phase CRLF				Supplemental
Terrestrial Applications	Leopard Frog (*Rana pipiens*)	LOAEC: 1.9 mg formulation/L	46650501/2004	No NOAEC
Indirect Toxicity to	Water flea			Both studies

Table 4.2 Freshwater Aquatic Toxicity Profile for Glyphosate Formulations				
Assessment Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID # /Date**	**Comment**
Aquatic-Phase CRLF via Acute Toxicity to Freshwater Invertebrates (i.e. prey items)	(*Daphnia magna*) for both application types			acceptable
Terrestrial Applications		48-hr EC_{50}: 3 ppm formulation	00162296/1979	30.3% Glyphosate IPA
Aquatic Applications		48-hr EC_{50}: 164.3 ppm formulation	45374003/1999	27.25% Glyphosate (360 g/L SL formulation
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Non-vascular Aquatic Plants Terrestrial and Aquatic Applications	Freshwater diatom (*Navicula pelliculosa*)	96-hr EC_{50}: 0.39 ppm formulation	45666701/2001	Acceptable Glyphosate (glyphos) 31.0%
Indirect Toxicity to Aquatic-Phase CRLF via Toxicity to Vascular Aquatic Plants	Duckweed (*Lemna gibba*) for both application types			
Terrestrial Applications		14-day EC_{50}: 4.9 ppm formulation	44125714/1984	Supplemental Glyphosate IPA salt (Roundup 41%)
Aquatic Applications		7-day EC_{50}: 25 ppm formulation	45666704/2001	Glyphosate (glyphos) 31.0% Acceptable

Toxicity to aquatic fish and invertebrates is categorized using the system shown in **Table 4.3** (U.S. EPA, 2004). Toxicity categories for aquatic plants have not been defined.

Table 4.3 Categories of Acute Toxicity for Aquatic Organisms	
LC_{50} (ppm)	**Toxicity Category**
< 0.1	Very highly toxic
> 0.1 – 1	Highly toxic
> 1 – 10	Moderately toxic
> 10 - 100	Slightly toxic
> 100	Practically nontoxic

4.1.1 Toxicity to Freshwater Fish and Aquatic-Phase Amphibians

Glyphosate toxicity data are available for both freshwater fish and aquatic-phase amphibians. The freshwater fish data were used as a surrogate to estimate direct acute risks to the CRLF because the endpoints from the fish data are more conservative. For chronic risk, the amphibian endpoint is utilized; however, it is noted that both the fish and

frog NOAECs are non-definitive (i.e., no effects were observed at the highest concentration tested and there was no LOAEC). In addition, the frog study is classified as supplemental. This study had some significant uncertainties associated with water quality and high mortality rates in the controls.

Freshwater fish toxicity data were also used to assess potential indirect effects of glyphosate to the CRLF. Effects to freshwater fish resulting from exposure to glyphosate have the potential to indirectly affect the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant, 1985).

A tabular summary of acute and chronic freshwater fish data, including data from the open literature, is provided below in Sections 4.1.1.1 through 4.1.1.3. Many acute toxicity studies are available for glyphosate formulations, with LC_{50}'s ranging from 1 to > 1000 mg/L. Because the number of fish studies on formulations is so extensive, only those studies which are referenced in the document are provided here. The remainder of the studies are summarized in tables in **Appendix J**. Acute toxicity data on the degradate, AMPA and two surfactants are also summarized.

4.1.1.1 Freshwater Fish: Acute Exposure (Mortality) Studies

Glyphosate and Its Salts Technical Material

Table 4.4 summarizes acute toxicity studies with freshwater fish on technical glyphosate and its salts. Study data are available for bluegill sunfish, rainbow trout, fathead minnow and channel catfish and are expressed in terms of glyphosate acid equivalents for comparison purposes. The data from these studies are so variable within each species that it is not possible to determine a range of sensitivities.

Table 4.4. Freshwater Fish Acute Toxicity for Technical Glyphosate and Its Salts					
Species	**% Active Ingredient***	**96-hour LC_{50} NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Bluegill sunfish (*Lepomis macrochirus*)	95.6	LC_{50}: **43 (30.6 - 53.5)[3]** NOAEC: 30.6 Slope: Not available	Slightly toxic	44320630/1995	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	83	LC_{50}: 99.6 (92.1 - 107.9)[1] NOAEC: 83 Slope: Not available	Slightly toxic	00108205/1978	Acceptable
Bluegill sunfish (*Lepomis macrochirus*)	96.7	LC_{50}: 100.2 (78.7 - 114.5)[4] NOAEC not reported Slope: Not available	Practically nontoxic	00162296/1979	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	83	LC_{50}: 71.4 (58.1-84.8) NOAEC: 34.9 Slope: Not available	Slightly toxic	00136339/1978	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	96.7	LC_{50}: 100.2 (85.9 - 121.6)[4] NOAEC not reported Slope: Not available	Practically nontoxic	00162296/1979	Acceptable
Rainbow trout (*Oncorhynchus mykiss*)	95.6	LC_{50}: 128.1 (95.6 - 172.1) NOAEC: 30.6 Slope: Not available	Practically nontoxic	44320629/1995	Acceptable
Fathead minnow (*Pimephales promelas*)	96.7	LC_{50}: 69.4 (56.5 - 85.9)[4] NOAEC not reported Slope: Not available	Slightly toxic	00162296/1979	Acceptable
Channel catfish (*Ictalurus punctatus*)	96.7	LC_{50}: 93 (78.7 - 114.5)[4] NOAEC not reported Slope: Not available	Slightly toxic	00162296/1979	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent

[1] Range is 95% confidence interval for endpoint

[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic

[3] **Bold** and shaded value will be used to calculate risk quotients

[4] Study conducted with the isopropylamine salt

Glyphosate and Its Salts Formulations

Table 4.5 summarizes selected acute toxicity studies on freshwater fish with several glyphosate and glyphosate salt formulations. Submitted data on glyphosate formulations indicate that some of the formulations are more toxic to freshwater fish than technical glyphosate itself. Studies have indicated that one surfactant, polyethoxylated tallowamine (referred to as polyoxy ethylene fatty amine or POEA) is probably the reason for the increased toxicity of some of the glyphosate formulations (Giesy, 2000; USDA, 2003; MRID 00162296). For example, in one study (MRID 00162296), fathead minnows were exposed to either technical isopropylamine salt of glyphosate (IPA), a glyphosate IPA formulation or the POEA surfactant. The resultant acute LC_{50}s were 69.4, 1.7 and 2.0 mg/L, respectively.

For the studies selected for the quantitative assessment of risk, the units for the formulations are expressed in both acid equivalents and in mg/L formulation. As stated previously, for terrestrial uses, the most conservative endpoint from all the active formulations was selected. For aquatic uses, the endpoint was selected from a study conducted with a formulation that is currently labeled for aquatic use.

The acute toxicity values between freshwater fish species are not sufficiently consistent to determine a range of sensitivities for freshwater fish. For example, one review indicates that the salmonids are more sensitive to glyphosate than other species of fish (USDA, 2003); however, the available data here do not necessarily support this statement. Data from the open literature (ECOTOX) provide some information on sublethal effects (see Section 4.1.1.3).

Also stated previously, the form of glyphosate (acid or salt) and the surfactants present in each of the formulations tested are either ambiguously reported or not reported at all. However, the Roundup® formulations generally have the IPA salt, a surfactant and water (Geisy, 2000). The formulations of Roundup® that have been tested often contain the POEA surfactant.

Note that when the acute LC_{50}s for the formulations are expressed in terms of glyphosate acid equivalents, they are not identical to the LC_{50} values for the same studies considered in previous risk assessments or reviews. The LC_{50} values are normally lower when expressed in terms of acid equivalents.

Table 4.5. Freshwater Fish Acute Toxicity for Glyphosate Formulations						
Chemical (Active Ingredient)	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup)*	Rainbow trout (*Oncorhynchus mykiss*)	30	LC_{50}: 1 (0.8 - 1.2)[2] (**3.17** mg formulation/L) NOAEC: N.R.* Slope:N.R.	Highly toxic	40098001/1986	Supplemental
Glyphosate (360 g/L SL)	Rainbow trout (*Oncorhynchus mykiss*)	27	LC_{50}: 224.5 (160.1 - 280.0) (**824** mg formulation/L) NOAEC: 160 Slope:N.R.	Practically non-toxic	45374001/1999	Supplemental
Glyphosate IPA (Roundup with POEA surfactant)	Fathead minnow (*Pimephales promelas*)	30	LC_{50}: 1.7 (1.4 - 2.1) NOAEC: N.R. Slope:N.R.	Moderately toxic	00162296/1979	Supplemental
Glyphosate IPA (Roundup)	Bluegill sunfish (*Lepomis macrochirus*)	31	LC_{50}: 1.8 (1.4 - 2.6) NOAEC: 0.7 Slope:N.R.	Moderately toxic	00124760/1982	Acceptable
Glyphosate IPA (Roundup)	Rainbow trout (*Oncorhynchus mykiss*)	31	LC_{50}: 2.5 (2.0 - 3.1) NOAEC: 1.8 Slope:N.R.	Moderately toxic	00124761/1982	Supplemental
Glyphosate IPA (Roundup)	Fathead minnow (*Pimephales promelas*)	41	LC_{50}: 2.9 (1.7 - 4.9) NOAEC: 1.7 Slope:N.R.	Moderately toxic	00070896/1980	Acceptable
Glyphosate IPA	Bluegill sunfish (*Lepomis macrochirus*)	30	LC_{50}: 3 (2.4 - 3.7) NOAEC: N.R. Slope:N.R.	Moderately toxic	40098001/1986	Supplemental
Glyphosate IPA (Roundup)	Bluegill sunfish (*Lepomis macrochirus*)	41	LC_{50}: 4.3 (2.7 - 7.3) NOAEC: 2.7 Slope:N.R.	Moderately toxic	00070897/1980	Acceptable
Glyphosate IPA (Roundup)	Channel catfish (*Ictalurus punctatus*)	41	LC_{50}: 4.9 (2.9 - 8.0) NOAEC: 2.9 Slope:N.R.	Moderately toxic	00070894/1980	Supplemental

Table 4.5. Freshwater Fish Acute Toxicity for Glyphosate Formulations						
Chemical (Active Ingredient)	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup)	Rainbow trout ((*Salmo gairdneri*)	36	LC_{50}: 5.5 - 9.2 (4.2 - 13) NOAEC: 4.2 Slope:N.R.	Moderately toxic	40579203/1986	Supplemental
Glyphosate IPA (Roundup)	Chinook Salmon (*Oncorhynchus tschawytscha*)	36	LC_{50}: 7.1 (5.9 - 9.7) NOAEC: <1.3 Slope:N.R.	Moderately toxic	40579201/1986	Not classified 10% mortality at 1.3 (loss of equilibrium and mobility)
Glyphosate IPA (Roundup)	Coho Salmon (*Oncorhynchus kisutch*)	36	LC_{50}: 8.2 (4.2 – 13.4) NOAEC: 3.42 Slope:N.R.	Moderately toxic	40579202/1986	Supplemental
Glyphosate IPA with X-77 surfactant	Rainbow trout (*Oncorhynchus mykiss*)	5	LC_{50}: 9.4 (7.0 - 12.4) NOAEC: 7 Slope:N.R.	Moderately toxic	00078664/1980	Acceptable
Glyphosate IPA with Geronol CF/AR surfactant	Rainbow trout (*Oncorhynchus mykiss*)	45	LC_{50}: > 450 (N.A.) mg a.e./L or > 1000 mg formulation/L NOAEC: 1000 mg formulation/L Slope:N.A.	Practically non-toxic	44738201/1996	Not classified

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; NR = not reported; NA = not available
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint
[3] **Bolded** and shaded values will be used to calculate risk quotients

Table 4.6 summarizes submitted acute toxicity studies on freshwater fish with two surfactants, POEA and geronol, an alkyl polyoxy ethylene phosphoric acid ester. The studies with POEA indicate that it is slightly to highly toxic with similar toxicity values in rainbow trout, fathead minnows and channel catfish and slightly less toxic to bluegill sunfish. Geronol does not appear to be toxic to zebra fish.

Table 4.6. Freshwater Fish Acute Toxicity for Surfactants Used with Glyphosate Formulations

Chemical	Species	% a.i.[1]	96-hour LC_{50}/NOAEC (mg/L)/Slope	Toxicity Category[2]	MRID #/Year	Study Classification
Polyoxy ethylene fatty amine (POEA)	Rainbow trout (*Oncorhynchus mykiss*)	100	LC_{50}: 1 (1.2 - 1.7)[3] NOAEC and slope not reported	Highly toxic	00162296/1979	Acceptable
Polyoxy ethylene fatty amine (POEA)	Fathead minnow (*Pimephales promelas*)	100	LC_{50}: 2 (1.5 - 2.7) NOAEC and slope not reported	Moderately toxic	00162296/1979	Acceptable
Polyoxy ethylene fatty amine (POEA)	Channel catfish (*Ictalurus punctatus*)	100	LC_{50}: 3 (2.5 - 3.7) NOAEC and slope not reported	Moderately toxic	00162296/1979	Acceptable
Polyoxy ethylene fatty amine (POEA)	Bluegill sunfish (*Lepomis macrochirus*)	100	LC_{50}: 13 (10.0 - 17.0) NOAEC and slope not reported	Slightly toxic	00162296/1979	Acceptable
Surfactant Geronol CF/AR (alkyl polyoxy ethylene phosphoric acid ester)	Zebra fish (*Brachydanio rerio*)	100	LC_{50}: >100 (N.A.) NOAEC and slope not reported	Practically non-toxic	44738201/ Summary from another study	Not classified

[1] a.i. = active ingredient, assumed 100% for technical material
[2] Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

The acute toxicity study with rainbow trout (**Table 4.7**) indicates that the degradate, aminomethyl phosphonic acid (AMPA) is less toxic to freshwater fish than the parent glyphosate.

Table 4.7. Freshwater Fish Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate

Chemical	Species	% a.i.[1]	96-hour LC_{50}/NOAEC (mg/L)/Slope	Toxicity Category[2]	MRID #/Year	Study Classification
AMPA	Rainbow trout (Oncorhynchus mykiss)	94.38	LC50: 499 (391 - 647) NOAEC: 174 Slope: 6.42	Practically nontoxic	43334713/1991	Acceptable

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

4.1.1.2 Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

No effects were observed at the highest level tested, 25.7 mg a.e./L in a life cycle study with technical glyphosate in fathead minnows. No other chronic studies were found with freshwater fish, including in the open literature; however, subchronic studies were found in the open literature. Sublethal effects from these studies are summarized in Section 4.1.1.3. No appropriate chronic toxicity data for either the surfactants or the degradate have been located.

Table 4.8. Freshwater Fish Chronic Toxicity for Technical Glyphosate and Its Salts

Species	% Active Ingredient	NOAEC/LOAEC (mg acid equivalent/L)	MRID #/Year	Study Classification
Fathead minnow (*Pimephales promelas*)	87.3	25.7/>25.7[1]	00108171/1975	Acceptable

4.1.1.3 Freshwater Fish: Sublethal Effects and Additional Open Literature Information

None of the open literature data provided more conservative endpoints that may be used in a quantitative estimate of risk. Several studies were published that concentrated on potential sublethal effects following glyphosate exposure, particularly on a microscopic and biochemical level. In addition, at least one study examined potential behavioral effects. Any sublethal effects observed in the submitted acute toxicity studies on the technical material are also summarized in **Table 4.9**. Observed sublethal effects in the

chronic studies are already summarized in other sections of the ecological effects characterization section. For freshwater fish, sublethal data from the open literature and submitted studies are available for tilapia, topmouth gudgeon, rainbow trout, north African catfish and Lee Koh. The formulations, Roundup®, Vision® and glyphosate with several different surfactants and glyphosate were tested. The NOAECs for sublethal effects range from 8 ppb to 30.6 ppm. The lowest NOAEC is 8 ppb, based on an increase in wigwag behavior in rainbow trout at the LOAEC of 46 ppb following exposure to Vision®, a formulation containing the toxic surfactant, POEA. The highest NOAEC is 30.6 ppm, based on dark coloration in rainbow trout at the LOAEC of 53.6 ppm following exposure to 95.6% glyphosate. Other studies show sublethal effects on several organs (gills, liver and kidneys) and various systemic enzymes, plus some behavioral and neurophysiological changes. In addition, in a fish mutagenicity study, Roundup induced erythrocyte micronuclei at 42, 85 and 170 mg/kg. Unless they can be quantitatively associated with mortality, growth or reproduction, sublethal effects are not included in the quantitative assessment of risk; however, they are discussed in the risk description.

Table 4.9. Freshwater Fish Sublethal Effects From Submitted and Open Literature Studies

Species	Chemical	NOAEC	LOAEC:Effects	MRID/ECOTOX Reference No.
Nile tilapia (*O. niloticus*)	Roundup (48% a.e.)	Not determined	5 ppm: gills: filament cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting, and aneurysm. Liver: vacuolation of hepatocytes and nuclear pyknosis. Kidneys: dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cells. Significant increase in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities. Decreased activity.	E096917 – **This study used to determine Action Area**
Nile tilapia (*O. niloticus*)	Roundup (48% a.e.)	5 ppm	15 ppm: gills: mucosal cells of laminar epithelium - loss of microridges and appearance of intercellular spaces; thickening of primary epithelium, edema, lifting and fusion of secondary lamellae – may impair respiratory function. Liver: progressive reduction and fragmentation of RER; swollen mitochondria; increases in number and sizes of lysosomes and lipid droplets; infiltration of leukocytes; increased hepatocyte size with pyknotic nuclei and presence of vacuoles. Kidney: degeneration of nuclear membrane; mitochondrial contraction and/or swelling; accumulation of large electron dense particles; increase in number and size	E096937

Table 4.9. Freshwater Fish Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LOAEC:Effects**	**MRID/ECOTOX Reference No.**
			of lysosomes and apical vacuoles; some cellular necrosis. Increased plasma aspartate and alanine aminotransferase and alkaline phosphatase activities at 15 ppm.	
Topmouth gudgeon (*pseudorasobora parva*)	Glyphosate IPA salt (41%)	Not determined	1 ppm: Initial possible inhibition of liver esterase activity and then possible induction of enzyme activity. Not dose dependent.	E097111
Rainbow trout (*O. mykiss*)	Vision (356 g/L glyphosate acid with surfactant)	8 ppb	45.75 ppb: increase in wigwag behavior (one of agonistic behaviors). No effects on growth, foraging variables or antagonistic activity; no evidence of neoplasia or melanomacrophages and no increase in gill lesions at 45.75 ppb (highest concentration tested).	E097714
Rainbow trout (*O. mykiss*)	Glyphosate (assumed technical) and combinations with surfactants R-11 and Target Prospeador Acitvator	1.25 ppm (glyphosate alone)	Rainbow trout vitellogenin assay. Estrogenic effects. No effects with glyphosate alone. When combined with surfactants at 1.25 ppm, trends indicated elevated vitellogenin.	E080643
North African catfish (*Clarius gariepinus*)	Roundup (no other identification)	Not determined	3.9 ppm: Increased plasma AST, ALP, ALT levels.	E097133
Rainbow trout (*O. mykiss*)	Technical glyphosate 95.6%	30.6 ppm	53.6 ppm: dark coloration	MRID 44320629
T. rendalli	Roundup® (480g/l) and surfactant	No NOAEL	42 mg/kg. Fish erythrocyte micronucleus assay. Pesticide applied by injection. Roundup induced micronuclei at 42, 85 and 170 mg/kg	E074478
Rainbow trout *(O. mykiss)*	Roundup® 143 g/L	0.01 ppm	0.1 ppm. Olfactory-mediated behavioral and neurophysiological response. Over a concentration range that does not result in acute toxicity, trout detect Roundup but do not avoid it. Above that concentration, they avoid it (≥ 10 ppm). Study found that behavioral responses may be more sensitive tox. endpoints than neurophysiological responses.	E089625 Tierney 2007
Rainbow trout *(O. mykiss)*	Roundup® 356 g/L glyphosate IPA MON 02139	30 ppm	40 ppm. Fish tend to avoid concentrations that are lethal (40 ppm and above). 96-hr LC_{50} 54.8 in the lab and 52 in the field. No mortality at 2.2 kg a.e./ha, 10x and 100x field dose.	E010471
Rainbow trout *(O. mykiss)*	Vision® 356 g a.e./L with either 10% or	Avoidance: 27 ppm	96 hr LC_{50}: 100 ppm (7.5%); 75 ppm (10%); 27 ppm (15%).	E05182

Table 4.9. Freshwater Fish Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LOAEC:Effects**	**MRID/ECOTOX Reference No.**
	15% surfactant (POEA). 7.5% surfactant tested in acute study	(15%) & 75 ppm (10%) Other behavior 6.75 ppm (15%) & 18.75 ppm (10%)	Avoidance behavior LOAEC: 150 ppm (10%); 54 ppm (15%) Other behavior LOAEC: Erratic swimming & rapid respiration 13.5 ppm (15%); erratic swimming & labored respiration 37.5 ppm (10%)	
Tilapia (*Oreochromis niloticus*) Lee Koh (*Cyprinus carpio*)	Roundup® 30.5% w/w glyphosate	0.31 ppm for tilapia 1.7 ppm for Lee Koh	Tilapia: 0.55 ppm: erratic swimming. 96-hr LC_{50}: 2.3 ppm. Lee Koh: LC_{50}: 3.1 ppm. LOAEC not provided.	E03296

4.1.1.4 Aquatic-phase Amphibian: Acute and Chronic Studies

Acute and chronic studies have been conducted on glyphosate, both technical and formulations with various frog species. These studies indicate that the frog is generally either equally or less susceptible to glyphosate toxicity than fish. **Tables 4.10 – 4.14** summarize the submitted frog studies for technical glyphosate, its salts, and formulations. Data are also available on the surfactant, POEA. MRID 46650501 tested the green frog (*Rana clamitans*, Gosner stage 25) with technical glyphosate (isopropylamine salt (IPA)), an IPA formulation with 15% POEA, and POEA. The acute LC_{50}'s were >17.9, 2.0 and 2.2 mg/L, respectively, with technical IPA and the IPA formulation expressed in terms of glyphosate acid equivalents. This study indicates that aquatic amphibians are also susceptible to POEA toxicity.

Forty-two day studies with leopard frog (*Rana pipiens*) larvae indicate that a formulation containing 15% POEA and the POEA surfactant itself are more toxic to the frogs than the technical IPA salt (MRID 46650501).

Table 4.10 Aquatic-Phase Amphibian Acute Toxicity for Technical Glyphosate and Its Salts					
Species	**% Active Ingredient***	**96-hour LC_{50} NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Australian tree frog (*Litoria moorei*) Tadpole	96	LC50: 103.2 (43.2 - 172.8)[1] NOAEL: N.R.* Slope: N.R.	Practically nontoxic	43839601/1995	Supplemental
Australian frog (*Crinia insignifera*) Adult	96	LC50: 75 (60.4-92.7) NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Green Frog (*Rana clamitans*) Gosner Stg 25	Tech[4]	LC50: >17.9 (NR) NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = not reported
[1] Range is 95% confidence interval for endpoint
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Study conducted with the isopropylamine salt

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate- IPA (Cosmo Flux Coca mix)	African clawed frog (*Xenopus laevis*) Larvae	18	LC50: 1.1 (0.56 - 2.3) or 10 mg/L formulation NOAEL: 0.14 Slope: 4.92	Moderately toxic	46873601/2006	Supplemental
Glyphosate IPA (Cosmo Flux Poppy mix)	African clawed frog (*Xenopus laevis*) Larvae	0.0205	LC50: 1.3 (0.92 - 1.8) or 16 mg/L formulation NOAEL: 0.43 Slope: NA*	Moderately toxic	46873602/2006	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 2 (1.9-2.2) or 6.5 mg/L formulation NOAEL: NR* Slope: NR	Moderately toxic	46650501/2001	Supplemental

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup Transorb with 15% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 2.2 (2.1-2.4) or 7.2 mg/L formulation NOAEL: NR Slope: NA	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Leopard Frog (*Rana pipiens*) Gosner Stg 25	NR	LC50: 2.9 (NR) or 9.2 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/2000	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	American toad (*Bufo americanus*) Gosner Stg 25	NR	LC50: <4.0 (NR) or < 12.9 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup with 15% POEA)	Wood Frog (*Rana sylvatica*) Gosner Stg 25	NR	LC50: 5.1 (4.9-5.4) or 16.5 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup 360)	Australian tree frog (*Litoria moorei*) Tadpole	30.3	LC50: 5.6 (4.4 - 7.1) or 18.5 mg/L formulation NOAEL: N.R. Slope: N.R.	Moderately toxic	43839601/1995	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Leopard Frog (*Rana pipiens*) Gosner Stg 20	NR	LC50: 6.5 (6.1-6.8) or 20.9 mg/L formulation NOAEL: NR Slope: NA	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	Green frog (*Rana clamitans*) Gosner Stg 20	NR	LC50: 7.1 (6.6-7.6) or 22.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Roundup Original with 15% POEA)	American toad (*Bufo americanus*) Gosner Stg 20	NR	LC50: 8 (NR) or 25.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup Original with 15% POEA)	Wood Frog (*Rana sylvatica*) Gosner Stg 20	NR	LC50: > 8 (NR) or > 25.8 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/1994	Supplemental
Glyphosate IPA (Glyphos AU with 3-7% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: 8.9 (8.6-9.2) or 28.6 mg/L formulation NOAEL: NR Slope: NR	Moderately toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup Biactive with 10-20% unspecified surfactant)	Green frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: >17.9 (NR) or > 57.7 mg/L formulation NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental
Glyphosate IPA (Glyphos BIO with 3-7% POEA)	Green Frog (*Rana clamitans*) Gosner Stg 25	NR	LC50: >17.9 (NR) or >57.7 mg/L formulation NOAEL: NR Slope: NR	Slightly toxic	46650501/2001	Supplemental
Glyphosate IPA (Roundup 360)	Australian frog (*Crinia insignifera*) Adult	30.3	LC50: 30.4 (0-infinity) or 100.2 mg/L formulation NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Glyphosate IPA (Roundup 360)	Australian frog (*Crinia insignifera*) Tadpole	30.3	48 hr LC50: 38.2 (30.2 - 48.8) or 125.9 mg/L formulation NOAEL: N.R. Slope: N.R.	Slightly toxic	43839601/1995	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	45	LC50: >450 (N.A.) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (Roundup Biactive))	Common froglet (*Ranidella signifera*) Tadpole	36	LC50: >360 (N.A) or >1000 mg/L formulation NOAEL: <800 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental

Table 4.11 Aquatic-Phase Amphibian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**96-hour LC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	36	LC50: >360 (N.A) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental
Glyphosate IPA (with surfactant Geronol CF/AR	Common froglet (*Ranidella signifera*) Tadpole	10	LC50: >100 (N.A.) or >1000 mg/L formulation NOAEL: 1000 Slope: N.A.	Practically nontoxic	44738201/1996	Supplemental

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt, N.A. = not available, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

Table 4.12. Aquatic-Phase Amphibian Acute Toxicity for POEA Surfactant Used with Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**96-hour LC_{50}/NOAEC (mg/L)/Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Polyoxy ethylene fatty amine (POEA or MON 0818)	Green Frog (*Rana clamitans*) Gosner Stg 25	69-73	LC50: 2.2 (2.1-2.4) NOAEC: NR* Slope: NR	Moderately toxic	46650501/2001	Supplemental

* NR = not reported
[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint.

Table 4.13. Aquatic Phase Amphibian Chronic Toxicity for Technical Glyphosate IPA Salt and IPA Salt Formulations				
Species	**% Active Ingredient**	**NOAEC/LOAEC (mg acid equivalent/L)**	**MRID #/Year**	**Study Classification**
Leopard Frog (*Rana pipiens*)	Tech IPA (assumed 100%)	NOAEC/LOAEC: **1.8/>1.8**[1]	46650501/2004	Supplemental
Leopard Frog (*Rana pipiens*)	Roundup Original & Transorb 15% POEA	NOAEC/LOAEC: **0.6/1.8**[1] decr. percentage larvae surviving to reach Stage 42 and length at metamorphosis. Incr. time to metamorphosis, mixed-sex gonads and tail damage. Gosner stage 25, larvae treated with Roundup® Original at 1.8 mg a.e/L or with Roundup® Transorb at 0.6 and 1.8 mg a.e./L exhibited significantly higher thyroid hormone mRNA expression than controls.	46650501/2004	Supplemental
[1] **Bold** and shaded value will be used to calculate risk quotients				

Table 4.14 Aquatic-Phase Amphibian Chronic Toxicity for POEA Surfactant Used with Glyphosate Formulations					
Chemical	**Species**	**% a.i.**[1]	**NOAEC/ LOAEC (mg a.i./L)**	**MRID #/Year**	**Study Classification**
Polyoxy ethylene fatty amine (POEA or MON 0818)	Leopard Frog (*Rana pipiens*) Larvae	Tech	NOAEC/ LOAEC: 0.6/1.8	46650501/2004	Supplemental
[1] a.i. = active ingredient, assumed 100% for technical material					

4.1.1.5 Aquatic Amphibian Sublethal Effects and Additional Open Literature Information

Some of the open literature studies on amphibians provide additional information that may be of use in the risk characterization for glyphosate. These studies are summarized in **Table 4.15**.

Table 4.15. Aquatic Amphibian Sublethal Effects From Submitted and Open Literature Studies

Species	Chemical	NOAEC	LC_{50} or LOAEC:Effects	MRID/ ECOTOX Ref. No.
Green frog *(Rana pipiens)*	Vision® (contains POEA surfactant)	Not determined for mortality	LOAEC for mortality: 0.75 ppm a.e. at pH 7.5. Note: higher pH (7.5) versus 5.5 increases acute toxicity	E072794
African clawed frog (*Xenopus laevis*)	Rodeo® (480 g a.e./L no surfactant) Roundup® (356 g ae/L with POEA surfactant)	5 ppm a.e. (Roundup®) and 2000 ppm a.e. (Rodeo®)	Frog embryo teratogenesis assay. LC_{50}'s: POEA (6.8 ppm), Roundup® (9.3 ppm a.e.), Rodeo® (7297 ppm a.e.). No significant increases in embryo malformations for either formulation.	E053090
Crinia insignifera, Heleioporus eyrei, Limnodynastes dorsalis,and Litoria moorei	Glyphosate, glyphosate IPA, Roundup®, Touchdown® and Roundup® Biactive	N/A	48-hr acute LC_{50}'s (formulations) for tadpoles, metamorphs and adults between 2.9 and >360 mg a.e./L with Roundup® (MON 2139) as the most toxic formulation to Roundup® Biactive as the least toxic formulation. Glyphosate IPA salt alone (LC_{50}: 466 mg a.e./L) less toxic than glyphosate acid (LC_{50}: 81.2 – 121 mg a.e./L), probably due to acid intolerance. Slight differences in species sensitivity *L moorei* tadpoles more sensitive than other tadpoles; adult and new metamorphs less sensitive than tadpoles.	E071857
Leopard frog (*Rana pipiens*), Green frog, (*Rana clamitans*) American toad, (*Bufo americanus*), African clawed frog (*Xenopus laevis*)	Vision® (contains POEA surfactant)	N/A	96-hr acute studies. Toxicity enhanced by elevated pH with Surfactant POEA (15%) hypothesized as major source of pH interaction. LC_{50}'s (mga.e./L) pH 6.0 pH 7.5 Leopard frog embryo* 15.1 7.5 Leopard frog larvae* 1.8 1.1 Green frog embryo 5.3 4.1 Green frog larvae 3.5 1.4 American toad embryo 4.8 6.4 American toad larvae 2.9 1.7 African clawed frog embryo 15.6 7.9 African clawed frog larvae 2.1 0.88 *Gosner 8-25 = embryo, Gosner 25 = larvae Growth inhibition in surviving frogs observed with clawed frog, green frog and leopard frog	E072795
Scinax nasicus tadpoles Gosner stages 25-26 (prometamorphic)	Glyfos (48% IPA + 15% POEA)	N/A	96-hr acute LC_{50}: 2.64 mg glyphos/L (1.95 mg a.e./L). Malformations (craniofacial and mouth deformities, eye abnormalities and bent curved tails) increase with increased time and mortality.	E071969

Table 4.15. Aquatic Amphibian Sublethal Effects From Submitted and Open Literature Studies				
Species	**Chemical**	**NOAEC**	**LC_{50} or LOAEC:Effects**	**MRID/ ECOTOX Ref. No.**
Western chorus frog (*Pseudacris triseriata*) and Plains leopard frog (*Rana blairi*) tadpoles Gosner stage 25	Kleeraway Grass and Weed Killer RTU (IPA 0.75%, surfactant – ethoxylated tallowamine).		Concentration levels 750, 75, 7.5 or 0.75 ppm IPA. 24-hr exposure period. No frogs survived 7.5 – 750 ppm. Western chorus frogs slightly more sensitive. No effect on growth or final Gosner stage.	E61464
Rana cascadae larvae	Roundup® 50.2%	Not determined for time to metamorph-osis	LOAEL 1 ppm. Concentration levels 0.96 and 1.94 ppm for 43 days. None survived to metamorphosis at 1.94 ppm (mean time 7.5 days). Bent tails and slow swimming ability before death. Metamorphosis occurred more rapidly in treated frogs with decreased size and mass. Unclear from this study as to whether or not LOAEL is in terms of a.e..	E096423

4.1.2 Toxicity to Freshwater Invertebrates

Freshwater aquatic invertebrate toxicity data were used to assess potential indirect effects of glyphosate to the CRLF. Effects to freshwater invertebrates resulting from exposure to glyphosate have the potential to indirectly affect the CRLF via reduction in available food items. As discussed in Section 2.5.3, the main food source for juvenile aquatic- and terrestrial-phase CRLFs is thought to be aquatic invertebrates found along the shoreline and on the water surface, including aquatic sowbugs, larval alderflies and water striders.

A summary of acute and chronic freshwater invertebrate data, including data published in the open literature, is provided below in Sections 4.1.2.1 through 4.1.2.3.

4.1.2.1 Freshwater Invertebrates: Acute Exposure Studies

The acute toxicity endpoint for aquatic invertebrates is taken from the study on early fourth instar midge larvae, maintained in laboratory cultures. As with freshwater fish, many studies are available on formulations. Because the number of studies on formulations is so extensive, only a few of the studies are summarized here. The remainder of the studies are summarized in tables in **Appendix J**. One study (MRID 00162296) tested glyphosate technical, a glyphosate IPA formulation and the surfactant, POEA on the midge. The EC_{50}'s were: 53.2, 13.3 and 13 mg/L. The EC_{50}'s for the technical material and the formulation are expressed in terms of glyphosate acid equivalents. As with freshwater fish and amphibians, this study indicates that the increased toxicity of the formulations with the surfactant, POEA are probably due to the surfactant.

For formulations, as with freshwater fish, for terrestrial uses, the most conservative endpoint from all the active formulations was selected. For aquatic uses, the endpoint

was selected from a study that was conducted with a formulation that is currently labeled for aquatic use.

Table 4.16. Freshwater Invertebrates Acute Toxicity for Technical Glyphosate*

Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Midge (*Chironomus plumosus*)	96.7	LC_{50}: **53.2 (30.0 - 93.8)**[3] NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable
Water flea (*Daphnia magna*)	95.6	EC_{50}: 128.1 (95.6 - 172.1) NOAEC: 95.6 Slope: N.R.	Practically nontoxic	44320631/1995	Acceptable
Water flea (*Daphnia magna*)	83	EC_{50}: 647.4 (577.7 - 725.4) NOAEC: 464.8 Slope: N.R.	Practically nontoxic	00108172/1978	Acceptable

* No technical glyphosate salts were tested; a.i. = active ingredient; a.e. = acid equivalent, N.R. = not reported
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] **Bolded** and shaded value will be used to calculate risk quotients
[3] Range is 95% confidence interval for endpoint

Table 4.17. Freshwater Invertebrates Acute Toxicity for Glyphosate Formulations

Chemical	Species	% a.i.*	48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope	Toxicity Category[1]	MRID #/Year	Study Classification
Glyphosate IPA (Roundup with POEA surfactant)	Water flea (*Daphnia magna*)	30.3	EC50: 2.2 (1.9 - 2.5); **formulation: 3** NOAEC: N.R. Slope: N.R.	Moderately toxic	00162296/1979	Acceptable
Glyphosate (360 g/L SL formulation)	Water flea (*Daphnia magna*)	27.25	EC50: 44.8 (38.0 - 52.0); **formulation: 164.3** NOAEC: 26 Slope: 7.6	Slightly toxic	45374003/1999	Acceptable

Table 4.17. Freshwater Invertebrates Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**48-hour EC_{50} - LC_{50}/ NOAEC (mg a.e./L)*/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Glyphosate IPA (Roundup)	Water flea (*Daphnia magna*)	41.36	EC50: 1.6 (1.4 - 1.9)[2] NOAEC: 0.6 Slope: 5.4	Moderately toxic	00070893/1980	Acceptable
Glyphosate IPA (Roundup)	Crayfish (*Orconectes nais*)	30.3	LC50: 5.2 (4.1 - 6.4) NOAEC: N.R. Slope: N.R.	Moderately toxic	40098001/1986	Supplemental
Glyphosate IPA (Roundup)	Scud (*Gammarus pseudolimnaeus*)	31	LC50: 13 (9.6 - 19.2) NOAEC: 1.4 Slope: 2.33	Slightly toxic	00124762/1982	Supplemental
Glyphosate IPA (Roundup with POEA surfactant)	Midge (*Chironomus plumosus*)	30.3	LC50: 13.3 (7.0 - 23.7) NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable
Glyphosate IPA (no surfactant)	Water flea (*Daphnia magna*)	62.4	EC50: 401.3 (347.7 - 470.5) NOAEC: 147.8 Slope: 7.6	Practically nontoxic	00078663/1981	Acceptable
Glyphosate IPA with surfactant Geronol CF/AR	Water flea (*Daphnia carinata*)	36	EC50: 220 (194 – 252) (610 (540 - 700) mg formulation/L) NOAEC: 49 or 135 mg formulation/L Slope: N.R.	Practically nontoxic	44738201/1996	Not classified

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint
[3]**Bolded** and shaded value will be used to calculate risk quotients

Table 4.18. Freshwater Invertebrates Acute Toxicity for Surfactants Used with Glyphosate Formulations						
Chemical	**Species**	**% a.i.***	**48-hour EC_{50} - LC_{50}/ NOAEC (mg/L)/ Slope**	**Toxicity Category[1]**	**MRID #/Year**	**Study Classification**
Surfactant Geronol CF/AR (alkyl polyoxy ethylene phosphoric acid)	Daphnia (*Daphnia magna*)	Tech.	EC50: 48 NOAEC: Slope: N.A.	Slightly toxic	44738201/1996	Not classified
MON 0818 (POEA)	Midge (*Chironomus plumosus*)	100	LC50: 13 (7.1-24.0)[2] NOAEC: N.R. Slope: N.R.	Slightly toxic	00162296/1979	Acceptable

* a.i. = active ingredient, assumed 100% for technical.
[1]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[2] Range is 95% confidence interval for endpoint

The acute toxicity study with the water flea (Table 4.19) indicates that the degradate, aminomethyl phosphonic acid (AMPA) is less toxic to freshwater invertebrates than the parent glyphosate.

Table 4.19. Freshwater Invertebrates Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**48-hour LC_{50}/NOAEC (mg/L)/Slope**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
AMPA	Water flea (*Daphnia magna*)	94.38	EC50: 683 (553 - 1010) NOAEC: 320 Slope: N.A.	Practically nontoxic	43334715/1994	Acceptable

[1] a.i. = active ingredient, assumed 100% for technical material
[2]Based on LC_{50} (mg/L): < 0.1 very highly toxic; 0.1-1 highly toxic; >1-10 moderately toxic; >10-100 slightly toxic; >100 practically nontoxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available

4.1.2.2 Freshwater Invertebrates: Chronic Exposure Studies

Table 4.20. Freshwater Invertebrates Chronic Toxicity for Technical Glyphosate IPA Salt				
Species	**% Active Ingredient**	**NOAEC/LOAEC (mg acid equivalent/L)**	**MRID #/Year**	**Study Classification**
Water flea (*Daphnia magna*)	99.7	**49.9**/95.7[1]	00124763/1982	Acceptable
[1]**Bold** value will be used to calculate risk quotients				

4.1.2.3 Freshwater Invertebrates: Open Literature Data

There are additional freshwater invertebrate toxicity data, including sublethal effects information, available in the open literature (for references and other details see **Appendices G and H**). None of the toxicological endpoints identified in the open literature studies are more sensitive than the most sensitive acute and chronic endpoints available in the submitted studies (see Sections 4.1.2.1 – 4.1.2.2).

4.1.3 Toxicity to Aquatic Plants

Aquatic plant toxicity studies were used as one of the measures of effect to evaluate whether or not glyphosate has the potential to affect primary production and the availability of aquatic plants as food for CRLF tadpoles. Primary productivity is essential for indirectly supporting the growth and abundance of the CRLF.

Two types of studies were used to evaluate the potential of glyphosate to affect aquatic plants. Laboratory and field studies were used to determine whether or not glyphosate has the potential to cause direct effects to aquatic plants. A tabular summary of the laboratory data and freshwater field studies for aquatic plants is provided in Sections 4.1.3.1 and 4.1.4.

4.1.3.1 Aquatic Plants: Laboratory Data

For aquatic vascular plants, the endpoint is selected from a duckweed study (MRID 44320638). This study does not fulfill guideline requirements because it needs phytotoxicity data; however, this is a 14-day study and it has a lower EC_{50} value than any of the other studies. Therefore, this study is selected for the vascular plant endpoint. For aquatic non-vascular plants, the endpoint is selected from a toxicity study on green algae (MRID 40236901). This study appears to have fewer uncertainties than MRID 40236904. Therefore, the endpoint is selected from this study. Again, as with other aquatic species, some of the formulations appear to be more toxic than the technical material.

Table 4.21. Aquatic Vascular and Nonvascular Freshwater Plant Toxicity Studies for Technical Glyphosate				
Species	**% Active Ingredient***	**EC_{50} NOAEC (mg a.e./L)*/ Slope**	**MRID #/Year**	**Study Classification**
Vascular Plants				
Duckweed (*Lemna gibba*)	95.6	14-day EC50: **11.9 (9.4-14.9)** NOAEC: 1.3 Slope: N.R.	44320638/1996	Supplemental
Duckweed (*Lemna gibba*)	96.8	7-day EC50: 23.2 (20.3 - 27.1) NOAEC: 7.3 Slope: 2.91	45773101/2002	Acceptable
Duckweed (*Lemna gibba*)	96.6	14-day EC50: 20.8 (N.R.) NOAEC: <1.8 Slope: N.R.	40236905/1987	Acceptable
Non-vascular Plants				
Green algae (*Selenastrum capricornutum*)	96.6	4-day EC50: **12.1 (11.5 - 12.9)** NOAEC: N.R. Slope: 12	40236901/1987	Acceptable
Bluegreen algae (*Anabaena flos-aquae*)	96.6	4-day EC50: 11.4 (10.5 - 12.1) NOAEC: N.R. Slope: 3.53	40236904/1987	Acceptable
Green algae (*Selenastrum capricornutum*)	95.6	5-day EC50: 13.4 (9.6 - 19.1) NOAEC: 9.6 Slope: N.R.	44320637/1995	Acceptable
Bluegreen algae (*Anabaena flos-aquae*)	95.6	5-day EC50: 14.3 (9.3 - 25.8) NOAEC: 11.5 Slope: N.R.	44320639/1996	Acceptable
Freshwater diatom (*Navicula pelliculosa*)	95.6	5-day EC50: 16.3 (11.5 - 22.9) NOAEC: 1.7 Slope: N.R.	44320641/1996	Acceptable
Freshwater diatom (*Navicula pelliculosa*)	96.6	7-day EC50: 37.3 (34.8 - 41.5) NOAEC: 18.5 Slope: 5.87	40236902/1987	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported
[1] Range is 95% confidence interval for endpoint
[2] **Bold** value will be used to calculate risk quotients

Table 4.22. Aquatic Vascular and Nonvascular Freshwater Plant Toxicity Studies for Glyphosate Formulations					
Chemical	**Species**	**% a.i.***	**EC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**MRID #/Year**	**Study Classification**
Vascular Plants					
Glyphosate IPA salt* (glyphos (glyphosate product))	Duckweed (*Lemna gibba*)	31.0	7-Day EC50: 7.7 (7.1 - 8.3) [1] Formulation: **25** NOAEC: 0.29 Slope: 4.76	45666704/2001	Acceptable
Glyphosate IPA salt (Roundup 41%)	Duckweed (*Lemna minor*)	30.3	14-day EC50: 1.5 (N.R.) ; for formulation: **4.9** NOAEC: N.R. Slope: N.R.	44125714/1984	Supplemental
Glyphosate IPA salt (TEP Roundup)	Duckweed (*Lemna minor*)	NR	48 hr. EC50: >16.91 (N.A.) NOAEC: 16.91 Slope: N.A.	44125713/1989	Supplemental
Glyphosate IPA salt (Roundup, % not reported)	Duckweed (*Lemna minor*)	N.R.	14-day EC50: 2.0 (N.R.) NOAEC: N.R. Slope: N.R.	44125714/1984	Supplemental
Nonvascular Plants					
Glyphosate monoammonium salt	Green algae (*Selenastrum capricornutum*)	68.5	72-hr EC50: 1.85 (1.3 - 2.3) NOAEC: 0.61 Slope: N.R.	45777403/1999	Supplemental
Glyphosate monoammonium salt	Green algae (*Selenastrum capricornutum*)	64.9	72-hr EC50: 11.2 (10 - 12.6) NOAEC: 1.58 Slope: N.R.	45767102/2002	Supplemental
Glyphosate IPA salt with surfactant Geronol CF/AR	Green algae (*Selenastrum capricornutum*)	36	72-hr EC50: 97 (85 - 111) NOAEC: 73 Slope: N.A.	44738201/1996	Supplemental
Glyphosate IPA salt with surfactant Geronol CF/AR	Green algae (*Selenastrum capricornutum*)	36	72-hr EC50: 39 (33 - 45) NOAEC: 16 Slope: N.A.	44738201/1996	Supplemental
Glyphosate (glyphos)	Freshwater diatom (*Navicula pelliculosa*)	31.0	96-hr EC50**: 0.12 (0.11 – 0.13)** [2]; for formulation: **0.39** NOAEC: 0.082 Slope: 8.78	45666701/2001	Acceptable
Glyphosate IPA salt (glyphos (glyphosate product))	Green algae (*Selenastrum capricornutum*)	31.0	96-hr EC50: 0.68 (0.57 - 0.81) NOAEC: 0.43 Slope: 4.47	45666702/2001	Acceptable

* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; NR = not reported; NA = not available
[1] Range is 95% confidence interval for endpoint
[2] **Bolded** and shaded value will be used to calculate risk quotients

Table 4.23. Aquatic Nonvascular Freshwater Plant Toxicity Studies on Glyphosate Mixtures					
Chemical	**Species**	**% a.i.***	**EC_{50}/ NOAEC (mg a.e.*/L)/ Slope**	**MRID #/Year**	**Study Classification**
Nonvascular Plants					
Glyphosate acid-equivalent (IPA)/Oxyfluorfen mix	Green algae (*Selenastrum capricornutum*)	32	96-hr EC50: 0.0026 (0.0021 – 0.0033)[1] NOAEC: 0.00045 Slope: 3.96	45906008/2001	Acceptable
* a.i. = active ingredient; a.e. = acid equivalent; IPA = isopropylamine salt; [1]Range is 95% confidence interval for endpoint					

4.1.3.2 Aquatic Plants: Open Literature Data

Three studies on 3 different species of green algae were conducted which provide lower 96-hr EC_{50}'s based on cell counts (growth) correlated with absorbance over time for 96 hours on a Shimadzu UV-2401 PC Spectrophotometer. All of these studies were performed by the same group of scientists and published in different papers. In the first study, conducted with 95% technical material (not stated if glyphosate or the IPA of glyphosate), the 96-hr EC_{50} was 3.530 mg/L for *Chlorella pyrenoidosa* (Ma et.al 2001, ECOTOX reference 61983). In the second study (Ma et al., 2002, ECOTOX reference 65938), the 96 hr. EC_{50} for *Chlorella vulgaris* was 4.70 mg/L. This was again conducted with a 95% technical product. The study authors used the CAS number for glyphosate, not IPA, so it is assumed that this is the acid. The third study, conducted with *Raphidocelis subcapitata (Selenastrum capricornutum)* (Ma et al., 2006, ECOTOX ref. 83543), the 96 hr. acute toxicity value is 5.56 mg/L. Again, the study was conducted with 95% technical product, which is presumed to be the glyphosate acid. The results from these studies are discussed and compared to the aquatic exposure values in the risk characterization section (Section 5.2.2.1).

4.1.4 Freshwater Field/Mesocosm Studies

A study was conducted to examine the effects of glyphosate on the biomass of predators, tadpoles/small herbivores, zooplankton and periphyton, the survival of predators, the abundance of zooplankton, and survival of tadpole species in mesocosm study units (1200L tanks (Relyea, ECOTOX ref. 89112)). A simulated application rate of 6.4 mL/m^2 with a 25.2% formulation was used, providing a nominal concentration of 3.8 mg a.i.L. Species used in the mesocosms were reported to be naturally co-occurring and at loading rates similar to what are found in the field. The study was conducted for 13 days under static conditions following a single spray application. Under the conditions tested, species richness was reduced by 22% with Roundup®. Roundup® completely eliminated two species of tadpoles (leopard frogs and gray tree frogs) and nearly eliminated wood frogs (98% mortality), resulting in a 70% decline in the species richness of tadpoles. It is not clear from the methods section which specific formulation of the pesticide was used; however, the study authors state that the formulation of glyphosate (Roundup) contains

polyethoxylated tallowamine (POEA). Although Roundup appeared to be associated with a high mortality rate in amphibian larvae, amphibian mortality in controls ranged from approximately 30 to 80%. The relatively high mortality rate with control tadpole species was likely due to predation from spotted salamanders and predacious beetles; however, it is difficult to interpret glyphosate-related mortality given the extent of mortality in controls for some tadpole species. It is noteworthy that while increased mortality of amphibian larvae appeared to be associated with glyphosate treatment, red-spotted salmanders were not affected.

A study was conducted with glyphosate to determine whether or not glyphosate plus the surfactant, polyethoxylated tallow amine (POEA) affects survival of anurans, either in aquatic environments (mesocosms) and/or terrestrial environments (semi-dry tanks; Relyea, ECOTOX Ref. 86885). The pesticide was applied by a direct overspray. In an aquatic larvae study, a factorial combination of glyphosate present or absent with three different soil treatments (no soil, sand, and loam) was tested. The concentration of glyphosate was reportedly based on the label recommended application rate (i.e., a nominal concentration of 3.8 mg a.i./L (simulated application rate of 1.6 mL a.i./m^2)). Roundup® Weed and Grass Killer was tested (25.2% active ingredient plus POEA surfactant). For the terrestrial juvenile study, glyphosate with POEA surfactant was tested in comparison to a control. The nominal amount tested was 6.5 mL at a rate of 1.6 mg a.i./m^2. There were three replicates, each time with a different amphibian species.

The results of the study suggested that exposure to nominal concentrations of Roundup® Weed and Grass Killer at a rate equivalent to 1.6 mg a.i./m^2 (3.8 mg a.i./L) for 20 days, decreased survival of leopard frogs, American toads and gray tree frogs [aquatic phase] larvae by over 73%. American toad larvae were the most sensitive with only 20% survival followed by gray tree frog (50% survival) and leopard frog (75%) survival compared to controls with >80% survival). It is not clear whether the toxicity can be attributed to glyphosate alone, the surfactant polyethoxylated tallowamine (POEA) alone, or to the combination of glyphosate and POEA. Although the study suggests that presence of soil did not decrease the toxicity of Roundup®, it is also not clear whether the amount of soil added to each of the study units was adequate to test this hypothesis. Exposure of juvenile [terrestrial phase] wood frogs, tree frogs and American toads to Roundup at a rate of 1.6 mg a.i./m^2 resulted in over 64% decrease in survival across species after 24 hours. It is not clear how the terrestrial exposure of Roundup® to terrestrial-phase juvenile frogs relates to conditions that may exist in the field. The moist paper towel would likely prolong exposure beyond what may typically be encountered in the field.

A mesocosm study was conducted with a glyphosate formulation (13% a.i.) applied to 1,200L outdoor cattle troughs containing three aquatic-phase amphibian species (leopard frog, gray tree frog and the American toad) with and without predators (red-spotted newt or *Dytiscus* beetles). Exposure was static for 23 days (Relyea et. al, ECOTOX Ref. 86886). Although there was uncertainty associated with the application rates and the specific formulation used, study units were apparently treated at a nominal concentration of 1.3 mg glyphosate/L. Glyphosate treatment reduced overall tadpole survival and biomass. American toad larvae were the most sensitive with only 20% survival followed

by gray tree frog (50% survival) and leopard frog (75%) survival compared to controls with >80% survival). Glyphosate had no effect on the survival of red-spotted newts. The study design is not sufficient to determine whether the decreased survival/biomass associated with exposure to Roundup is due to glyphosate or to some other component of the formulated product. While the study authors speculate on the potential role of the surfactant, polyethoxylated tallowamine (POEA), in causing the observed effects on anuran larvae, the study does not test this potential relationship.

Chemical and biological monitoring studies were conducted in 51 different wetlands to quantify the magnitude of contamination by glyphosate formulation Vision® (Thompson et. al, ECOTOX Ref. 72797). Wetlands were classified as over-sprayed, adjacent, or buffered in relation to the operational target spray blocks. Aqueous concentrations of glyphosate in buffered wetlands were below the level of detection (<0.02 mg a.i./L) in 14 of the 16 buffered wetlands. Mean glyphosate concentrations in the buffered wetlands (0.03 mg a.i./L) were significantly ($p<0.05$) less than that of either adjacent (0.18 mg a.i./L) or over-sprayed wetlands (0.33 mg a.i./L). Biomonitoring of caged amphibians larvae showed no significant effect on mean 48-hr mortality of either green leopard frogs (*Rana pipiens*) or green frogs (*R. clamitans*) exposed *in situ*. Percent mortality was not significantly correlated with exposure concentrations. The authors conclude that there were no statistically significant differences in mortality between treatment sites; however, leopard frog and green frog larvae had 14.2% and 35.6% mortality in over-sprayed areas. Buffered areas with the lowest mean concentrations (0.03 mg a.i./L) of glyphosate had larval mortality for leopard frog larvae (15%) and green frog larvae (25.7%) roughly similar to oversprayed areas. The authors conclude that glyphosate exposures typically occurring in forest wetlands are insufficient to induce significant acute mortality in native amphibian larvae. No raw data were included in the study; however, the results suggest that there was a large amount of variability that could have obscured detecting treatment effects especially given that these were naturally occurring wetlands that represented a range of environmental conditions. Additionally, since concentrations of the surfactant (MON0818) were not measured, it is uncertain as to the extent that this co-formulant was present in any of the aquatic habitats studied.

Open Literature Studies

Aquatic vascular plants

For most of the studies on vascular plants, there are insufficient details in the articles to accurately determine concentration levels tested. For other studies, the endpoints were higher than those found in the submitted studies.

Aquatic nonvascular plants

Of the available open literature studies from which data may be extracted for comparing the results with the submitted studies, 3 studies, on 3 different species of green algae provide lower 96-hr EC_{50}'s based on cell counts (growth) correlated with absorbance over time for 96 hours on a Shimadzu UV-2401 PC Spectrophotometer. All of these studies were performed by the same group of scientists and published in different papers.

The papers were not thoroughly reviewed for acceptability according to Agency guidelines; however, they are discussed in this section and compared to the highest aquatic EEC. In the first study, conducted with 95% technical material (not stated if glyphosate or the IPA of glyphosate), the 96-hr EC_{50} was 3.530 mg/L for *Chlorella pyrenoidosa* (Ma et.al 2001, ECOTOX reference 61983). Comparing that value to the highest EEC of 222.9 ppb, the RQ would be 0.06, significantly lower than the LOC for aquatic plants. In the second study (Ma et al., 2002, ECOTOX reference 65938), the 96 hr. EC_{50} for *Chlorella vulgaris* was 4.70 mg/L. This was again conducted with a 95% technical product. The study authors used the CAS number for glyphosate, not IPA, so it is assumed that this is the acid. The resulting highest RQ from this study would be 0.05. The third study, conducted with *Raphidocelis subcapitata (Selenastrum capricornutum)* (Ma et al., 2006, ECOTOX ref. 83543), the 96 hr. acute toxicity value is 5.56 mg/L with a resulting RQ of 0.04. Again, the study was conducted with 95% technical product, which is presumed to be the glyphosate acid. Even with these lower endpoints, the LOC for aquatic plants would not be exceeded.

4.2 Toxicity of Glyphosate to Terrestrial Organisms

Tables 4.24 and 4.25 summarize the most sensitive terrestrial toxicity endpoints for the CRLF, based on an evaluation of both the submitted studies and the open literature. A brief summary of submitted and open literature data considered relevant to this ecological risk assessment for the CRLF is presented below.

Table 4.24 Terrestrial Toxicity Profile for Glyphosate and/or Its Salts

Endpoint	Species	Toxicity Value Used in Risk Assessment	Citation MRID#/Date	Comment
Acute Direct Toxicity to Terrestrial-Phase CRLF (LD_{50})	Bobwhite quail (*Colinus virginianus*)	LD_{50}: >1912 mg/kg bw	44320626/1997	Acceptable
Acute Direct Toxicity to Terrestrial-Phase CRLF (LC_{50})	Bobwhite quail (*Colinus virginianus*)	LC_{50}: >4971.2 PPM	44320628/1997	Acceptable
Chronic Direct Toxicity to Terrestrial-Phase CRLF	Bobwhite quail (*Colinus virginianus*)	Reproduction study NOAEC: 830 PPM	108207/1978	Acceptable LOAEC: >830 PPM (highest concentration tested).
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to mammalian prey items)	Rat (*rattus norvegicus*)	LD_{50} >4800 mg/kg bw	43728003/1989	Acceptable
Indirect Toxicity to Terrestrial-Phase CRLF (via	Rat (*rattus norvegicus*)	NOAEL: 500 mg/kg bw/day; NOAEC: 10000 ppm	41621501/1990	Acceptable Reproduction study parental/pup LOAEL: 1500 mg/kg

Table 4.24 Terrestrial Toxicity Profile for Glyphosate and/or Its Salts				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
chronic toxicity to mammalian prey items)				bw/day; LOAEC: 30000 ppm (soft stools, decreased body weight gain and food consumption in parents and decreased body weight gain during lactation in pups).
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to terrestrial invertebrate prey items)	Honey bee (*Apis mellifera*)	48 hr LD_{50} (O): >100 µg/bee	00026489/1972	Acceptable
Indirect Toxicity to Terrestrial- and Aquatic-Phase CRLF (via toxicity to terrestrial plants)	Seedling Emergence Monocots	EC25: >5 LB/A	40159301/1987	Acceptable
	Seedling Emergence Dicots	EC25: > 5 LB/A	40159301/1987	Acceptable
	Vegetative Vigor Monocots	EC25**:** 0.16 LB/A	44125715/45045101/ 1995	Acceptable
	Vegetative Vigor Dicots	EC25: 0.074 LB/A	44320636/1996	Acceptable

For birds and mammals, the endpoints following acute exposure are not discrete and a quantitative estimate of risk could not be done. However, for registered formulation products, there is one avian study and 4 mammalian studies with discrete values. For estimation of risk, these studies were matched with the specific labeled rates and uses. Endpoints for these studies are summarized in **Table 4.25**.

Table 4.25 Terrestrial Toxicity Profile for Glyphosate Formulations				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
Acute Direct Toxicity to Terrestrial-Phase CRLF (LD_{50})	Bobwhite quail (*Colinus virginianus*)	LD_{50}: 1651mg/kg bw	45777402/1999	Acceptable Glyphosate monoammonium salt (MON 14420)
Indirect Toxicity to Terrestrial-Phase CRLF (via acute toxicity to	Rat (*rattus norvegicus*)	LD_{50}: 3750 mg/kg bw	41305404/1989	Acceptable

Table 4.25 Terrestrial Toxicity Profile for Glyphosate Formulations				
Endpoint	**Species**	**Toxicity Value Used in Risk Assessment**	**Citation MRID#/Date**	**Comment**
mammalian prey items)	Rat (*rattus norvegicus*)	LD_{50}: 5000 mg/kg bw	41142304/1989	Acceptable
	Rat (*rattus norvegicus*)	LD_{50}: 5827 mg/kg bw	44615502/1998	Acceptable
	Rat (*rattus norvegicus*)	LD_{50}: 3803 mg/kg bw	44918601/1999	Acceptable

Acute toxicity to terrestrial animals is categorized using the classification system shown in **Table 4.4** (U.S. EPA, 2004). Toxicity categories for terrestrial plants have not been defined.

Table 4.26 Categories of Acute Toxicity for Avian and Mammalian Studies		
Toxicity Category	**Oral LD_{50}**	**Dietary LC_{50}**
Very highly toxic	< 10 mg/kg	< 50 ppm
Highly toxic	10 - 50 mg/kg	50 - 500 ppm
Moderately toxic	51 - 500 mg/kg	501 - 1000 ppm
Slightly toxic	501 - 2000 mg/kg	1001 - 5000 ppm
Practically non-toxic	> 2000 mg/kg	> 5000 ppm

4.2.1 Toxicity to Birds

As specified in the Overview Document, the Agency uses birds as a surrogate for terrestrial-phase amphibians when amphibian toxicity data are not available (U.S. EPA, 2004). No terrestrial-phase amphibian data are available for glyphosate; therefore, acute and chronic avian toxicity data are used to assess the potential direct effects of glyphosate to terrestrial-phase CRLFs.

4.2.1.1 Birds: Acute Exposure (Mortality) Studies

Acute toxicity data on selected avian species are available for technical glyphosate, several formulations and the AMPA degradate. Based on the available studies, glyphosate is at the most, only slightly toxic. It does not appear that the formulations are any more toxic than the technical material. The AMPA degradate is not more toxic than the parent either. **Tables 4.27 – 4.29** summarize these studies.

Table 4.27. Avian Acute Toxicity for Technical Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	LD50: >**3196** mg a.e./kg bw	Slightly toxic	00108204	Acceptable No treatment-related mortalities.
Glyphosate	Mallard duck (*Anas platyrhynchos*)	98.5	LC50: >4570 (N.A.) PPM NOAEC: 4570.4	Slightly toxic	108107/37765/1973	Acceptable No mortalities at any concentration
Glyphosate	Bobwhite quail (*Colinus virginianus*)	98.5	LC50: >4570 (N.R.) PPM NOAEC: 4570	Slightly toxic	00076492/1973	Acceptable No mortalities at any concentration
Glyphosate	Bobwhite quail (*Colinus virginianus*)	95.6	LD50: >1912 (N.A.) mg/kg bw NOAEL: 1912	Slightly toxic	44320626/1997	Acceptable No mortalities at any dose
Glyphosate	Mallard duck (*Anas platyrhynchos*)	95.6	LC50: >4971 (N.A.) PPM NOAEC: 4971.2	Slightly toxic	44320627/1998	Acceptable No mortalities at any concentration
Glyphosate	Bobwhite quail (*Colinus virginianus*)	95.6	LC50: >**4971** (N.A.) PPM NOAEC: 4971.2	Slightly toxic	44320628/1997	Acceptable No mortalities at any concentration

[1] a.i. = active ingredient; a.e. = acid equivalent

[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic

[3] Range is 95% confidence interval for endpoint, N.A. = not available, N.R. = not reported

[4] **Bolded** and shaded value will be used to calculate risk quotients.

Table 4.28 Avian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Trisodium diglyphosate/Urea (Polado formula; MON 8000)	Bobwhite quail (*Colinus virginianus*)	75	LD50: >780 (N.R.) PPM NOAEC: 780	Slightly toxic	00085638/1980	Supplemental
Trisodium diglyphosate/Urea (Polado formula; MON 8000)	Bobwhite quail (*Colinus virginianus*)	75	LC50: >1770 (N.R.) PPM NOAEC: 1770	Slightly toxic	00085639/1981	Supplemental
Trisodium diglyphosate/Urea (Polado formula; MON 8000)	Mallard duck (*Anas platyrhynchos*)	75	LC50: >1770 (N.R.) PPM NOAEC: 315	Slightly toxic	00085640/1980	Supplemental
Glyphosate monoammonium salt (MON 14420)	Bobwhite quail (*Colinus virginianus*)	68.5	LD50: 1131 (925 - 1541) mg/kg bw (**1651** mg formulation/ kg bw)[4] NOAEL: 333	Slightly toxic	45777402/1999	Acceptable
Glyphosate isopropylamine salt (MON65005)	Mallard duck (*Anas platyrhynchos*)	31.32	LC50> 1760 (N.A.) PPM NOAEC: 1760	Slightly toxic	44465701/1997	Acceptable
Glyphosate isopropylamine salt (MON65005)	Bobwhite quail (*Colinus virginianus*)	31.32	LC50> 1760 (N.A.) PPM NOAEC: 1760	Slightly toxic	44465702/1997	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[3] Range is 95% confidence interval for endpoint, N.A. = not available, N.R. = not reported
[4] **Bolded** and shaded value will be used to calculate risk quotients.

Table 4.29. Avian Acute Toxicity for Aminomethyl Phosphonic Acid (AMPA) Degradate of Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)/ Slope[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
AMPA	Bobwhite quail (*Colinus virginianus*)	87.8	LD50: >1976 (N.A.) mg/kg NOAEL: 1185 Slope: N.A.	Slightly toxic	43334709/1991	Acceptable
AMPA	Bobwhite quail (*Colinus virginianus*)	87.8	LC50: >4934 (N.A.) PPM NOAEC: 4934 Slope: N.A.	Slightly toxic	43334710/1994	Acceptable
AMPA	Mallard duck (*Anas platyrhynchos*)	87.8	LC50: >4934 (N.A.) PPM NOAEC: 4934 Slope: N.A.	Slightly toxic	43334711/1994	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LC_{50} (ppm): < 50 very highly toxic; 50 - 500 highly toxic; 501 - 1000 moderately toxic; 1001-5000 slightly toxic; >5000 practically non-toxic; based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic
[4] Range is 95% confidence interval for endpoint, N.A. = not available

4.2.1.2 Birds: Chronic Exposure (Growth, Reproduction) Studies

Neither reproductive effects nor effects on growth were observed following chronic exposure to either mallards or bobwhite quail.

Table 4.30. Avian Chronic Toxicity for Technical Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**Toxicity Category[2]**	**MRID #/Year**	**Study Classification**
Glyphosate	Mallard duck (*Anas platyrhynchos*)	90.4	LOAEC: >27 (N.A.) PPM NOAEC: 27	N.A	00036328/113457/1975	Supplemental
Glyphosate	Mallard duck (*Anas platyrhynchos*)	83	LOAEC: >830 (N.A.) PPM NOAEC: 830	N.A.	111953/1978	Acceptable
Glyphosate	Bobwhite quail (*Colinus virginianus*)	83	LOAEC: >830 (N.A.) PPM NOAEC: **830**	N.A.	108207/1978	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable
[3] **Bolded** shaded value will be used to calculate risk quotients.

4.2.1.3 Birds: Open Literature Data

There are additional avian toxicity data, including sublethal effects information, available in the open literature (for details see **Appendix H**). None of the toxicological endpoints identified in the open literature studies are more sensitive than the most sensitive acute and chronic endpoints available in the submitted avian toxicity studies (see Sections 4.2.1.1 – 4.2.1.2).

There was one subchronic study on the effects of the formulation, Roundup "(360 g/l of glyphosate, 480 g/l of IPA salt and 684 g/l of other inert ingredients)" on the epididymal region of drakes (*Anas platyrhynchos*) (Oliveira et. al. 2007, ECOTOX Reference No. 97136). The formulation was administered by gavage to three groups of 6 adult drakes for 15 days at 0 (distilled water), 5 and 100 mg/kg bw. There was a significant reduction (90%, $p \leq 0.05$) in plasma testosterone levels after treatment at both dose levels when compared to the control group. The report stated that "alterations in the structure of the testis and epididymal region…with changes in the expression of androgen receptors restricted to the testis" were observed. The authors also stated that "the effects were mostly dose dependent, indicating that this herbicide may cause disorder in the morphophysiology of the male genital system of animals". Further studies would be needed to determine whether or not these observed effects would affect avian (or, in this case, terrestrial-phase amphibian) reproduction.

4.2.1.4 Terrestrial-phase Amphibian Acute and Chronic Studies

No toxicity studies on glyphosate are available for terrestrial-phase amphibians.

4.2.2 Toxicity to Mammals

Mammalian toxicity data are used to assess potential indirect effects of glyphosate to the terrestrial-phase CRLF. Effects to small mammals resulting from exposure to glyphosate have the potential to also indirectly affect the CRLF via reduction in available food. As discussed in Section 2.5.3, over 50% of the prey mass of the CRLF may consist of vertebrates such as mice, frogs, and fish (Hayes and Tennant, 1985).

4.2.2.1 Mammals: Acute Exposure (Mortality) Studies

The acute toxicity studies on the technical material indicate that glyphosate is practically non-toxic to mammals. Hundreds of studies are available on formulations. Most of the LD_{50}'s are greater than the highest dose tested. Only a small sample of the studies on the formulations is presented here. The rest of the studies are presented in **Appendix J**.

Table 4.31. Mammalian Acute Toxicity for Technical Glyphosate						
Chemical	**Species**	**% a.i.[1]**	**LD_{50} (mg a.e./kg bw)[1]**	**Toxicity Category[2]**	**MRID No.**	**Study Classification**
Glyphosate	Rat (*Rattus norvegicus*)	96	**>4800**	Practically non-toxic	43728003	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	95	>4750	Practically non-toxic	45058306	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	97.2	>4860 up and down	Practically non-toxic	46760505	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	88	>4400	Practically non-toxic	44320604	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	95	>4750 up and down	Practically non-toxic	46998805	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	76	>3800	Practically non-toxic	41400601	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	96	>1920	Slightly toxic or less	44142104	Acceptable
Glyphosate	Rat (*Rattus norvegicus*)	95.4	>4770 up and down	Practically non-toxic	46816107	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic.
[3] **Bolded** shaded value will be used to calculate risk quotients.

Table 4.32 Mammalian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**LD_{50} (mg a.e./kg bw a.e.)[1]**	**Toxicity Category[2]**	**MRID No.**	**Study Classification**
HM-2028 (Glyphosate: 11.4%)	Rat (*Rattus attus norvegicus*)	11.4	357	Moderately toxic when reported as a.e. due to low percentage of a.i.	46714802	Acceptable – **not registered in California**
MON 20033	Rat (*rattus norvegicus*)	63	3150 (**5000** mg formulation/ kg)	Practically nontoxic	41142304	Acceptable
MON 77063	Rat (*rattus norvegicus*)	65.4	2599 (**5827** mg formulation/ kg)	Practically nontoxic	44615502	Acceptable

Table 4.32 Mammalian Acute Toxicity for Glyphosate Formulations						
Chemical	**Species**	**% a.i.[1]**	**LD_{50} (mg a.e./kg bw a.e.)[1]**	**Toxicity Category[2]**	**MRID No.**	**Study Classification**
Glyphosate IPA	Rat (*Rattus norvegicus*)	22.9	724 (**3803** mg formulation/ kg)	Slightly toxic	44918601	Acceptable
MON 20047	Rat (*rattus norvegicus*)	18.4	460 – 690 (**3750** mg formulation/ kg)	Moderately toxic when reported as a.e. due to low percentage of a.i.	41305404	Acceptable
ClearOut 41 (41% glyphosate IPA)	Rat (*Rattus norvegicus*)	30.3	>606	Slightly toxic	44883104	Acceptable
Clearout 62 (62% glyphosate IPA)	Rat (*Rattus norvegicus*)	62	>1240	Slightly toxic	45657801	Acceptable
GF-1667 (62.1% glyphosate dimethylammonium salt)	Rat (*Rattus norvegicus*)	49	>2450	Practically nontoxic	46730705	Acceptable
HM-0548 5905-LTE Mixture of ammonium salt (19.68%) and IPA (13.36%)	Rat (*Rattus norvegicus*)	25	>1250	Slightly toxic	47236803	Acceptable
MON 60696 (70.1% monoammonium salt)	Rat (*Rattus norvegicus*)	54	>2700	Practically nontoxic	43049302	Acceptable
MON 78634 (71.8% ammonium salt)	Rat (*Rattus norvegicus*)	65.2	>1304	Slightly toxic	46087001	Acceptable
Nufarm RUP0532 (41% Glyphosate as IPA and ammonium salts)	Rat (*Rattus norvegicus*)	30.3	>1515	Slightly toxic	45386802	Acceptable
56077-LL - Phoss-8	Rat (*Rattus norvegicus*)	80	>4000	Practically nontoxic	45044402	Acceptable
Roundup L&G Ready to Use (glyphosate IPA)	Rat (*Rattus norvegicus*)	0.85	>40	Highly toxic when reported as a.e. due to low percentage of a.i.	41395601	Acceptable
Spray–Charlie (44% GLY-41 (524-475 with 41% IPA)	Rat (*Rattus norvegicus*)	15.2	>760	Slightly toxic	45929403	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2]Based on LD_{50} (mg/kg bw): < 10 very highly toxic; 10 - 50 highly toxic; 51 - 500 moderately toxic; 501-2000 slightly toxic; >2000 practically non-toxic.
[3] **Bolded** shaded value will be used to calculate risk quotients.

4.2.2.2 Mammals: Chronic Exposure (Growth, Reproduction) Studies

The chronic mammalian endpoint is selected from a 2-generation reproduction study in the rat. In this dietary study, the parental/systemic NOAEL is 500 mg/kg/day in both sexes and the LOAEL is 1500 mg/kg/day based on soft stools, decreased body weight gain and food consumption. The reproductive NOAEL is ≥ 1500 mg/kg/day (HDT) in both sexes. The offspring NOAEL is 500 mg/kg/day in both sexes with a LOAEL of 1500 mg/kg/day based on decreased body weight gain during lactation.

There is a lower endpoint based on maternal mortality in the rabbit developmental toxicity study. The maternal NOAEL is 175 mg/kg bw/day and the maternal LOAEL is 350 mg/kg/day based on mortality, diarrhea, soft stools, and nasal discharge. The chronic mammalian endpoint was not selected from this study because it is believed that the effects may be acid effects from glyphosate acid, administered as a bolus dose by gavage. It may not occur through the diet with mammals. Several of the deaths were due to gastroenteritis and/or caecal ulcerations. Similar effects (stomach hemorrhages) were observed in the rat developmental toxicity study at higher dose levels.

Table 4.33. Mammalian Chronic Toxicity for Technical Glyphosate					
Chemical	**Species**	**% a.i.[1]**	**NOAEL/ NOAEC (mg a.e./kg bw or ppm a.e.)[1]**	**MRID #/Year**	**Study Classification**
Glyphosate	Rat (*rattus norvegicus*)	97.67	2-generation reproduction study **Parental/Systemic NOAEL: 500 mg/kg/day (10,000 ppm)** **LOAEL: 1500 mg/kg/day (30,000 ppm)** Reproductive NOAEL: 1500 mg/kg/day (HDT) **Offspring NOAEL: 500 mg/kg/day (10,000 ppm)** **LOAEL: 1500 mg/kg/day**	41621501/1990	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	100%	3-generation reproduction study Parental/Systemic, Offspring and Reproductive NOAELs: 30 mg/kg/day (highest dose tested).	00081674; 00105995 1981; 1982	Acceptable
Glyphosate	Rabbit (*Oryctolagus cuniculus*)	98.7	Developmental toxicity study Maternal NOAEL = 175 mg/kg/day LOAEL = 350 mg/kg/day based on mortality, diarrhea, soft stools, and nasal discharge. Developmental NOAEL = 350 mg/kg/day (HDT) LOAEL = not established.	00046363/1980	Acceptable
Glyphosate	Rat (*rattus norvegicus*)	98.7	Maternal NOAEL = 1000 mg/kg/day LOAEL = 3500 mg/kg/day based on inactivity, mortality, stomach hemorrhages and reduced body weight gain. Developmental NOAEL = 1000 mg/kg/day LOAEL = 3500 mg/kg/day based on increased incidence in the number of fetuses and litters with unossified sternebrae and decreased fetal body weight.	00046362/1980	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.A. = not applicable
[3] **Bolded** shaded value will be used to calculate risk quotients.

4.2.3 Toxicity to Terrestrial Invertebrates

Terrestrial invertebrate toxicity data are used to assess potential indirect effects of glyphosate to the terrestrial-phase CRLF. Effects to terrestrial invertebrates resulting from exposure to glyphosate have the potential to also indirectly affect the CRLF via reduction in available food.

4.2.3.1 Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

Studies on terrestrial invertebrates are available on both the technical material and on formulations. The studies indicate that glyphosate does not appear to be very toxic to terrestrial invertebrates. The formulations do not appear to be more toxic than the technical material.

Table 4.34. Acute Toxicity Studies on Terrestrial Invertebrates for Technical Glyphosate

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate	Honey bee (*Apis mellifera*)	98.5	48 hr LD50 (O): **>100** (N.R.)[2] **µg/bee**[3] NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate	Honey bee (*Apis mellifera*)	98.5	48 hr LD50 (C): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent
[2] Range is 95% confidence interval for endpoint, N.R. = not reported; O = oral study; C = contact study
[3] **Bolded** shaded value will be used to calculate risk quotients.

Table 4.35. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations

Chemical	Species	% a.i.[1]	LD_{50}/ LC_{50} NOAEL/ NOAEC	MRID #/Year	Study Classification
Glyphosate monoammonium salt (MON78568)	Honey bee (*Apis mellifera*)	65.6	48 hr LD50 (C): >100 (N.A.)[2] µg/bee NOAEL: 100 Slope: N.R.	45767104/2001	Not classified
Glyphosate monoammonium salt (MON78568)	Honey bee (*Apis mellifera*)	65.6	48 hr LD50 (O): >76.23 (N.A.) µg a.e./bee NOAEL: <76.23 µg a.e./bee Slope: N.R.	45767104/2001	Not classified

Table 4.35. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC**	**MRID #/Year**	**Study Classification**
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	7 D LD50 (C): 1200 (839-1786) g a.e./ha (1.1 lb/A) NOAEL: 216 Slope: N.R.	45767105/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	7 D LD50 (C): >4320 (N.R.) g/ha (>3.85 lb/A) NOAEL: 216 Slope: N.R.	45767106/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Predatory mite (*Typhlodromus pyri*)	64.9	14 - 21 D LD50 (C): N.A. (N.A.) g/ha NOAEL: 216 or <119 (no dose-response) (<0.11 lb/A) Slope: N.A.	45767106/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Earthworm (*Eisenia fetida*)	64.9	14 D LD50 (C): >6560 (N.A.) mg/kg soil NOAEL: 6560 Slope: N.R.	45767109/2001	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >108 (N.R.) g a.e./ha (>0.096 lb/A) NOAEL: Not established Slope: N.R.	45767107/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >4320 (N.R.) g/ha (>3.86 lb/A) NOAEL: 4320 Slope: N.R.	45767107/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Parasitic wasp (*Aphidius rhopalosiphi*)	64.9	48 hr - 13 days LD50 (C): >4320 (N.R.) g a.e./ha (>3.86 lb/A) NOAEL: 4320 Slope: N.R.	45767108/2002	Not classified
Glyphosate monoammonium salt (MON78568)	Lacewing (*Chrysoperla carnia*)	64.9	Up to 10 days LD50 (C): >4320 (N.R.) g/ha (>3.86 lb/A) NOAEC: 4320 Slope: N.R.	45767110/2002	Not classified
Glyphosate IPA salt (MON 2139)	Honey bee (*Apis mellifera*)	36	48 hr LD50 (O): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable
Glyphosate IPA salt (MON 2139)	Honey bee (*Apis mellifera*)	36	48 hr LD50 (C): >100 (N.R.) µg/bee NOAEL: N.R. Slope: N.R.	00026489/1972	Acceptable

Table 4.35. Acute Toxicity Studies on Terrestrial Invertebrates for Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**LD_{50}/ LC_{50} NOAEL/ NOAEC**	**MRID #/Year**	**Study Classification**
Glyphosate IPA salt (MON65005)	Honey bee (*Apis mellifera*)	31.32	48 hr LD50 (C): >31.3 (N.A.) µg a.e./bee NOAEL: 319 Slope: N.A.	44465703/1997	Acceptable
Glyphosate IPA salt (MON 77360)	Honey bee (*Apis mellifera*)	30.0	48 hr LD50 (C): >30 (NA) µg/bee NOAEL: 30 Slope: NA	45370301/2001	Acceptable
Glyphosate IPA salt (MON 77360)	Honey bee (*Apis mellifera*)	30.0	48 hr LD50 (O): >30 (NA) µg/bee NOAEL: 15 Slope: NA	45370302/2001	Supplemental

[1] a.i. = active ingredient; a.e. = acid equivalent/ IPA = isopropylamine; N.R. = not reported; O = oral study; C = contact study
[2] Range is 95% confidence interval for endpoint,

4.2.3.2 Terrestrial Invertebrates: Open Literature Studies

Open literature data on glyphosate, its salts and/or formulations included a large number of efficacy studies which were not useful for a quantitative assessment of risk. Those studies which could possibly be used were either tested at lower concentrations than the submitted studies with no effects or insufficient information was provided on the test material to determine the concentration levels tested for either the active ingredient and/or the glyphosate acid equivalent.

4.2.4 Toxicity to Terrestrial Plants

Terrestrial plant toxicity data are used to evaluate the potential for glyphosate to affect riparian zone and upland vegetation within the action area for the CRLF. Impacts to riparian and upland (i.e., grassland, woodland) vegetation have the potential to result in indirect effects to both aquatic- and terrestrial-phase CRLFs, as well as modification to designated critical habitat PCEs via increased sedimentation, alteration in water quality, and reduction in of upland and riparian habitat that provides shelter, foraging, predator avoidance and dispersal for juvenile and adult CRLFs.

Plant toxicity data from both registrant-submitted studies and studies in the scientific literature were reviewed for this assessment. Registrant-submitted studies are conducted under conditions and with species defined in EPA toxicity test guidelines. Sub-lethal endpoints such as plant growth, dry weight, and biomass are evaluated for both monocots and dicots, and effects are evaluated at both seedling emergence and vegetative life stages. Guideline studies generally evaluate toxicity to ten crop species. A drawback to these tests is that they are conducted on herbaceous crop species only, and extrapolation

of effects to other species, such as the woody shrubs and trees and wild herbaceous species, contributes uncertainty to risk conclusions.

Commercial crop species have been selectively bred, and may be more or less resistant to particular stressors than wild herbs and forbs. The direction of this uncertainty for specific plants and stressors, including glyphosate, is largely unknown. Homogenous test plant seed lots also lack the genetic variation that occurs in natural populations, so the range of effects seen from tests is likely to be smaller than would be expected from wild populations.

The results of the Tier II seedling emergence and vegetative vigor toxicity tests on non-target plants are summarized below in **Tables 4.36 and 4.37.**

Table 4.36 Vegetative Vigor Study on Terrestrial Plants with Technical Glyphosate

Chemical	Species	% a.i.[1]	EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]	MRID #/Year	Study Classification
Monocots					
Glyphosate	Oat (*Avena sativa*)	96.6	21 D EC25: 0.4 (N.R.) LB/A NOAEC/EC05: 0.14 Slope: 2.3	43088701/1994	Acceptable
Glyphosate	Corn (*Zea mays*)	96.6	21 D EC25: 0.43 (N.R.) LB/A NOAEC/EC05: 0.07 Slope: 3.7	43088701/1994	Acceptable
Glyphosate	Onion (*Allium cepa*)	96.6	21 D EC25: 0.83 (N.R.) LB/A NOAEC/EC05: 0.56 Slope: 2.4	43088701/1994	Acceptable
Glyphosate	Ryegrass (*Lolium perenne*)	96.6	21 D EC25: 0.98 (N.R.) LB/A NOAEC/EC05: 0.56 Slope: 4.9	43088701/1994	Acceptable
Dicots					
Glyphosate	Tomato (*Lycopersicon esculentum*)	96.6	21 D EC25: 0.11 (N.R.) LB/A NOAEC/EC05: 0.035 Slope: 3.4	43088701/1994	Acceptable
Glyphosate	Cucumber (*Cucumis sativus*)	96.6	21 D EC25: 0.46 (N.R.) LB/A NOAEC/EC05: 0.14 Slope: 2.6	43088701/1994	Acceptable
Glyphosate	Lettuce (*Lactuca sativa*)	96.6	21 D EC25: 0.4 (N.R.) LB/A NOAEC/EC05: 0.28 Slope: N.R.	43088701/1994	Acceptable
Glyphosate	Cabbage (*Brassica oleracea*)	96.6	21 D EC25: 0.3 (N.R.) LB/A NOAEC/EC05: 0.14 Slope: N.R.	43088701/1994	Acceptable
Glyphosate	Soybean (*Glycine max*)	96.6	21 D EC25: 0.42 (N.R.) LB/A NOAEC/EC05: 0.28 Slope: N.R.	43088701/1994	Acceptable

Table 4.36 Vegetative Vigor Study on Terrestrial Plants with Technical Glyphosate					
Chemical	**Species**	**% a.i.[1]**	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]**	**MRID #/Year**	**Study Classification**
Glyphosate	Radish (*Rhaphanus sativus*)	96.6	21 D EC25: 0.14 (N.R.) LB/A NOAEC/EC05: 0.035 Slope: N.R.	43088701/1994	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported
[2] **Bold** value will be used to calculate risk quotients.

Studies on Formulations

Table 4.37 Terrestrial Plant Studies with Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]**	**MRID #/Year**	**Study Classification**
Seedling Emergence Studies					
Monocots					
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	75	29 D EC25: >4.5 (N.R.) NOAEC/EC05: 3.6 Slope: N.R.	44125712/1996	Acceptable
Glyphosate IPA salt CP-70139	Oat (*Avena sativa*), Rice (*Oryza sativa*), Sorghum (*Sorghum bicolor*), Barnyard grass (*Echinochloa crusgalli*)	50	14 D EC25: >5 (N.R.) NOAEC/EC05: N.R. Slope: N.R.	40159301/1987	Acceptable
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	48.3	4WKS EC25: >4 (N.A.) NOAEC/EC05: 4 Slope: N.A.	44320635/1996	Acceptable
Dicots					
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	75	29 D EC25: >4.5 (N.R.) NOAEC/EC05: 3.6 Slope: N.R.	44125712/1996	Acceptable
Glyphosate IPAsalt CP-70139	Soybean, Sugarbeet, Buckwheat, Cocklebur, Crabgrass, Panicum grass, Downy brome, Velvetleaf, Smartweed, Morning glory, Lambsquarter, Hemp	50	14 D EC25: >5 (N.R.) NOAEC/EC05: N.R. Slope: N.R.	40159301/1987	Acceptable
Glyphosate(80WDG formulation)	Veg.Crops(10 Sp.) (Monocots & Dicots)	48.3	4WKS EC25: >4 (N.A.) NOAEC/EC05: 4 Slope: N.A.	44320635/1996	Acceptable
Vegetative Vigor Studies					
Monocots					

Table 4.37 Terrestrial Plant Studies with Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]**	**MRID #/Year**	**Study Classification**
Glyphosate(80WDG formulation)	Onion (*Allium cepa*)	75	27 D EC25: 0.28 (N.R.) NOAEC/EC05: 0.14 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sorghum (*Sorghum bicolor*)	75	27 D EC25**: 0.16** (N.R.) NOAEC/EC05: 0.07 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Wheat (*Triticum aestivum*)	75	27 D EC25: 0.22 (N.R.) NOAEC/EC05: 0.1 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Corn (*Zea mays*)	75	27 D EC25: 0.35 (N.R.) NOAEC/EC05: 0.18 Slope: N.R.	44125715/45045 101/1996	Acceptable
Glyphosate(80WDG formulation)	Corn (*Zea mays*)	48.3	48WKS EC25: 0.227 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Purple nutsedge (*Cyperus rotundus*)	48.3	4WKS EC25: 0.805 (N.R.) NOAEC/EC05: 0.445 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Wheat (*Triticum aestivum*)	48.3	4WKS EC25: 0.176 (0.138-0.183 a.e.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Oat (*Avena sativa*)	48.3	4WKS EC25: 0.201 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Dicots					
Glyphosate(80WDG formulation)	Garden pea (*Pisum sativum*)	75	27 D EC25: 0.89 (N.R.) NOAEC/EC05: 0.45 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sugarbeet (*Beta vulgaris*)	75	27 D EC25: 0.21 (B.R.) NOAEC/EC05: 0.12 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sunflower (*Helianthus annus*)	75	27 D EC25: 0.16 (N.R.) NOAEC/EC05: 0.08 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Radish (*Rhaphanus sativus*)	75	27 D EC25: 0.09 (N.R.) NOAEC/EC05: 0.02 Slope: N.R.	44125715/45045 101/1995	Acceptable

Table 4.37 Terrestrial Plant Studies with Glyphosate Formulations					
Chemical	**Species**	**% a.i.[1]**	**EC_{25}/ NOAEC (EC_{05}) (lbs a.e./Acre[1]**	**MRID #/Year**	**Study Classification**
Glyphosate(80WDG formulation)	Soybean (*Glycine max*)	75	27 D EC25: 0.32 (N.R.) NOAEC/EC05: 0.12 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Cucumber (*Cucumis sativus*)	75	27 D EC25: 0.45 (N.R.) NOAEC/EC05: 0.16 Slope: N.R.	44125715/45045 101/1995	Acceptable
Glyphosate(80WDG formulation)	Sugarbeet (*Beta vulgaris*)	48.3	4WKS EC25: 0.277 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Radish (*Rhaphanus sativus*)	48.3	4WKS EC25: 0.235 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Soybean (*Glycine max*)	48.3	4WKS EC25: 0.126 (N.R.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Lettuce (*Lactuca sativa*)	48.3	4WKS EC25: 0.217 (N.R.) NOAEC/EC05: 0.148 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Cucumber (*Cucumis sativus*)	48.3	4WKS EC25: **0.074** (N.R.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Rape (*Brassica compestris*)	48.3	4WKS EC25: 0.098 (0.065-0.084) NOAEC/EC05: 0.049 Slope: N.A.	44320636/1996	Acceptable
Glyphosate(80WDG formulation)	Okra (*Hibiscus esculentus*)	48.3	4WKS EC25: 0.172 (N.R.) NOAEC/EC05: 0.049 Slope: N.R.	44320636/1996	Acceptable

[1] a.i. = active ingredient; a.e. = acid equivalent; N.R. = Not reported; IPA = isopropylamine
[2] **Bolded** shaded value will be used to calculate risk quotients.

4.3 Use of Probit Slope Response Relationship to Provide Information on the Endangered Species Levels of Concern

The Agency uses the probit dose response relationship as a tool for providing additional information on the potential for acute direct effects to individual listed species and aquatic animals that may indirectly affect the listed species of concern (U.S. EPA, 2004). As part of the risk characterization, an interpretation of acute RQ for listed species is discussed. This interpretation is presented in terms of the chance of an individual event (i.e., mortality or immobilization) should exposure at the EEC actually occur for a species with sensitivity to glyphosate on par with the acute toxicity endpoint selected for RQ calculation. To accomplish this interpretation, the Agency uses the slope of the dose response relationship available from the toxicity study used to establish the acute toxicity measures of effect for each taxonomic group that is relevant to this assessment. The individual effects probability associated with the acute RQ is based on the mean estimate of the slope and an assumption of a probit dose response relationship. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available.

Individual effect probabilities are calculated based on an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). The model allows for such calculations by entering the mean slope estimate (and the 95% confidence bounds of that estimate) as the slope parameter for the spreadsheet. In addition, the acute RQ is entered as the desired threshold.

4.4 Incident Database Review

A review of the EIIS database for ecological incidents involving glyphosate and its salts (PC Codes 417300, 103601, 103603, 103604 and 103607) was completed on 08/11/2008. The results of this review for terrestrial, plant, and aquatic incidents are discussed below in Sections 4.4.1 through 4.4.3, respectively. A complete list of the incidents involving glyphosate and its salts, including associated uncertainties is included as **Appendix K**.

4.4.1 Terrestrial Incidents

One incident report for technical glyphosate was filed on 6/13/2006. The certainty code was classified as **unlikely**. This incident was for a registered use on sunflowers, broadcast spray. It was reported that 1 american kestrel, 1 robin, 5 grackels, 597 horned larks, an unknown number of kangaroo rats, a few lark buntings, 1633 mourning doves, 5 red-winged blackbirds, 12 sparrows, 150 unknown birds and 5 western meadowlarks were killed upon ingestion of the herbicide.

Five incident reports for glyphosate isopropylamine salt were filed, 2 in 1993, 1 in 1994, 1 in 1996 and 1 in 2004 for uses on corn, field, home/lawn and a tree farm. One report did not file a specific use. The certainty indices were from unlikely to probable. The

unlikely report was for incapacitation of a duck and mortality in 2 geese following inhalation. The possible reports were for mortality in an unknown quantity of birds from drift, mortality in 3 birds from drift and mortality in several dogs from runoff. The probable report was for incapacitation of two iguanas following ingestion of glyphosate.

4.4.2 Plant Incidents

For glyphosate, 63 incidents were reported for mostly plant damage to a wide variety of plants from either direct treatment or spray drift. The reports were filed from 1992 – 2008 with the certainty code ranging from possible to highly probable. The majority of the reports were either probable or highly probable.

For the isopropylamine salt of glyphosate, 443 incident reports were filed for a wide variety of terrestrial plants, particularly agricultural crops and grass. There were only a few incidents of trees being damaged or killed. The majority of the reports were rated as probable but there were some highly probable incidents and a number of possible incidents. The reports were filed from 1990 – 2006 with a large number of accidental misuses and of unknown legality. Plant damage and mortality were the main issues with drift as the main exposure route.

4.4.3 Aquatic Incidents

For glyphosate, two incident reports were filed in which 1 carp and 1 catfish were incapacitated and 20 goldfish were killed upon ingestion of glyphosate. The certainty index was possible for both incidents. The reports were filed in 2003.

For the isopropylamine salt of glyphosate, 16 incident reports were filed from 1990 – 2003. The certainty indices ranged from unlikely to highly probable. There was one accidental misuse in which thousands of shad were killed upon ingestion. It was not stated what the application method was, but this was the one report that was rated highly probable. Three other misuses were reported and the remainder were either registered uses (majority) or unknown. Eight of the reports were from runoff, 2 ingestion, 1 pond treatment and 1 skin contact. The others were either unknown or not reported. Fifteen reported mortality and 2 reported incapacitation. All of the reports were on fish. The numbers of fish killed ranged from 9 to thousands.

5. Risk Characterization

Risk characterization is the integration of the exposure and effects characterizations. Risk characterization is used to determine the potential for direct and/or indirect effects to the CRLF or for modification to its designated critical habitat from the use of glyphosate in California. The risk characterization provides an estimation (Section 5.1) and a description (Section 5.2) of the likelihood of adverse effects. In addition, it includes risk assessment assumptions, limitations, and uncertainties as well as a comprehensive conclusion regarding the likelihood of adverse effects to the CRLF or its designated

critical habitat (i.e., "no effect," "likely to adversely affect," or "may affect, but not likely to adversely affect").

5.1 Risk Estimation

Risk is estimated by calculating the ratio of exposure to toxicity. This ratio is the risk quotient (RQ), which is then compared to pre-established acute and chronic levels of concern (LOCs) for each category evaluated (**Appendix C**). For acute exposures to the CRLF and its animal prey in aquatic habitats, as well as terrestrial invertebrates, the LOC for listed species is 0.05. For acute exposures to the CRLF and mammals in terrestrial habitats, the LOC for listed species is 0.1. The LOC for chronic exposures to CRLF and its prey, as well as acute exposures to plants is 1.0.

Risk to the aquatic-phase CRLF is estimated by calculating the ratio of exposure to toxicity using 1-in-10 year EECs (**Tables 3.3 – 3.5**) based on the label-recommended glyphosate usage scenarios summarized in **Table 2.5** and the appropriate aquatic toxicity endpoint from **Tables 4.1 and 4.2**. Risks to the terrestrial-phase CRLF and its prey (*e.g.* terrestrial insects, small mammals and terrestrial-phase frogs) are estimated based on exposures resulting from applications of glyphosate (**Tables 3.8 and 3.9**) and the appropriate toxicity endpoint from **Tables 4.24 and 4.25**. Exposures are also derived for terrestrial plants, as discussed in Section 3.3 and summarized in **Table 3.10**, based on the highest application rates of glyphosate use within the action area.

5.1.1 Exposures in the Aquatic Habitat

As stated in the Ecological Effects Characterization Section (**Section 4.1**), although glyphosate appears to be less toxic to amphibians than to freshwater fish, an endpoint from the amphibian studies would be used as a conservative estimate if the amphibian endpoint could conceivably be lower than the one selected from the fish studies. This is the case with the chronic endpoint for direct effects to the CRLF. Both the fish and amphibian chronic studies show no toxicity, with the NOAEC at the highest concentration tested. The NOAEC from the amphibian study is lower than the NOAEC from the fish study. Therefore, as a conservative estimate of risk, the chronic endpoint for direct effects to the CRLF was selected from the amphibian study.

Also noted in **Section 4.1**, some formulations have been found to be more toxic to aquatic organisms than glyphosate on an acid equivalent basis. For assessment of risk following exposure to formulations, the most conservative endpoints from all available formulation data were selected for terrestrial uses where the POEA surfactant is allowed and separate endpoints were selected from studies on formulations without POEA for aquatic uses where this surfactant is not allowed. Wherever possible, endpoints for aquatic uses were selected from studies conducted with formulations that are currently labeled for aquatic use. For aquatic plants, due to a similarity in the product label name, it could not be determined from the aquatic plant studies whether or not they were conducted with a formulation labeled for terrestrial uses or with a formulation labeled for aquatic uses. The two formulations are different in terms of the inerts; nevertheless, the

formulation for terrestrial uses does not have the POEA surfactant in it. Therefore, as a conservative estimate, these studies were utilized for the assessment of risk to aquatic plants following exposure to formulations labeled for aquatic use. Exposure to the formulations is expressed in terms of EEC of the formulation rather than to the glyphosate acid equivalent. For consistency of units, the toxicity endpoints are also expressed in terms of concentration of formulation rather than the glyphosate acid equivalent.

Data from several studies indicate that the toxicity of glyphosate in aquatic environments, particularly for some of the formulations, is pH and temperature dependent. This may be enhanced by the presence of surfactants. These two potential factors are not accounted for in this assessment.

5.1.1.1 Direct Effects to Aquatic-Phase CRLF

Glyphosate

Direct effects to the aquatic-phase CRLF are based on the highest peak aquatic EEC and the lowest acute and chronic toxicity values for freshwater fish and/or amphibians. There are no acute or chronic LOC exceedances. The highest aquatic EEC (210 ppb) was generated from the registered use for management of aquatic plants at 3.75 lbs a.e./A and was calculated by assuming direct application to water by a simple dilution. As a conservative estimate, the RQs following chronic exposure were calculated from the peak EEC. EECs for chronic exposure would only have been estimated if the chronic RQs exceeded the chronic LOC for aquatic animals using the conservative peak value. The highest acute RQ is < 0.01, using the lowest EC_{50} value of 43000 ppb a.e. from the acute toxicity study with Bluegill sunfish (*Lepomis macrochirus*; MRID 44320630). This value is less than the acute LOC of 0.05 for listed aquatic animals. For mortality following acute exposure, the probability of an individual effect at the acute RQ is 1 in 5.0E+24 (1 in 4.8E+05 to 7.0E+94) using a default slope assumption of 4.5 with lower and upper 95% confidence intervals of 2 and 9, respectively (Urban and Cook, 1986).

The highest chronic RQ is 0.12, using the lowest NOAEC of 1800 ppb a.e. (highest concentration tested, no LOAEC) from the chronic toxicity study in the leopard frog (*Rana pipiens*; MRID 46650501). It is noted that there is considerable uncertainty associated with this study due to the relatively high rate of mortality in the control groups (38%) and insufficient analysis of the water quality; however, the study does provide the most conservative estimate of risk. The RQ is less than the chronic aquatic LOC of 1 for aquatic animals.

Based on the highest acute RQ of less than 0.01 and the highest chronic RQ of 0.12, glyphosate is not expected to directly affect the aquatic-phase of the CRLF when the risks are estimated from the toxicity endpoints with the technical material. The preliminary effect determination is “no effect.”

Formulations

Risk from Terrestrial Uses

Aquatic EECs for formulations were estimated from spray drift only for each potential scenario (see **section 3.2.1.3**). The most conservative acute toxicity LC_{50} value for a formulation is 3170 ppb formulation from a study on rainbow trout (MRID 40098001). Using the highest peak aquatic EEC for formulations (95.2 ppb for forestry (aerial, 34 lbs formulation/A)), the highest acute RQ for freshwater fish is 0.03, which is less than the acute aquatic listed species LOC of 0.05. There are no exceedances for any of the other uses. There are no acceptable chronic toxicity studies on formulations with freshwater fish; however, there was one report of a 42-day chronic study conducted with several formulations on leopard frog larvae (MRID 46650501). These formulations contain the toxic surfactant, POEA. The LOAEC is 1900 ppb formulation based on decreased length at metamorphosis and percentage of larvae surviving to reach Stage 42 and increased time to metamorphosis, mixed-sex gonads and tail damage. Again, it is noted that there is considerable uncertainty associated with this study. Nevertheless, as a conservative estimate, a comparison of the LOAEC of 1900 ppb with the peak aquatic EEC value of 95.2 ppb (the chronic 60-day EEC for formulations cannot be estimated), the chronic RQ would be a value that is greater than 0.05. For the chronic LOC of 1 to be exceeded, the NOAEC for the study would have to be less than 95.2 ppb or 20 times less than the LOAEC. Due to the fact that some of the results are highly variable with lack of statistical significance in some key parameters, the study data are only being used as a bounding value for potential risk to the aquatic-phase CRLF following exposure to formulations used in terrestrial scenarios. Therefore, based on the weight of the evidence, including the use of the highest peak EEC for a chronic EEC value, the preliminary effect determination for formulations (direct effect: terrestrial uses) is "no effect."

Risk from Aquatic Uses

For accessing acute risk to formulations labeled for aquatic use, the endpoint was selected from a fish study for which there was a matching label which has aquatic uses (MRID 45374001, rainbow trout study with a glyphosate SL formulation, Reg. No. 100-1135) with an LC_{50} value of 824 ppm or 824000 ppb formulation). There was also a bluegill sunfish study with a non-discrete LC_{50} that was greater than 183700 ppb formulation; however, the rainbow trout study was selected because the LC_{50} is a discrete value. The peak aquatic EEC estimated on a formulation basis for direct application to water (use on aquatic plants at 32.9 lbs formulation/A) is 1840 ppb. Comparing the peak aquatic EEC with the toxicity endpoint of 824000 ppb, the RQ for direct application to water is < 0.01. This is less than the aquatic listed species LOC of 0.05. Therefore, for freshwater fish, surrogate for the aquatic phase CRLF, the preliminary effect determination (direct effect: aquatic uses) is "no effect."

5.1.1.2 Indirect Effects to Aquatic-Phase CRLF via Reduction in Prey (non-vascular aquatic plants, aquatic invertebrates, fish, and frogs)

Non-vascular Aquatic Plants

Glyphosate

Indirect effects of glyphosate to the aquatic-phase CRLF (tadpoles) via reduction in non-vascular aquatic plants in its diet are based on peak EECs from the standard pond and the lowest acute toxicity value (EC_{50}) for aquatic non-vascular plants. With the highest peak EEC of 210 ppb and the most conservative 96-hr EC_{50} of 12100 ppb from a study on green algae (MRID 40236901), the highest RQ for non-vascular plants would be 0.02. This is less than the LOC of 1 for aquatic plants. Therefore, glyphosate is not expected to indirectly affect the aquatic-phase of the CRLF through the diet (tadpoles) or habitat from aquatic non-vascular plants. The preliminary effect determination for glyphosate is "no effect."

Formulations

Risk from Terrestrial Uses

As with fish, it is noted that some formulations can be considerably more toxic to non-vascular aquatic plants. The study with the lowest 96-hr EC_{50} on a formulation basis (390 µg glyphos/L) was conducted with freshwater diatom (*Navicula pelliculosa*; MRID 45666701). Using the highest peak EEC for formulations registered for terrestrial uses (95.2 ppb), the highest RQ for non-vascular plants is 0.24, which is less than the LOC of 1 for aquatic plants. Therefore, the preliminary effect determination for formulations (indirect effect: diet or habitat terrestrial uses) is "no effect."

Risk from Aquatic Uses

As with freshwater fish, formulations containing the toxic surfactant, POEA are not allowed to be used in aquatic applications. Therefore, for accessing risk to nonvascular aquatic plants, a study was selected in which the formulation was known not to contain POEA. This study is the same one as selected above with an EC_{50} of 390 µg glyphos/L. As stated previously, a glyphos product is available for aquatic uses. It is unclear as to whether or not this study was conducted with the exact formulation because there are glyphos products for terrestrial uses and glyphos products for aquatic uses. This study was selected as the most conservative endpoint, assuming that the product tested was for aquatic uses.

The peak aquatic EEC estimated on a formulation basis for direct application to water (use on aquatic plants) is 1840 ppb. The RQ is direct application to water is 4.7. This is higher than the LOC for aquatic plants of 1. Therefore, with the formulations, the preliminary effect determination (indirect effect: diet or habitat aquatic uses) is "may affect."

Aquatic Invertebrates

Glyphosate

Indirect acute effects to the aquatic-phase CRLF via effects to prey (invertebrates) in aquatic habitats are based on the highest peak EECs from the registered uses (aquatic plant management) and the lowest acute toxicity value for freshwater invertebrates. For chronic risks, as with freshwater fish, the peak EEC and the lowest chronic toxicity value for invertebrates are used to derive RQs. There are no LOC exceedances with risk estimations based on the highest peak (210 ppb) EECs generated from the registered uses (management of aquatic plants). The highest acute RQ is < 0.01, using the lowest EC_{50} value of 53200 ppb a.e. from the acute toxicity study with the midge (*Chironomus plumosus*; MRID 00162296). This value is less than the acute LOC of 0.05 for listed aquatic animals. The highest chronic RQ is also <0.01, using the most conservative NOAEC of 49900 ppb a.e. from the chronic toxicity study in daphnia (*Daphnia magna*; MRID 00124763). This value is less than the chronic LOC of 1 for aquatic animals. Therefore, glyphosate is not expected to indirectly affect the aquatic-phase of the CRLF via direct effects on aquatic invertebrates and the preliminary effect determination is "no effect."

Formulations

Risk from Terrestrial Uses

As with fish and aquatic non-vascular plants, it is noted that some formulations can be considerably more toxic to freshwater invertebrates. The most conservative EC_{50} on a formulation basis is 3 mg/L formulation (3000 ppb) (*daphnia magna* with a 41% glyphosate IPA formulation, MRID 00162296). Using the highest peak EEC for formulations registered for terrestrial use (95.2 ppb), the highest RQ for aquatic invertebrates is 0.03, which is less than the listed species LOC of 0.05 for aquatic invertebrates. None of the other uses exceed any of the acute aquatic invertebrate LOCs. There are no acceptable chronic toxicity studies on formulations with freshwater invertebrates. Therefore, no RQs were estimated. The preliminary effect determination for formulations (indirect effect: reduction in prey - terrestrial uses) is "no effect."

Risk from Aquatic Uses

An acute toxicity study was found on a freshwater invertebrate for which there was a matching label with aquatic uses (MRID 45374003; daphnia study with a glyphosate SL formulation; 360g/L; Reg. No. 100-1135) with an EC_{50} value of 164.3 ppm formulation (164300 ppb). The peak formulation EEC for aquatic uses is 1840 ppb. This provides an RQ of 0.01 following acute exposure, which does not exceed the acute aquatic LOC for listed species. Therefore, for formulations (indirect effect: reduction in prey - aquatic uses) is "no effect."

Fish and Frogs

Fish and frogs also represent potential prey items of adult aquatic-phase CRLFs. RQs associated with acute and chronic direct toxicity to the CRLF are used to assess potential indirect effects to the CRLF based on a reduction in freshwater fish and frogs as food items. Based on an acute RQ of <0.01 and a chronic RQ of 0.12 for the aqueous-phase CRLF, glyphosate is not expected to indirectly affect the adult aquatic-phase CRLFs when the risks are estimated from the toxicity endpoints with the technical material. The preliminary effect determination is "no effect." For acute risk from formulations, the highest acute RQ from terrestrial applications is 0.03, which is less than the acute LOC of 0.05 for listed aquatic animals. The highest chronic RQ is a value that would be greater than 0.05 based on the conservative peak aquatic EEC value of 95.2 ppb and a chronic LOAEC of 1900 ppb. As stated previously, for the chronic LOC of 1 to be exceeded, the NOAEC for the study would have to be 20 times less than the LOAEC. In addition, some of the results are highly variable with lack of statistical significance in some key parameters. Therefore, for formulations (terrestrial uses), the preliminary effect determination is "no effect". For formulations labeled for aquatic use, the highest acute RQ is < 0.01. Therefore, the preliminary effect determination for formulations (aquatic uses) is also "no effect".

5.1.1.3 Indirect Effects to CRLF via Reduction in Habitat and/or Primary Productivity (Freshwater Aquatic Plants)

Glyphosate

Indirect effects to the CRLF via direct toxicity to aquatic plants are estimated using the most sensitive non-vascular and vascular plant toxicity endpoints. Because there are no obligate relationships between the CRLF and any aquatic plant species, the most sensitive EC_{50} values, rather than NOAEC values, were used to derive RQs. For both non-vascular and vascular plants, the LOC for aquatic plants is not exceeded with the highest peak EEC generated from the registered uses (management of aquatic plants). The risk to non-vascular plants is summarized in **Section 5.1.2.2.** The highest RQ for non-vascular plants is 0.02. For vascular plants, the highest RQ is also 0.02, based on the peak EEC of 210 ppb and an EC_{50} of 11900 ppb a.e. (MRID 44320638) for duckweed. Glyphosate is not expected to indirectly affect the aquatic-phase CRLF through habitat from aquatic vascular and non-vascular plants. The preliminary effect determination is "no effect."

Formulations

Risk from Terrestrial Uses

As stated previously, the EC_{50} of 390 µg glyphos/L with freshwater diatom, an aquatic non-vascular plant, provides an RQ of 0.24 with the highest peak EEC of 95.2 ppb for a formulation. This is less than the LOC of 1 for aquatic plants. The EC_{50} for aquatic vascular plants was selected from a duckweed study (MRID 44125714): 4.9 ppm (4900

ppb) on a formulation basis. The resulting RQ is 0.02, which is less than the LOC of 1 for aquatic plants.

Therefore, the preliminary effect determination for formulations (indirect effect: habitat and/or primary productivity - terrestrial uses) is "no effect."

Risk from Aquatic Uses

As stated previously, the same endpoint for non-vascular plants (390 ppb) is used for aquatic uses. For vascular plants, only a 7-day study is available on a formulation which does not contain POEA. As with the non-vascular plants, this study was conducted with glyphos, which has a formulation for aquatic uses. The endpoint for the duckweed study is 25 ppm or 25000 ppb (MRID 45666704). Again, the peak aquatic EEC estimated on a formulation basis for direct application to water (use on aquatic plants) is 1840 ppb. The RQ for vascular plants, direct application to water is 1840/25000 or 0.07. This is lower than the LOC for aquatic plants of 1. However, the RQ for non-vascular plants is 4.7. Therefore, with some of the formulations, the preliminary effect determination (indirect effect: habitat and/or primary productivity - aquatic uses) is "may affect."

5.1.2 Exposures in the Terrestrial Habitat

5.1.2.1 Direct Effects to Terrestrial-phase CRLF

Glyphosate

As previously discussed in **Section 3.3**, potential direct effects to terrestrial-phase CRLFs are based on foliar applications of glyphosate. Potential direct acute effects to the terrestrial-phase CRLF are derived by considering dose- and dietary-based EECs modeled in T-REX for a small bird (20 g) consuming small invertebrates (**Table 3.8**) and acute oral and subacute dietary toxicity endpoints for avian species. There were no mortalities in any of the available acute avian studies. Therefore, no RQs were calculated. The highest dose/concentration tested in the acute avian studies were >3196.3 mg a.e./kg bodyweight (83% technical) and >4971.2 mg a.e./kg diet (95.6% technical), both with bobwhite quail.

Potential direct chronic effects of glyphosate to the terrestrial-phase CRLF are derived by considering dietary-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates. Chronic effects are estimated using the lowest available toxicity data for birds. EECs are divided by toxicity values to estimate chronic dietary-based RQs.

Table 5.1 shows that the chronic avian LOC is exceeded for birds consuming small invertebrates for the following uses: forestry (7.95 lbs a.e./A, aerial); uses on areas with impervious surfaces (ground: i.e., agricultural/farm structures/buildings and equipment, commercial storage/warehouse premises, household/domestic dwellings outdoor premises, industrial areas, non-agricultural outdoor buildings/structures, path/patios, paved areas (private roads/sidewalks) and urban areas (7.95 lbs a.e./A, ground) and for

rights of way uses (7.5 lbs a.e./A, aerial). None of the other uses exceed the chronic avian LOC. It is noted that there were no effects in the chronic avian study at the highest concentration tested; however, the preliminary effect determination is "may affect."

Table 5.1 Summary of Chronic RQs* Used to Estimate Direct Effects to the Terrestrial-phase CRLF (non-granular application)

Use (Application Rate)	Dietary-based Chronic RQ[1]
Forestry and Areas with Impervious Surfaces 7.95 lbs a.e./A (aerial), 1 application/year	**1.29**
California Rights of Way 7.5 lbs a.e./A (aerial), 1 application/year	**1.22**
Almond, fruit, grape and olive 3.84 lbs a.e./A, 2 applications/year, 14 day application interval	0.78

* = LOC exceedances (chronic RQ $\geq$ 1) are bolded and shaded.
[1] Based on avian NOAEC of 830 ppm a.e. (MRID 00108207)

Formulations

Acute oral and acute dietary avian studies have been conducted on some formulations. As with the technical material, most of the LD_{50}/LC_{50}'s are higher than the highest dose/concentrations tested. There is one study in which there is a definitive LD_{50}. This study was conducted with bobwhite quail on the glyphosate monoammonium salt (MON 14420: 68.5% w/w glyphosate, MRID 45777402). The LD_{50} is 1651 mg formulation/kg bodyweight on a formulation basis. For this formulation, there is a specific label with application rates ranging from 5.5 to 1.1 lbs formulation/A. These uses were modeled with this particular LD_{50} for this particular formulation, assuming one application per year and a half-life of 7 days. The RQs (diet of small invertebrates) for all use scenarios exceed the acute avian LOC of 0.1 for listed species (see **Table 5.2** below). With this particular formulation, any application rate of 0.8 lbs formulation/A and above will exceed the acute avian LOC of 0.1 for listed species.

For other formulations, RQs were not calculated because the LD_{50}'s were higher than the highest dose/concentration tested. The highest dose/concentration tested in these studies were: >2510 mg formulation/kg bodyweight (MRID 00085638) and >5620 mg formulation/kg diet (MRID 00085639) on bobwhite quail with trisodium diglyphosate/Urea (Polado formula (MON 8000)).

Table 5.2. Summary of Acute RQs* on Formulations Used to Estimate Direct Effects to the Terrestrial-phase CRLF (non-granular application)	
Use (Application Rate)	**Dose-based acute RQ[1]**
MON 14420[2]	
Industrial areas outdoor (5.5 lbs formulation/A)	**0.71**
Ornamental lawns and turf (2.2 lbs formulation/A)	**0.28**
Ornamental lawns and turf (1.1 lbs formulation/A)	**0.14**
* = LOC exceedances (acute RQ ≥ 0.1) are bolded and shaded. [1] Based on avian LD_{50} of 1651 mg formulation/kg bw [2] Registration Number 524-424	

Therefore, the preliminary effect determination for formulations (direct effect on terrestrial-CRLF) is "may affect."

5.1.2.2 Indirect Effects to Terrestrial-Phase CRLF via Reduction in Prey (terrestrial invertebrates, mammals, and frogs)

5.1.2.2.1 Terrestrial Invertebrates

In order to assess the risks of glyphosate to terrestrial invertebrates, which are considered prey of CRLF in terrestrial habitats, the honey bee is used as a surrogate for terrestrial invertebrates. The toxicity value for terrestrial invertebrates is calculated by multiplying the lowest available acute contact LD_{50} of >100 µg a.i./bee (MRID 00026489) by 1 bee/0.128g, which is based on the weight of an adult honey bee. EECs (µg a.i./g of bee) calculated by T-REX for small and large insects are then divided by the calculated toxicity value for terrestrial invertebrates (>781.25 µg a.i./g of bee) to estimate the RQ. Although the acute LD_{50} value is not a discrete value, it is noted that there is 27% mortality at this dose level. Since mortality was observed, the T-REX model was used to estimate upper bound RQs for terrestrial invertebrates. The results show that for small insects, all of the RQs for all uses could exceed the LOC of 0.05 for listed terrestrial invertebrates. Uses on forestry, areas with impervious surfaces at 7.95 lbs a.e./A and rights of way could exceed the acute LOC of 0.5 for non-listed species. For large insects, uses on forestry, areas with impervious surfaces and rights of way could exceed the acute LOC of 0.05 for listed species. None of the other uses exceed the acute LOC for listed species. Due to the fact that the acute LD_{50} value for the honey bee is not a discrete value and that there is mortality at the single limit dose tested, the RQs could exceed the acute LOC for listed species at all application rates for small insects and at the higher application rates for large insects. In addition, the RQs could exceed the acute LOC for non-listed species at the higher application rates for small insects. Therefore, there is an uncertainty for terrestrial invertebrate species. **Table 5.3** summarizes the results.

Table 5.3. Summary of Upper-Bound RQs Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Terrestrial Invertebrates as Dietary Food Items		
Use	**Small Insect RQ***	**Large Insect RQ***
Forestry and Areas with Impervious Surfaces 7.95 lbs a.e./A	**<1.4**	**<0.15**
California rights of way 7.5 lbs a.e./A	**<1.3**	**<0.14**
California corn and wheat 0.75 lbs a.e./A	**<0.17**	<0.02
California rangeland 1.54 lbs a.e./A	**<0.35**	<0.04
California rangeland 0.387 lbs a.e./A	**<0.1**	<0.01
* = LOC exceedances (RQ $\geq$ 0.05) are bolded and shaded. Because a definitive endpoint was not established for terrestrial invertebrates (i.e., the value is greater than the highest test concentration), the RQ represents an upper bound value.		

Potential risk to terrestrial invertebrates is further discussed in the risk description section. The preliminary effect determination is "may affect."

5.1.2.2.2 Mammals

Glyphosate

Risks associated with ingestion of small mammals by large terrestrial-phase CRLFs are derived for dietary-based and dose-based exposures modeled in T-REX for a small mammal (15g) consuming short grass. Acute and chronic effects are estimated using the most sensitive mammalian toxicity data. EECs are divided by the toxicity value to estimate acute and chronic dose-based RQs as well as chronic dietary-based RQs. There were no mortalities in any of the available acute mammalian studies. Therefore, no RQs were calculated.

Table 5.4 summarizes the risk quotients for small mammals eating short grass with chronic exposure to glyphosate. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for use rates of 3.84 lb/A and above.

Table 5.4. Summary of Chronic RQs* Used to Estimate Indirect Effects to the Terrestrial-phase CRLF via Direct Effects on Small Mammals as Dietary Food Items (non-granular application)		
Use Scenario Application Rate (# Applications per year/Interval (days))	**Chronic RQ**	
	Dose-based Chronic RQ[1]	**Dietary-based Chronic RQ[2]**
Forestry (aerial) and areas with impervious surfaces 7.95 lbs a.e./A (1)	**1.66**	0.19
Right of way 7.5 lbs a.e./A (1)	**1.56**	0.18
Citrus, cole crop, lettuce, melon, onion, potato and wine grape 3.85 first application, 2.3 second application lbs a.e./A (2/14)	0.6 – **1.00**	0.07 – 0.12
Almond, fruit, grape and olive 3.84 lbs a.e./A (2/14)	**1.00**	0.12
Corn, cotton, garlic, impervious surfaces, row crop, strawberry and wheat 3.75 first application, 2.25 second application lbs a.e./A (2/14)	0.59 – 0.98	0.07 - 0.11
Alfalfa, avocado, forestry, nursery, rangeland, residential and turf 3.75 lbs a.e./A (2/14)	0.98	0.11
Right of way 3.69 lbs a.e./A (2/14)	0.96	0.11
* = LOC exceedances (chronic RQ $\geq$ 1) are bolded and shaded. [1] Based on dose-based EEC and glyphosate rat NOAEL of 500 mg/kg-bw (MRID 41621501). [2] Based on dietary-based EEC and glyphosate rat NOAEC = 10000 mg/kg-diet.		

Formulations

Many acute oral toxicity studies have been conducted on formulations with the rat. As with the technical material, most of the LD_{50}'s are higher than the highest dose/concentrations tested. There are six submitted studies in which there are definitive LD_{50}'s. Label matches were conducted for each of these products and estimates were made as to how much of the formulated product could be applied in pounds per acre before exceeding the acute LOC for listed mammals. A label match-up with one of these products (MRID 46714802) determined that it is not registered in California (Registration number 5905-560). Other labels state that "not all products recommended on this label are registered for use in California". The specific formulation uses that are not allowed in California are not detailed here. For the five formulations in which there are definitive acute mammalian LD_{50}'s available and in which at least some of the uses may be allowed in California, **Table 5.5** provides that application rates in terms of pounds formulated product per acre that would exceed the acute mammalian listed species LOC for that product. An assumption is made that the product is applied only once per season. It is noted that for many labels, there are other products that are submitted to the Agency that use the same acute toxicity studies for their labels.

Table 5.5. Application Rates with Formulations Exceeding the Acute Mammalian LOC for Listed Species for Specific Formulations with Definitive Acute Mammalian LD_{50} Values – Small Mammals Eating Short Grass		
Registration Number	**Acute Mammalian LD_{50} mg/kg bw (MRID No.)**	**Application Rate Exceeding Dose-Based Acute RQ (lb formulation/A)[1,2]**
524-440	3750 (41305404)	3.5
62719-323	3803 (44918601)	3.5
524-504	5827 (44615502)	5.5
524-435	5000 (41142304)	5
524-424	2686 (40853903)	2.5
[1]LOC exceedances (acute RQ ≥ 0.1) [2] Assuming only 1 application per season		

The preliminary effect determination for both glyphosate and formulations (indirect effect on terrestrial-CRLF: diet) is "may affect."

5.1.2.2.3 Frogs

An additional prey item of the adult terrestrial-phase CRLF is other species of frogs. In order to assess risks to these organisms, dietary-based and dose-based exposures modeled in T-REX for a small bird (20g) consuming small invertebrates are used. See **Section 5.1.2.1** and associated table (**Table 5.1**) for results. No acute RQs were calculated for birds because there were no mortalities in any of the available acute avian studies.

Since the chronic avian LOC was exceeded for birds consuming small invertebrates for forestry and rights of way uses (aerial application) and for uses with areas with impervious surfaces, the preliminary effect determination is "may affect."

5.1.2.3 Indirect Effects to CRLF via Reduction in Terrestrial Plant Community (Riparian and Upland Habitat)

Potential indirect effects to the CRLF resulting from direct effects on riparian and upland vegetation are assessed using RQs from terrestrial plant seedling emergence and vegetative vigor EC_{25} data as a screen (the most sensitive EC_{25}'s were used rather than the NOAEC or EC_{05} because there are no obligate relationships between the CRLF and any terrestrial plant species). The most sensitive toxicity thresholds are 0.16 (monocot – dry weight) and 0.074 (dicot - phytotoxicity) lb ae/acre from the vegetative vigor studies. No effects were observed in the seedling emergence studies. RQs were estimated using the Terrplant (Version 1.2.2) model for the various uses of glyphosate in California.

Tables 5.6 and 5.7 summarize the risks to monocots and dicots from glyphosate uses with both ground and aerial spray applications. None of the RQs for terrestrial plants

living in either dry or semi-aquatic areas exposed to the combined deposition estimates from runoff and spray drift exceed the terrestrial plant LOC of 1. The terrestrial plant LOC is exceeded for both monocots and dicots when they are exposed to glyphosate via spray drift for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A. The preliminary effect determination is "may affect". An example output from TerrPlant v.1.2.2 is provided in **Appendix F.**

Table 5.6 RQs* for Monocots Inhabiting Dry and Semi-Aquatic Areas Exposed to Glyphosate via Runoff and Drift

Use Scenario	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift RQ	Dry area RQ	Semi-aquatic area RQ
Alfalfa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	3.75	Ground	1	0.23	<0.1	<0.38
Almond, fruit, grape, olive	3.84	Ground	1	0.24	<0.1	<0.39
Citrus	3.85	Ground	1	0.24	<0.1	<0.39
Cole crop, lettuce, melon, onion, potato, wine grape	3.85	Aerial	5	**1.20**	<0.1	<0.42
Corn	0.75	Aerial	5	0.23	<0.1	<0.1
Forestry	7.95	Aerial	5	**2.48**	<0.16	<0.87
Impervious	7.95	Ground	1	0.5	<0.1	<0.81
Nursery, rangeland, sugar beet, tomato, turf	3.75	Aerial	5	**1.17**	<0.1	<0.41
Rangeland	1.54	Ground	1	<0.1	<0.1	<0.16
Rangeland	0.387	Aerial	5	0.12	<0.1	<0.1
Rights of way	7.5	Aerial	5	**2.34**	<0.15	<0.83
Rights of way	3.69	Ground	1	0.23	<0.1	<0.38
Wheat	0.75	Ground	1	<0.1	<0.1	<0.1

* = LOC exceedances (RQ ≥ 1) are bolded and shaded.

Table 5.7 RQs* for Dicots Inhabiting Dry and Semi-Aquatic Areas Exposed to Glyphosate via Runoff and Drift

Use Scenario	Application rate (lbs a.i./A)	Application method	Drift Value (%)	Spray drift RQ	Dry area RQ	Semi-aquatic area RQ
Alfalfa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	3.75	Ground	1	0.51	<0.1	<0.38
Almond, fruit, grape, olive	3.84	Ground	1	0.52	<0.1	<0.39
Citrus	3.85	Ground	1	0.52	<0.1	<0.39
Cole crop, lettuce, melon, onion, potato, wine grape	3.85	Aerial	5	**2.60**	<0.1	<0.42
Corn	0.75	Aerial	5	0.51	<0.1	<0.1
Forestry	7.95	Aerial	5	**5.37**	<0.16	<0.87
Impervious	7.95	Ground	1	**1.07**	<0.1	<0.81
Nursery, rangeland, sugar beet, tomato, turf	3.75	Aerial	5	**2.53**	<0.1	<0.41
Rangeland	1.54	Ground	1	0.21	<0.1	<0.16
Rangeland	0.387	Aerial	5	0.26	<0.1	<0.1
Rights of way	7.5	Aerial	5	**5.07**	<0.15	<0.83
Rights of way	3.69	Ground	1	0.50	<0.1	<0.38
Wheat	0.75	Ground	1	0.10	<0.1	<0.1
* = LOC exceedances (RQ ≥ 1) are bolded and shaded.						

5.1.3 Primary Constituent Elements of Designated Critical Habitat

5.1.3.1 Aquatic-Phase (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.
- Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.,* algae).

The preliminary effects determination for aquatic-phase PCEs of designated habitat related to potential effects on aquatic and/or terrestrial plants is "may affect", based on the risk estimation provided in **Sections 5.1.1.2, 5.1.1.3**, and **5.1.2.3**.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." To assess the impact of glyphosate on this PCE, acute and chronic freshwater fish and invertebrate toxicity endpoints, as well as endpoints for aquatic non-vascular plants are used as measures of effects. RQs for these endpoints were calculated in **Sections 5.1.1.1** and **5.1.1.2**. For freshwater fish and invertebrates, there are no acute or chronic aquatic LOC exceedances for glyphosate or for formulations with the highest peak EECs generated from the registered uses (aquatic weed management). The LOC for aquatic plants is not exceeded with the highest peak EEC generated from the uses involving direct application to water (aquatic weed management) for glyphosate a.e. but is exceeded for direct application to water for formulations. Based on an acute RQ of 4.7 for formulations for aquatic non-vascular plants, the preliminary effect determination for the PCE, "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source" is "no effect" for glyphosate and "may affect" for formulations.

5.1.3.2 Terrestrial-Phase (Upland Habitat and Dispersal Habitat)

Two of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the CRLF are related to potential effects to terrestrial plants:

- Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance
- Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal

The preliminary effects determination for terrestrial-phase PCEs of designated habitat related to potential effects on terrestrial plants is "may affect", based on the risk estimation provided in **Section 5.1.2.3**.

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of glyphosate on this PCE, acute and chronic toxicity endpoints for birds, mammals, and terrestrial invertebrates are used as measures of effects. RQs for these endpoints were calculated in **Section 5.1.2.2**. There were no mortalities for glyphosate a.e. in either the acute avian or the acute mammalian studies. Therefore, no RQs were calculated. The chronic avian LOC is exceeded for birds consuming small invertebrates for forestry uses with aerial application, for uses with areas with impervious surfaces and for rights of way (aerial

application). The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for every use rate of 3.84 lbs a.e./A and above. The LOC for listed terrestrial invertebrates is exceeded for all uses for small insects. The LOC for listed terrestrial invertebrates for large insects is exceeded for all uses at 7.5 lbs a.e./A and above (see **Table 5.4** for application rates and scenarios). Therefore, the preliminary effect determination is "may affect."

The fourth terrestrial-phase PC is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source. Direct acute and chronic RQs for terrestrial-phase CRLFs are presented in **Section 5.2.1.2**. There were no mortalities in the acute avian studies. Therefore, no RQs were calculated. The chronic avian LOC is exceeded for birds consuming small invertebrates for forestry uses with aerial application, for uses with areas with impervious surfaces and for rights of way (aerial application). Therefore, the preliminary effect determination is "may affect."

5.2 Risk Description

The risk description synthesizes an overall conclusion regarding the likelihood of adverse impacts leading to an effects determination (*i.e.*, "no effect," "may affect, but not likely to adversely affect," or "likely to adversely affect") for the CRLF and its designated critical habitat.

If the RQs presented in the Risk Estimation (**Section 5.1**) show no direct or indirect effects for the CRLF, and no modification to PCEs of the CRLF's designated critical habitat, a "no effect" determination is made, based on glyphosate's use within the action area. However, if direct or indirect effect LOCs are exceeded or effects may modify the PCEs of the CRLF's critical habitat, the Agency concludes a preliminary "may affect" determination for the FIFRA regulatory action regarding glyphosate. A summary of the results of the risk estimation (*i.e.*, "no effect" or "may affect" finding) is provided in **Table 5.8** for direct and indirect effects to the CRLF and in **Table 5.9** for the PCEs of designated critical habitat for the CRLF.

Table 5.8 Preliminary Effects Determination Summary for Glyphosate - Direct and Indirect Effects to CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
Aquatic Phase (eggs, larvae, tadpoles, juveniles, and adults)		
Survival, growth, and reproduction of CRLF individuals via direct effects on aquatic phases	No effect	No LOC exceedances for freshwater fish and/or aquatic-phase amphibians following either acute or chronic exposures to either glyphosate (a.e.) or to its formulations.
Survival, growth, and reproduction of CRLF individuals via effects to food supply (*i.e.*, freshwater invertebrates, non-vascular plants)	May affect	No LOC exceedances for freshwater invertebrates following either acute or chronic exposures and no LOC exceedances for aquatic non-vascular plants following acute exposure with glyphosate (a.e.). With formulations, no LOC exceedances for freshwater invertebrates following acute exposures from either terrestrial or aquatic uses and for non-vascular plants from terrestrial uses; however, there are LOC exceedances for aquatic non-vascular plants following acute exposures from aquatic uses (32.9 lbs formulation/A).
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat, cover, and/or primary productivity (*i.e.*, aquatic plant community)	May affect	No LOC exceedances for aquatic non-vascular and vascular plants with glyphosate a.e.. With formulations, no LOC exceedances for aquatic vascular and non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances with non-vascular plants following acute exposures from aquatic uses (32.9 lbs formulation/A).
Survival, growth, and reproduction of CRLF individuals via effects to riparian vegetation, required to maintain acceptable water quality and habitat in ponds and streams comprising the species' current range.	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses on areas with impervious surfaces at 7.95 lbs a.e./A.
Terrestrial Phase (Juveniles and adults)		
Survival, growth, and reproduction of CRLF individuals via direct effects on terrestrial phase adults and juveniles	May affect	Chronic avian LOC exceeded for forestry, uses on areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). Acute avian LOC for listed species exceeded for one formulation for application rates of 0.8 lbs formulation/A and above: Industrial areas outdoors and non-agricultural rights of way (5.5 lbs formulation/A), ornamental lawns and turf (1.1 – 2.2 lbs formulation/A
Survival, growth, and reproduction of CRLF individuals via effects on prey (*i.e.*, terrestrial invertebrates, small terrestrial mammals and terrestrial phase amphibians)	May affect	See box above for terrestrial phase amphibians. For small insects, LOC for listed species potentially exceeded for all uses (0.387 lbs a.e./A and above) and LOC for non-listed species potentially exceeded for forestry, areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). For large

Table 5.8 Preliminary Effects Determination Summary for Glyphosate - Direct and Indirect Effects to CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
		insects, LOC for listed species potentially exceeded forestry, areas with impervious surfaces and rights of way. Acute RQs for small mammals exceed acute mammal LOC for listed species with 4 formulations at 3.5 lbs formulation/A and above. Chronic RQs for small mammals exceed chronic mammalian LOC on a dose-basis for every use scenario at 3.84 lbs a.e./A and above.
Survival, growth, and reproduction of CRLF individuals via indirect effects on habitat (*i.e.*, riparian vegetation)	May affect	Terrestrial plant LOC exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A.

Table 5.9 Preliminary Effects Determination Summary for Glyphosate PCEs of Designated Critical Habitat for the CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
Aquatic Phase PCEs (Aquatic Breeding Habitat and Aquatic Non-Breeding Habitat)		
Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A. No LOC exceedances for aquatic non-vascular and vascular plants with glyphosate a.e.. With formulations, no LOC exceedances for aquatic vascular and non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances for non-vascular plants following exposures from aquatic uses (management of aquatic plants at 32.9 lbs formulation/A).
Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A. No LOC exceedances for aquatic non-vascular and vascular plants with glyphosate a.e.. With formulations, no LOC exceedances for aquatic vascular and non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances with non-vascular plants following exposures from aquatic uses (management of

Table 5.9 Preliminary Effects Determination Summary for Glyphosate PCEs of Designated Critical Habitat for the CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
		aquatic plants at 32.9 lbs formulation/A).
Alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source.	May affect	For glyphosate a.e.: no aquatic LOC exceedances for freshwater fish and invertebrates and aquatic plants with the highest peak EEC (management of aquatic plants). For formulations: no LOC exceedances for freshwater invertebrates and fish following acute exposure from either terrestrial or aquatic uses. No LOC exceedances for non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances following acute exposures from aquatic uses (management of aquatic plants at 32.9 lbs formulation/A).
Reduction and/or modification of aquatic-based food sources for pre-metamorphs (e.g., algae)	May affect	Glyphosate a.e.: no exceedance of aquatic plant LOC with the highest peak EEC (management of aquatic plants at 3.75 lbs a.e./A). Formulations: no LOC exceedances for aquatic non-vascular plants following acute exposures from terrestrial uses; however, there are LOC exceedances following exposures from aquatic uses (management of aquatic plants at 32.9 lbs formulation/A).
Terrestrial Phase PCEs (Upland Habitat and Dispersal Habitat)		
Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or dripline surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A.
Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal	May affect	The terrestrial plant LOC is exceeded for spray drift for both monocots and dicots for aerial uses at 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A.
Reduction and/or modification of food sources for terrestrial phase juveniles and adults	May affect	Chronic avian LOC exceeded for forestry, uses on areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). Acute avian LOC for listed species exceeded for one formulation for application rates of 0.8 lbs formulation/A and above. Acute RQs for small mammals exceed acute mammal LOC for listed species with 4 formulations at 3.5 lbs formulation/A and above. Chronic RQs for small

Table 5.9 Preliminary Effects Determination Summary for Glyphosate PCEs of Designated Critical Habitat for the CRLF		
Assessment Endpoint	**Preliminary Effects Determination**	**Basis For Preliminary Determination**
		mammals exceed chronic mammalian LOC on a dose-basis for every use scenario at 3.84 lbs a.e./A and above. For small insects, LOC for listed species potentially exceeded for all uses (0.387 lbs a.e./A and above) and LOC for non-listed species potentially exceeded for forestry, areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). For large insects, LOC for listed species potentially exceeded forestry, areas with impervious surfaces and rights of way.
Alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.	May affect	Chronic avian LOC exceeded for forestry, uses on areas with impervious surfaces and rights of way (7.5 lbs a.e./A and above). Acute avian LOC for listed species exceeded for one formulation for application rates of 0.8 lbs formulation/A and above.

Following a "may affect" determination, additional information is considered to refine the potential for exposure at the predicted levels based on the life history characteristics (*i.e.*, habitat range, feeding preferences, etc.) of the CRLF. Based on the best available information, the Agency uses the refined evaluation to distinguish those actions that "may affect, but are not likely to adversely affect" from those actions that are "likely to adversely affect" the CRLF and its designated critical habitat.

The criteria used to make determinations that the effects of an action are "not likely to adversely affect" the CRLF and its designated critical habitat include the following:

- Significance of Effect: Insignificant effects are those that cannot be meaningfully measured, detected, or evaluated in the context of a level of effect where "take" occurs for even a single individual. "Take" in this context means to harass or harm, defined as the following:
    - Harm includes significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering.
    - Harass is defined as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding, or sheltering.
- Likelihood of the Effect Occurring: Discountable effects are those that are extremely unlikely to occur.
- Adverse Nature of Effect: Effects that are wholly beneficial without any adverse effects are not considered adverse.

A description of the risk and effects determination for each of the established assessment endpoints for the CRLF and its designated critical habitat is provided in **Sections 5.2.1 through 5.2.3**.

5.2.1 Direct Effects

5.2.1.1 Aquatic-Phase CRLF

The aquatic-phase considers life stages of the frog that are obligatory aquatic organisms, including eggs and larvae. It also considers submerged terrestrial-phase juveniles and adults, which spend a portion of their time in water bodies that may receive runoff and spray drift containing glyphosate.

Glyphosate

The acute and chronic RQs for direct effects to the aquatic-phase CRLF are presented in **Section 5.1.1.1**. As stated previously, none of the RQs exceed either the acute or chronic LOCs for freshwater fish (surrogate for the CRLF).

The highest peak aquatic EEC for glyphosate is 210 ppb for aquatic plant management. Monitoring data (NAQWA) indicate surface water concentrations ranging from 0.1 – 7.46 ppb. These concentrations are more than an order of magnitude lower than the estimated concentration utilized in the risk estimations.

The probability of an individual effect at the RQ (<0.01) is <1 in 8.9E+18 (1 in 3.2E+04 to 1.0E+72). The acute fish studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability.

For freshwater fish, the data on the technical material from the acute toxicity studies are so variable within each species that it is not possible to provide a sensitivity analysis. For amphibians, acute toxicity data are available on 3 species: the Australian tree frog, Australian frog and the green frog. The data are not sufficient to indicate a range of sensitivities for frogs. The acute toxicity values range from > 17.9 to 103.2 ppm. The study with the lowest potential endpoint (green frog: >17.9 ppm) does not provide a definitive endpoint. For the other two species, the confidence interval for the Australian tree frog (LC_{50}: 103.2 (43.2 - 172.8)) overlaps with the confidence interval for the Australian frog (LC_{50}: 75 (60.4-92.7)). Therefore, for the technical material, a species sensitivity analysis could not be conducted.

Formulations

Terrestrial Uses

Acute risk from formulated products (terrestrial uses) were estimated using the most conservative LC_{50} and peak aquatic EEC. The acute aquatic LOCs were not exceeded. Therefore, the acute aquatic LOCs would not be exceeded for any of the uses. Risks

from chronic exposure were estimated from a chronic toxicity study on a formulated product with leopard frog larvae. As stated previously, this study has no NOAEC; however, the NOAEC would have to be 20 times less than the LOAEC for the chronic LOC for aquatic animals to be exceeded. Due to the fact that there was high variability in the results and either minimal or a lack of statistical significance for some of the key parameters, the NOAEC is probably closer to the LOAEC than a value that is 20 times less than the LOAEC.

Aquatic Uses

Acute risk from formulated products (aquatic uses) were estimated using a fish study that had a matching label which has aquatic uses and the peak aquatic EEC. The acute aquatic LOCs were not exceeded.

As stated in the Ecological Effects Characterization section (Section 4), submitted data on formulations indicate that some of the formulations are more toxic to freshwater fish than glyphosate itself, particularly those studies which tested formulations with one type of surfactant, polyethoxylated tallowamines (polyoxy ethylene fatty amine). Other surfactants do not appear to increase the toxicity of glyphosate except the X-77 surfactant with an acute LC_{50} value of 9.4 in rainbow trout when mixed with the isopropylamine salt (MRID 00078664). The data from the formulation studies do not provide a pattern consistent enough to determine a range of sensitivities for freshwater fish. One review indicates that the salmonids are more sensitive to glyphosate than other species of fish (USDA, 2003); however, the available data here are not sufficient to indicate that that is the case. The acute LC_{50}'s from studies conducted with formulations and freshwater fish range from 1 ppm (rainbow trout, MRID 40098001) for Roundup™ (most likely with the polyethoxylated tallowamine surfactant) to >1000 ppm (rainbow trout, geronol surfactant, MRID 44738201). The range of acute toxicity values are likely more related to the various formulations tested rather than to the sensitivities of the various freshwater fish species tested. The Roundup formulations have been tested the most and the acute LC_{50} values for the various fish species are all between 1 and 10 ppm. For example, submitted acute toxicity study values (LC_{50} in ppm) for freshwater fish species with Roundup formulations are: rainbow trout (1 - 9.2 (MRID 40098001, 00124761, 00162296 and 40579203)); bluegill sunfish (1.8 - 4.3 (MRID 00124760, 40098001 and 00070897)); fathead minnow (2.9 (MRID 00070896)); channel catfish (4.9 (MRID 00070894)); Chinook salmon (7.1 (MRID 40579201)) and Coho salmon (8 (MRID 40579202)). Formulations with other surfactants ("W", "X-77", "AA" and geronol provided a wide range of LC_{50} values, starting from 9.4 ppm to >1000 ppm.

For amphibians, a few studies were conducted on multiple formulations with several species of frogs. As with freshwater fish, the acute LC_{50}'s range from 1.1 ppm to 1000 ppm with the differences more likely related to the various formulations tested rather than to the sensitivities of the various frog species. For example, data on Roundup®, containing the polyethoxylated tallowamine surfactant provide LC_{50} values in a tight range from 2 ppm in the green frog (Gosner Stage 25) to 8 ppm with the wood frog (Gosner Stage 20). Again, as with the freshwater fish, these values are all between 1 and

10 ppm. In a series of studies reported by Howe et al. (MRID 46650501) which examined the acute and chronic effects of glyphosate alone, the surfactant polyethoxylated tallowamine (POEA) and glyphosate formulated products on 4 aquatic phase amphibian (anuran) species, the data indicated that younger amphibian larvae (Gosner stage 20) were less sensitive to acute lethality from the toxic surfactant-containing formulations than older larvae (Gosner stage 25). At stage 25, *R. clamitans* (green frog) was the most sensitive (96-hr LC_{50} = 6.5 mg/L or 2.0 mg a.e./L) and *R sylvatica* (wood frog) was the most tolerant (96-hr LC_{50} = 16.5 mg/L or 5.1 mg a.e./L).

Table 5.10. Aquatic-Phase Amphibian Acute Toxicity for Roundup Formulation

Chemical	Species	96-hour LC_{50} (mg a.e.*/L)	MRID #/Year
Glyphosate IPA (Roundup)	Green Frog (*Rana clamitans*) Gosner Stg 25	LC_{50}: 2 (1.9-2.2)[1]	46650501/2001
Glyphosate IPA (Roundup)	Leopard Frog (*Rana pipiens*) Gosner Stg 25	LC_{50}: 2.9 (NR)	46650501/2000
Glyphosate IPA (Roundup)	American toad (*Bufo americanus*) Gosner Stg 25	LC_{50}: 4.2 (NR)	46650501/1994
Glyphosate IPA (Roundup)	Wood Frog (*Rana sylvatica*) Gosner Stg 25	LC_{50}: 5.1 (4.9-5.4)	46650501/1994
Glyphosate IPA (Roundup)	Leopard Frog (*Rana pipiens*) Gosner Stg 20	LC_{50}: 6.5 (6.1-6.8)	46650501/1994
Glyphosate IPA (Roundup)	Green frog (*Rana clamitans*) Gosner Stg 20	LC_{50}: 7.1 (6.6-7.6)	46650501/1994
Glyphosate IPA (Roundup)	American toad (*Bufo americanus*) Gosner Stg 20	LC_{50}: 8 (NR)	46650501/1994
Glyphosate IPA (Roundup)	Wood Frog (*Rana sylvatica*) Gosner Stg 20	LC_{50}: 8 (NR)	46650501/1994

*a.e. = acid equivalent; IPA = isopropylamine salt, N.R. = not reported
[1] Range is 95% confidence interval for endpoint

Incident Data

Two incident reports were filed for glyphosate and 16 incidents were filed for the isopropylamine salt of glyphosate. For glyphosate, with a certainty index of possible, 2 fish were incapacitated and 20 fish were killed following exposure. For the isopropylamine salt of glyphosate, the certainty indices of the reports ranged from unlikely to highly probable. There was one accidental misuse in which thousands of shad were killed upon ingestion. Drums of Roundup were found floating in the water with the dead fish. This was the one report that was rated highly probable. The fish kill was more likely due to the surfactant in the Roundup formulation rather than from glyphosate itself. Eight of the incidents were from runoff, 2 from ingestion, 1 from pond treatment and 1 from skin contact. Fifteen reported mortality and 2 reported incapacitation. The numbers of fish killed ranged from 9 to thousands.

Open Literature Data and Sublethal Effects

For freshwater fish, as stated previously, none of the data from the open literature provided more conservative endpoints that could be used in a quantitative estimate of risk. Sublethal data are available for muliple fish species with technical glyphosate, the formulations, Roundup® and Vision® and with glyphosate with several different surfactants. The NOAECs for sublethal effects range from 8 ppb to 30.6 ppm. The lowest NOAEC/LOAEC is 8/46 ppb, based on an increase in wigwag behavior in rainbow trout following a 2 month exposure to Vision®, a formulation containing the toxic surfactant, POEA (E097714). In order to do a comparison of the NOAEC with the modeled chronic EEC for a formulation, only the EECs from terrestrial uses may be used because POEA is not allowed in formulations with aquatic uses. In addition, the results are expressed in terms of the acid equivalent, glyphosate. Therefore, based on the information provided in the paper, the lowest NOAEC/LOAEC may be converted to 25.8/148.3 ppb formulation. As stated previously, the chronic EEC for terrestrial formulations could not be estimated. If the peak EEC of 95.2 ppb for terrestrial formulations is used as a very conservative estimate, the comparison shows that the peak EEC is in between the NOAEC and the LOAEC for this behavioral effect. Therefore, there is an uncertainty as to whether or not this sublethal effect may occur in freshwater fish near areas where glyphosate is applied.

In amphibians, sublethal data from the open literature are available for green, African clawed, leopard, moaning, bull, motorbike, cascades and Western chorus frog; sign-bearing froglet; lesser snouted treefrog and the American toad. The lowest NOAEC is < 1 ppm and the highest is 4000 ppm. At a LOAEC of 1 ppm, metamorphosis occurred more rapidly in treated frogs with decreased size and mass when Cascade frogs were tested with Roundup®. In that study, no NOAEC was determined. At 6000 ppm with the Rodeo® formulation, there was a decrease in African clawed frog embryo growth. Other studies show malformations (craniofacial and mouth deformities, eye abnormalities and bent curved tails) and slow swimming abilities at lethal concentrations. Again, using the peak EEC of 95.2 ppb for terrestrial uses, a comparison of the LOAEC of 1000 ppb with the peak EEC shows that the concentration at which the LOAEC is observed is an order of magnitude higher than the peak EEC. The NOAEC could easily be at a concentration that is higher than the peak EEC.

For tables detailing these studies, see section 4.1.1.5 in the Ecological Effects Characterization section.

Effect Determination

Based on the weight of the evidence, the final effect determination is “no effect” for direct effects to the aquatic-phase CRLF. This determination is based on the lack of LOC exceedances following either acute or chronic exposures for both glyphosate and formulations, the low monitoring data in surface water when compared to the modeled concentrations and the low probability of an individual effect. The accidental misuse

where thousands of fish were killed involved Roundup, a formulation which often contains the toxic surfactant, POEA, which is not allowed for aquatic uses in California.

5.2.1.2 Terrestrial-Phase CRLF

Glyphosate

Acute RQs were not calculated because there were no mortalities up to and including the highest dose/concentration tested. There are also no sublethal effects in any of the avian studies on the technical material. The chronic avian studies also do not have any effects at the highest concentrations tested. If comparisons are made between the terrestrial EECs estimated from T-REX and the highest dose tested in the acute oral studies, the results show that all of the EEC values are lower, but at application rates above 0.75 lbs a.e./A, the EECs are greater than $1/10^{th}$ of the highest dose tested in the studies. For that reason, there is an uncertainty for listed avian species (the LOC for listed avian species is 0.1). For any of the uses at application rates of 7.5 lbs a.e./A and above, the EEC values are greater than half the highest dose/concentration tested in the acute avian studies. Therefore, for those applications, there is uncertainty for non-listed species (the LOC for non-listed species is 0.5). A similar situation holds true for the subacute dietary-based EECs. The acute dietary-based EEC's are greater than 10% of the highest concentration tested in the avian subacute feeding studies for application rates higher than 2.35 lbs a.e./A.

The probability of an individual effect at the LOC (0.1: no RQs) is <1 in 2.94E+05 (<1 in 4.40E+01 to <1 in 8.86E+18). The acute bird studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability.

The chronic avian study showed no effects at the highest concentration tested. As stated in the risk estimation section, uses with application rates of 7.5 lbs a.e./A and higher have EECs that are higher than the highest concentration tested in the chronic avian study. Following chronic exposure, the RQs exceed the chronic LOC of 1 for consumption of broadleaf plants and small insectivorous mammals at rates of 7.5 lbs a.e./A and above. The RQs drop below the chronic LOC at lower application rates. These RQ values are conservative because there is no LOAEL from the chronic avian study.

Formulations

As stated in the risk estimation section, most of the available avian studies on formulations indicate LD_{50}/LC_{50} values greater than the highest dose/concentration tested. For the one study which has a definitive acute toxicity value, the application rates from the specific label for which this study was submitted indicate an exceedance of the acute avian LOC for all use rates, including the highest rate (5.5 lbs formulation/A (industrial outdoors)) to the lowest rate (1.1 lbs formulation/A (ornamental lawns and turf)). The dose-based acute RQs are 0.71 and 0.14, respectively, for a diet of small invertebrates.

The probability of an individual effect for the formulation at the LOC would be the same as the probability for the technical material (see above). The probability of an individual effect at the RQ of 0.53 is 1 in 9.32 (1 in 3.44 to 1 in 1.53E+02) and at the RQ of 0.11 is 1 in 1.25E+05 (1 in 3.62E+01 to 1 in 3.19E+17). The acute bird study provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability. This model assumes a dose-response. The mortality in the acute oral study on the formulation did not provide a dose-response. There was one mortality at the second highest dose level and complete mortality at the highest dose level. Therefore, a significant uncertainty is associated with using this probit model for estimating the probability of an individual effect.

T-HERPS

As stated above, the acute avian LOC of 0.1 was exceeded on a dose-basis for all use rates from the specific label for the formulated product. Therefore, for direct effects to the terrestrial-phase CRLF following exposure to a formulation, the model, T-HERPS (v. 1.0) was used to further define the risk to herpetofauna following acute exposure to this formulated product on a dose-basis. Modeling the avian data in T-HERPS to estimate direct effects to the terrestrial-phase CRLF indicates that medium and large-sized herps eating small herbivorous mammals on a dose-basis may be at risk following acute exposure at the labelled application rate of 5.5 lb/A. At 2.2 lb/A, the risk for large-sized herps drops below the LOC; however, the risk to medium-sized herps remains above the LOC. At 1.1 lbs formulation/A, the risk to medium-sized herps continues to remain above the LOC.

The following tables provide the results from T-HERPS for industrial outdoor uses at 5.5 lbs formulation/A and for ornamental lawns and turf at 2.2 and 1.1 lbs formulation/A.

Table 5.11. Upper Bound Kenaga, Terrestrial Herpetofauna Dose-Based Risk Quotients Used to Estimate Direct Effects to the Terrestrial-Phase CRLF Following Acute Exposure to a Formulation on Industrial Outdoor Areas at 5.5 lb Formulation/A[1]

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1651.00	28.85	0.02	3.21	<0.01	N/A	N/A	N/A	N/A	N/A	N/A
37	1651.00	28.35	0.02	3.15	<0.01	822.79	**0.50**[2]	51.42	0.03	0.98	<0.01
238	1651.00	18.58	0.01	2.06	<0.01	127.91	**0.08**	7.99	<0.01	0.64	<0.01

[1]Registration Number 524-424

[2] **Bolded** values exceed the acute terrestrial LOC of 0.1 for listed species

Table 5.12. Upper Bound Kenaga, Terrestrial Herpetofauna Dose-Based Risk Quotients Used to Estimate Direct Effects to the Terrestrial-Phase CRLF Following Acute Exposure to a Formulation on Ornamental Lawns and Turf at 2.2 lb Formulation/A[1]

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1651.00	11.54	0.01	1.28	<0.01	N/A	N/A	N/A	N/A	N/A	N/A
37	1651.00	11.34	0.01	1.26	<0.01	329.12	**0.20**[2]	20.57	0.01	0.39	<0.01
238	1651.00	7.43	<0.01	0.83	<0.01	51.16	0.03	3.20	<0.01	0.26	<0.01

[1]Registration Number 524-424
[2] **Bolded** values exceed the acute terrestrial LOC of 0.1for listed species

Table 5.13. Upper Bound Kenaga, Terrestrial Herpetofauna Dose-Based Risk Quotients Used to Estimate Direct Effects to the Terrestrial-Phase CRLF Following Acute Exposure to a Formulation on Ornamental Lawns and Turf at 1.1 lb Formulation/A[1]

Size Class (grams)	Adjusted LD50	EECs and RQs									
		Broadleaf Plants/ Small Insects		Fruits/Pods/ Seeds/ Large Insects		Small Herbivore Mammals		Small Insectivore Mammal		Small Amphibians	
		EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ	EEC	RQ
1.4	1651.00	5.77	<0.01	0.64	<0.01	N/A	N/A	N/A	N/A	N/A	N/A
37	1651.00	5.67	<0.01	0.63	<0.01	164.56	**0.10**[2]	10.28	0.01	0.20	<0.01
238	1651.00	3.72	<0.01	0.41	<0.01	25.58	0.02	1.60	<0.01	0.13	<0.01

[1]Registration Number 524-424
[2] **Bolded** values either exceed or equal the acute terrestrial LOC of 0.1 for listed species

Open Literature Studies

No additional acute avian studies were found in the open literature to further inform this risk assessment on a quantitative basis. However, as stated previously, there was one 15-day gavage study in which the formulation, Roundup was observed to reduce plasma testosterone levels after treatment of 5 mg/kg bw and above. In addition, "alterations in the structure of the testis and epididymal region…with changes in the expression of androgen receptors restricted to the testis" were observed.

Incident Data

One incident report on glyphosate was categorized as unlikely. This report is not summarized here. For glyphosate isopropylamine salt, one incident connected with use on corn was reported where an unknown quantity of birds were killed following exposure through drift after a broadcast spray. This was an accidental misuse. This report was classified as possible. In a second incident, also categorized as possible, 3 birds (species

unknown) were killed following exposure through drift after an unknown application. The legality of the application is unknown.

Effect Determination

Based on the weight-of-evidence, the final effect determination is LAA. This is based on the following points:

- Although no effects were observed in the avian reproduction studies, the concentration levels tested were sufficiently low that at application rates of 7.5 lbs a.e./A and above, the terrestrial dietary EECs are greater than the highest concentration tested in the avian reproduction studies. This creates an uncertainty for direct effects following chronic exposure.
- An open literature study on the effects of the formulation, Roundup on the epididymal region of drakes indicates that there may be some potential effects on the morphophysiology of the male duck reproductive system at dose levels as low as 5 mg/kg bw. This study supports the uncertainty associated with the potential risk following chronic exposure at higher application rates.
- One formulation has a discreet LD_{50} value. Comparing that value to the terrestrial EECs generated from the specific label for that formulation indicates that the acute avian dose-based LOC is exceeded for all application rates (1.1 to 5.5 lb formulation/A) listed on the label.
- The T-HERPS model for the formulation mentioned above indicates that medium and large-sized herps eating small herbivorous mammals on a dose-basis may be at risk following acute exposure at the highest application rate. At the lower application rates on the label, the potential risk to medium-sized herps still remains.
- The incident data, although categorized as possible, indicates that if the acute exposure is sufficiently high, there may be some avian (and thus, CRLF) mortality following acute exposure through drift.
- As stated previously, between 1999 and 2006, glyphosate was reportedly used in all 58 counties in California with the total amount approximately 7.8 million pounds (a.i.) in 2006 (CDPR PUR). In addition, glyphosate has a number of residential and industrial uses that are not represented in these data. Landscape maintenance and rights of way are among the highest usages in the counties which may have some currently CRLF occupied areas (**Tables 2.7 – 2.9** and **Figure 2.2**).

5.2.2 Indirect Effects (via Reductions in Prey Base)

5.2.2.1 Algae (non-vascular plants)

Glyphosate

As stated in the risk estimation section, the highest RQ for non-vascular plants is 0.02. This was based on the lowest available EC_{50} of 12100 ppb for green algae and the highest

peak EEC of 210 ppb for management of aquatic plants. The preliminary effect determination was "no effect." Also stated previously, the monitoring data indicated the highest EEC of 7.46 ppb, at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

Formulations

Terrestrial Uses

Again, as stated in the risk estimation section, the highest RQ for non-vascular plants following terrestrial application of a formulation is 0.243 using the lowest 96-hr EC_{50} of 0.39 mg/L (390 µg/L) with the freshwater diatom and the highest peak EEC for formulations of 95.2 ppb. This is less than the LOC of 1 for aquatic plants.

Aquatic Uses

The highest RQ for non-vascular plants following aquatic application of a formulation is 4.7 using the lowest EC_{50} of 0.39 mg/L and the peak EEC for formulations following aquatic uses of 1.84 ppm. This is greater than the LOC of 1 for aquatic plants.

Open Literature Data

Of the available open literature studies from which data may be extracted for comparing the results with the submitted studies, 3 studies, on 3 different species of green algae provide lower 96-hr EC_{50}'s based on cell counts (growth) correlated with absorbance over time for 96 hours on a Shimadzu UV-2401 PC Spectrophotometer. All of these studies were performed by the same group of scientists and published in different papers. The papers were not thoroughly reviewed for acceptability according to Agency guidelines; however, they are discussed in this section and compared to the highest aquatic EEC. In the first study, conducted with 95% technical material (not stated if glyphosate or the IPA of glyphosate), the 96-hr EC_{50} was 3.530 mg/L for *Chlorella pyrenoidosa* (Ma et.al 2001, ECOTOX reference 61983). Comparing that value to the highest EEC of 222.9 ppb, the RQ would be 0.06, significantly lower than the LOC for aquatic plants. In the second study (Ma et al., 2002, ECOTOX reference 65938), the 96 hr. EC_{50} for *Chlorella vulgaris* was 4.70 mg/L. This was again conducted with a 95% technical product. The study authors used the CAS number for glyphosate, not IPA, so it is assumed that this is the acid. The resulting highest RQ from this study would be 0.05. The third study, conducted with *Raphidocelis subcapitata (Selenastrum capricornutum)* (Ma et al., 2006, ECOTOX ref. 83543), the 96 hr. acute toxicity value is 5.56 mg/L with a resulting RQ of 0.04. Again, the study was conducted with 95% technical product, which is presumed to be the glyphosate acid. Even with these lower endpoints, the LOC for aquatic plants would not be exceeded.

Based on the weight-of-evidence, final effects determination is LAA for indirect effects, reduction in prey base (aquatic non-vascular plants) following application of a formulation registered for aquatic uses. The effects determination is based on an

exceedance of the LOC for aquatic non-vascular plants. As with avian species, glyphosate is used in all 58 counties in California. Landscape maintenance and rights of way are among the highest usages in the counties which may have some currently CRLF occupied areas.

5.2.2.2 Aquatic Invertebrates

Glyphosate

The potential for glyphosate to elicit indirect effects to the CRLF via effects on freshwater invertebrate food items is dependent on several factors including: (1) the potential magnitude of effect on freshwater invertebrate individuals and populations; and (2) the number of prey species potentially affected relative to the expected number of species needed to maintain the dietary needs of the CRLF. Together, these data provide a basis to evaluate whether the number of individuals within a prey species is likely to be reduced such that it may indirectly affect the CRLF.

The acute and chronic RQs for indirect effects to the aquatic-phase CRLF (reduction in aquatic invertebrate prey) are presented in **Section 5.1.1.2**. As stated previously, none of the RQs exceed either the acute or chronic LOC for freshwater invertebrates. Monitoring data (NAQWA) indicate surface concentrations ranging from 0.1 – 7.46 ppb. These concentrations are at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

The probability of an individual effect at the RQ (<0.01) is <1 in 8.9E+18 (1 in 3.2E+04 to 1.0E+72). The acute aquatic invertebrate studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability. The percentage effect to the freshwater invertebrate population prey base is < 1.1E-17 percent.

Formulations

Terrestrial uses

As stated previously, comparing the most conservative EC_{50} of 3 mg formulation/L with the highest peak EEC for formulations of 95.2 ppb formulation, the highest RQ for aquatic invertebrates is 0.03, which is less than the LOC of 0.05 for aquatic invertebrates. Therefore, none of the other uses will exceed the acute aquatic invertebrate LOC.

Aquatic uses

Again, the LOC for aquatic animals is not exceeded with the acute toxicity study endpoint of 164.3 ppm formulation and the peak EEC of 1.84 ppm formulation.

Open Literature

There were no open literature studies on aquatic invertebrates that would further inform this assessment of risk. All of the studies provide endpoints that are greater than the most sensitive endpoints used in this assessment.

The acute and chronic RQs for glyphosate are below the acute and chronic LOC's for aquatic animals (highest acute and chronic RQ's are both < 0.01). For formulations, the acute LOC for listed aquatic invertebrates is not exceeded for either terrestrial or aquatic uses (acute RQs are 0.03 and 0.01, respectively). Again, as stated previously, monitoring data (NAQWA) indicate surface concentrations ranging from 0.1 – 7.46 ppb. These concentrations are at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

Based on the weight of the evidence, the final effect determination is "no effect" for indirect effects, reduction in prey base for the aquatic-phase CRLF. This determination is based on the lack of LOC exceedances following either acute or chronic exposures for both glyphosate and formulations, the low monitoring data in surface water when compared to the modeled concentrations, the low probability of an individual effect and the low percentage effect to the freshwater invertebrate population prey base.

5.2.2.3 Fish and Aquatic-phase Frogs

As stated previously, for both glyphosate and formulations, none of the RQs exceed the acute and/or chronic LOCs for freshwater fish (surrogate for the CRLF) and aquatic-phase amphibians. Risks from chronic exposure to formulations (terrestrial uses with the conservative peak EEC and the LOAEC from a leopard frog study) were not considered to be of a concern. The probability of an individual effect at the RQ (<0.01) is <1 in 8.9E+18 (1 in 3.2E+04 to 1.0E+72) and percentage effect to the freshwater fish/aquatic-phase amphibian population prey base is 1 in 1.1E-19.

Based on the weight-of-evidence, the final effects determination is "no effect" for this endpoint (indirect effects: fish and frogs - reduction in prey base). This determination is based on the lack of LOC exceedances following either acute or chronic exposures for both glyphosate and formulations, the low monitoring data in surface water when compared to the modeled concentrations, the low probability of an individual effect and the low percentage effect to the freshwater fish and amphibian population prey base.

5.2.2.4 Terrestrial Invertebrates

Glyphosate

When the terrestrial-phase CRLF reaches juvenile and adult stages, its diet is mainly composed of terrestrial invertebrates. As stated in the risk estimation section, the LD_{50} value for terrestrial invertebrates is not a discrete value (>781.25 µg a.i./g of bee). Using the terrestrial EECs estimated from the model, T-REX, this is equivalent to a rate of >

5.79 lb a.e./A. It is noted that there is 27% mortality at this dose level. Therefore, the terrestrial model, T-REX was used to estimate upper bound RQs for terrestrial invertebrates.

As stated previously, the results show that the RQs could exceed the acute LOC for listed species at all application rates for small insects and at the higher application rates for large insects. In addition, the RQs could exceed the acute LOC for non-listed species at the higher application rates for small insects. At the highest upper bound RQ (<1.4 with forestry uses), the chance of an individual effect to small insects is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with rangeland), the chance of an individual effect to large insects is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base.

Formulations

On an acid equivalent basis, the formulations were tested at lower concentrations than the technical material, ranging from 0.096 lbs a.e./A to 3.86 lbs a.e./A and/or 30 to 100 µg a.e./bee (technical material was tested at 100 µg/bee or as stated above, 5.79 lbs a.e./A.). With the following exception, all of the LD_{50} or LC_{50} values for formulations exceeded the highest dose/concentration tested. There was one formulation which had a discrete 7-day LD_{50} value for 1 – 2 day old predatory mites (*Typhlodromus pyri*) of 1.1 lb a.e./A. This is 7 times less than the highest application rate for glyphosate products on an acid equivalent basis. It is for glyphosate monoammonium salt (MRID 45767105; MON78568). There is no specific registered product in the United States with this name. Therefore, it remains an uncertainty whether or not currently registered glyphosate products may affect terrestrial invertebrates.

Open Literature Data

Open literature data on glyphosate, its salts and/or formulations included a large number of efficacy studies which were not useful for a quantitative assessment of risk. Those studies which could possibly be used were either tested at lower concentrations than the submitted studies with no effects or insufficient information was provided on the test material to determine the concentration levels tested for either the active ingredient and/or the glyphosate acid equivalent.

Based on the weight-of-evidence, the final effects determination is LAA for indirect effects on terrestrial invertebrates as dietary food items. The effects determination is based on a potential exceedance of the LOC for listed terrestrial invertebrates at all application rates (small invertebrates), for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above (small invertebrates) and for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above (large invertebrates). The probability of an individual effect and the percentage population effects are expected to be high at the higher application rates. As with avian species, glyphosate is used in all 58 counties in California. Landscape maintenance and rights of way are among the highest usages in the counties which may have some currently CRLF occupied areas.

5.2.2.5 Mammals

Glyphosate

Life history data for terrestrial-phase CRLFs indicate that large adult frogs consume terrestrial vertebrates, including mice. For glyphosate, acute RQs were not calculated for mammals because there were no mortalities up to and including the highest doses tested. In addition, no sublethal effects were reported in any of the acute mammalian studies on the technical material. If comparisons are made between the terrestrial EECs estimated from T-REX and the highest dose tested in the acute study, the results show that all of the EEC values are lower, but at application rates of 3.75 lbs a.e./A and above, the EECs are greater than $1/10^{th}$ of the highest dose tested in the acute mammalian studies. None of the EECs are higher than 20% of the highest dose tested. For that reason, there is an uncertainty for listed mammalian species but the uncertainty is considerably less for non-listed mammalian species. For example, for forestry uses at 7.95 lb a.e./A, the highest use rate, the dose-based EEC for small mammals eating short grass is 1819.13 mg a.e./kg bw. The adjusted acute LD_{50} for a 15 g herbivore mammal is 10549.59. One thousand eight hundred nineteen divided by 10549.59 is greater than 0.1, the LOC for listed species but less than 0.5, the LOC for non-listed species. The probability of an individual effect at the LOC (0.1: no RQs) is <1 in 2.94E+05 (<1 in 8.86E+18 to <1 in 4.40E+01). The acute mammal studies provided no slope. Therefore, a default slope of 4.5 (confidence limits 2 - 9) was used to estimate the probability. The percentage effect to the mammalian prey base would be <1%.

The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for use rates of 3.84 lbs a.e./A and above. This includes many crops, forestry, areas with impervious surfaces and rights of way. The RQs range from 0.16 to 1.66.

Formulations

As stated in the risk estimation section, most of the available mammalian studies on formulations indicate LD_{50} values greater than the highest dose tested. For the five studies which have definitive acute toxicity values, the application rates from the specific labels for which these studies were submitted indicate an exceedance of the acute mammalian LOC for use rates of 2.5 lbs formulation/A for one label, 3.5 lbs formulation/A and above for two labels, 5 lbs formulation/A for the third label and 5.5 lbs formulation/A for the fourth label.

The probability of an individual effect for the formulation at the LOC would be the same as the probability for the technical material (see above). Since the use rates from the labels for these products were not individually modeled, the probability at the RQ was not estimated. For these formulations, at the LOC (i.e., the application rates below which listed species would not expected to be affected), the percentage effect to the mammalian prey base would be <0.01%.

Note: There is a reproduction/developmental screening study on POEA, the toxic surfactant. This has a lower endpoint than the reproduction study on glyphosate (NOAEL: 300 ppm ((14.9 - 16.6 mg/kg bw/day (M) and 18.9 - 19.5 mg/kg bw/day (F)) and LOAEL 1000 ppm (52.8 – 56.1 mg/kg bw/day (M) and 64.9 – 66.6 mg/kg bw/day (F) based on increased mean number of unaccounted-for sites, litter loss, decreased mean number of pups born live, litter size and postnatal survival from birth to PND 4. The effects are not reproducible in second generation. This may impact risk to mammals following chronic exposure to one of the formulations containing the POEA surfactant.

Open Literature Data

No additional mammalian studies were found in the open literature to further inform this risk assessment on a quantitative basis. Most of the studies were field studies to observe indirect effects to various small mammal populations in forests following terrestrial plant reduction from glyphosate applications. These studies would be supportive of indirect effects related to changes in the riparian habitat of the CRLF.

Based on the weight-of-evidence, the final effects determination is LAA for indirect effects, reductions in prey base based on the potential risk to mammals following chronic exposure. The uncertainties associated with acute exposure to glyphosate and its formulations are considered to be insignificant because the CRLF does not have an obligate relationship with mammals, none of the EECs for acute exposure to glyphosate are higher than 20% of the highest dose tested (at which there was no effect) and the percentage effect to the mammalian prey base and the probability of an individual effect for both glyphosate and its formulations are considered to be low. Again, glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.

5.2.2.6 Terrestrial-phase Amphibians

Terrestrial-phase adult CRLFs also consume frogs. RQ values representing direct exposures of glyphosate to terrestrial-phase CRLFs are used to represent exposures of glyphosate to frogs in terrestrial habitats. Acute RQs for avian species (surrogate to CRLF) were not calculated because there were no mortalities up to and including the highest dose/concentrations tested; however, there is an uncertainty in the potential risk. Although all of the terrestrial EEC values are lower, many are greater than $1/10^{th}$ of the highest dose/concentration tested in the acute avian studies. The chronic avian study showed no effects at the highest concentration tested; however, again, there is an uncertainty in the potential risk with uses with application rates of 7.5 lbs a.e./A and higher because the terrestrial EECs that are higher than the highest concentration tested in the chronic avian study. T-HERPS indicated that herps eating broadleaf plants, small insects and small herbivorous mammals on a dietary-basis may be at risk following chronic exposure at application rates of 7.5 lb/A and above.

Based on the weight-of-evidence, the final effects determination is likely to adversely affect (LAA) (see section 5.2.1.2 for supporting statements).

5.2.3 Indirect Effects (via Habitat Effects)

5.2.3.1 Aquatic Plants (Vascular and Non-vascular)

Glyphosate

Aquatic plants serve several important functions in aquatic ecosystems. Non-vascular aquatic plants are primary producers and provide the autochthonous energy base for aquatic ecosystems. Vascular plants provide structure as attachment sites and refugia for many aquatic invertebrates, fish, and juvenile organisms, such as fish and frogs. In addition, vascular plants also provide primary productivity and oxygen to the aquatic ecosystem. Rooted plants help reduce sediment loading and provide stability to nearshore areas and lower streambanks. In addition, vascular aquatic plants are important as attachment sites for egg masses of CRLFs.

Potential indirect effects to the CRLF based on impacts to habitat and/or primary production were assessed using RQs from freshwater aquatic vascular and non-vascular plant data. For aquatic plants, the LOC is not exceeded for glyphosate in acid equivalents with the highest peak EEC generated from the registered uses (direct application to water). Based on an RQ of 0.02 for both vascular and non-vascular plants, glyphosate is not expected to indirectly affect the aquatic-phase of the CRLF through the diet (tadpoles) or habitat from aquatic non-vascular plants.

Risk to Emergent Aquatic Vegetation - Risk from Spray Drift Adjacent to Habitat Area

Sections 5.1.2.3 and 5.2.3.2 describe the risk to the terrestrial plant community. Risks to emergent plants following spray drift may be assessed using the same parameters. Using the most sensitive EC_{25} values for both dicots and monocots, the RQs range from <0.1 – 5.37 with application rates ranging from 0.387 (aerial) to 7.95 (aerial) lbs ae/A. Based on the EC_{25} ranges (see **Section 5.2.3.2**), those monocots and dicots with EC_{25} values of 0.4 lb a.e./A or greater will not exceed the terrestrial plant LOC with the highest terrestrial EECs from forestry uses at 7.95 lbs. a.e./A. Since some of the EC_{25}'s for both monocots and dicots are greater than 0.4 lb a.e./A, it is possible that not all emergent aquatic plants will be affected following spray drift alone. Spray drift buffers are estimated in Section 5.2.2.4.

Formulations

For formulations, again, the LOC for aquatic plants is not exceeded with the highest peak EEC generated from terrestrial applications for both non-vascular and vascular plants (highest RQs are 0.24 and 0.02, respectively). Following aquatic applications, the LOC is exceeded for non-vascular plants (RQ = 4.7) but is not exceeded for non-vascular plants (RQ = 0.07).

Open Literature Data

Open literature data for aquatic non-vascular plants are described in section 5.2.2.1. For most of the studies on vascular plants, there are insufficient details in the articles to accurately determine concentration levels tested. For other studies, the endpoints were higher than those found in the submitted studies.

As stated previously, monitoring data are at least an order of magnitude lower than the modeled concentrations utilized in the risk estimations.

Based on the weight-of-evidence, the final effects determination is LAA for aquatic plants (indirect effects: habitat) (see Section 5.2.2.1. for supporting evidence).

5.2.3.2 Terrestrial Plants

Terrestrial plants serve several important habitat-related functions for the CRLF. In addition to providing habitat and cover for invertebrate and vertebrate prey items of the CRLF, terrestrial vegetation also provides shelter for the CRLF and cover from predators while foraging. Terrestrial plants also provide energy to the terrestrial ecosystem through primary production. Upland vegetation including grassland and woodlands provides cover during dispersal. Riparian vegetation helps to maintain the integrity of aquatic systems by providing bank and thermal stability, serving as a buffer to filter out sediment, nutrients, and contaminants before they reach the watershed, and serving as an energy source.

As stated in the risk estimation section, none of the RQs for terrestrial plants living in either dry or semi-aquatic areas exposed to the combined deposition estimates from runoff and spray drift exceed the terrestrial plant LOC. The RQ values for monocots and dicots inhabiting dry and semi-aquatic areas are derived by comparing the combined deposition estimates from runoff and spray drift to adverse effect levels measured in seedling emergence studies. For glyphosate, there were no effects in the seedling emergence studies. Therefore, it follows that RQs estimated from seedling emergence values would be low. For estimation of risk from spray drift alone, the exposure from spray drift is compared to the more sensitive measure of effect, either seedling emergence or vegetative vigor. The results of these calculations are RQ values for monocots and dicots inhabiting adjacent and semi-aquatic areas and exposed to drift only. For aerial uses at application rates of 3.75 lbs a.e./A and above and for ground uses with impervious surfaces at 7.95 lbs a.e./A the RQs from spray drift for both monocots and dicots exceed the terrestrial plant LOC of 1.

The seedling emergence EC_{25} values for monocots and dicots are all greater than 4 lbs a.e./A. The RQs with the terrestrial uses of glyphosate for monocots and dicots inhabiting dry and semi-aquatic areas (runoff and spray drift), utilizing the seedling emergence EC_{25} values of > 4 lbs a.e./A range from < 0.1 to < 0.87.

For spray drift only, the RQs range from <0.1 – 5.37 with application rates ranging from 0.387 to 7.95 lbs ae/A. These values were derived from the most sensitive EC_{25} value of 0.074 lb ae/A (dicots). The EC_{25} values range from 0.074 to 0.89 lbs a.e./A for dicots and from 0.16 – 0.98 lbs a.e./A for monocots from the vegetative vigor studies. Based on these ranges, those monocots and dicots with EC_{25} values of 0.4 lb a.e./A or greater will not exceed the terrestrial plant LOC with the highest terrestrial EECs from forestry uses at 7.95 lbs. a.e./A. From the most sensitive vegetative vigor study this would include cucumber and garden pea for dicots and purple nutsedge for monocots. These risk estimates are based on terrestrial plant toxicity data for a limited set of agricultural plants. Therefore, there are uncertainties associated with potential toxicity to the wide variety of non-agricultural plants inhabiting the CRLF habitat. Even if glyphosate only kills the most sensitive terrestrial plants, the habitat may still be sufficiently modified to the point such that it is no longer viable CRLF habitat.

The glyphosate labels state that it is a postemergent, systemic herbicide. It is generally non-selective and gives broad-spectrum control of many annual weeds, woody brush and trees. For tree, vine and shrub crops, the general precautions state that extreme care must be exercised to avoid contact of herbicide solution, spray, drift or mist with foliage or green bark of trunk, branches, suckers, fruit or other parts of trees, canes and vines. Therefore, it is expected that glyphosate applications can affect both herbaceous and woody vegetation, especially when the exposure is via drift. This is supported by the incident data. For glyphosate, 63 incidents were reported for mostly plant damage to a wide variety of plants from either direct treatment or spray drift. For the isopropylamine salt of glyphosate, 443 incident reports were filed for a wide variety of terrestrial plants, particularly agricultural crops and grass. There were a few incidents of trees being damaged or killed. Plant damage and mortality were the main issues with drift as the main exposure route. Studies in the open literature were mainly efficacy studies or studies on fungi and were not useful as support for this risk assessment.

Based on the weight-of-evidence, the final effect determination is “LAA” for indirect effects: reduction in terrestrial plant community - riparian and upland habitat. This determination is based on LOC exceedances for terrestrial plants (both monocots and dicots) following spray drift at aerial application rates of 3.85 lbs a.e./A and above and at a ground application rates of 7.5 lbs a.e./A and above. Because the RQs for terrestrial plants are relatively low, sufficient buffers may mitigate the concern for the terrestrial habitat associated with the CRLF and reduce the determination to NLAA. Again, glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.

Spray Drift Buffer Analysis

As stated previously, the entire state of California is considered to be both the initial area of concern and the action area. Therefore, spray drift buffers can be estimated for a specific use; however, for aggregate uses, the widest buffer for both terrestrial and aquatic uses would be applied and would effectively be the entire state.

For a specific use, in order to determine terrestrial and aquatic habitats of concern due to glyphosate exposures through spray drift, it is necessary to estimate the distance that spray applications can drift from the treated area and still be present at concentrations that exceed levels of concern. The quantitative estimations of risk indicate that terrestrial plants generate the highest RQ risk values. Therefore, the spray drift analysis was conducted with the most sensitive endpoint for terrestrial plants. Using the most sensitive terrestrial plant endpoint with the AgDrift model in the Tier I aerial mode with the default droplet size distribution ASAE very fine to fine, the spray drift buffers for use rates of 7.5 lbs a.e./A and above exceed the 1,000 foot range. Therefore, the AgDrift Tier 3 model for aerial applications was used with a maximum downwind distance of 3000. This distance goes slightly beyond the maximum limit of the model and is thus an uncertainty.

In order to characterize the portion of the action area for a specific use that is relevant to the CRLF and specific to the area where the effects determination (*e.g.*, NLAA versus LAA) could be made, an analysis was conducted using the most sensitive non-listed plant EC_{25} of 0.074 lbs ai/acre. Typically the NOAEC is used when there is an obligate relationship between the species being assessed and listed plants (or other taxa). However, there is no obligate relationship between the CRLF and any listed plant; therefore, the LAA/NLAA determination would be based on the area defined by the non-listed species LOC (*e.g.*, EEC/EC_{25}).

For glyphosate uses, the maximum estimated distance is 2785 feet for aerial application on forestry at 7.95 lbs a.e./A. All of the estimations are based on a default droplet size distribution ASAE of very fine to fine. The next largest buffer is 2631 feet for forestry and rights of way aerial application at 7.5 lbs a.e./A. The remainder of the uses have reduced buffer distances for lower application rates. A summary of the modeled distances by application rate is presented in **Table 5.14.**

Table 5.14. Predicted Terrestrial Spray Drift Dissipation Distances for Glyphosate From AgDrift

Application Rate (lbs. a.e./A)/ Method	Uses Represented	Buffer Distance for Non-listed Plants Distance (ft)[1]
3.75 Ground	Alfafa, avocado, corn, cotton, forestry, garlic, impervious, residential, row crop, strawberry, wheat	125[2]
3.84 Ground	Almond, fruit, grape, olive	125[2]
3.85 Ground	Citrus	125[2]
3.85 Aerial	Cole crop, lettuce, melon, onion, potato, wine grape	1768[2]
0.75 Aerial	Corn	312[2]
7.5 Aerial	Forestry	2631[2]
7.95 Aerial	Forestry	2785[2,3]
7.95 Ground	Impervious	259

Table 5.14. Predicted Terrestrial Spray Drift Dissipation Distances for Glyphosate From AgDrift		
Application Rate (lbs. a.e./A)/ Method	**Uses Represented**	**Buffer Distance for Non-listed Plants Distance (ft)[1]**
3.75 Aerial	Nursery, rangeland, sugar beet, tomato, turf	1768[2]
1.54 Ground	Rangeland	53[2]
0.387 Aerial	Rangeland	135[2]
7.5 Aerial	Rights of way	2631[2]
3.69 Ground	Rights of way	125[2]
0.75 Ground	Wheat	25[2]

[1] The EC_{25} value is used to define the buffer associated with the relevant portion of the action area.
[2] AgDrift with droplet size distribution ASAE very fine to fine with high boom for ground applications and Tier 3 for aerial with maximum downwind distance of 3000 if needed (this is an uncertainty)
[3] Some of the forestry labels state: "do not use nozzles or nozzle configurations that dispense spray as fine spray droplets". In those cases, using the AgDrift Aerial Tier 3 model with a fine to medium droplet size would provide a reduced buffer distance of 1122 feet.

Open Literature

The open literature studies do not generally have endpoints that can be compared to those in the submitted studies. The studies are mostly on fungus and/or are efficacy studies.

5.2.4 Modification to Designated Critical Habitat

5.2.4.1 Aquatic-Phase PCEs

Three of the four assessment endpoints for the aquatic-phase primary constituent elements (PCEs) of designated critical habitat for the CRLF are related to potential effects to aquatic and/or terrestrial plants:

- Alteration of channel/pond morphology or geometry and/or increase in sediment deposition within the stream channel or pond: aquatic habitat (including riparian vegetation) provides for shelter, foraging, predator avoidance, and aquatic dispersal for juvenile and adult CRLFs.
- Alteration in water chemistry/quality including temperature, turbidity, and oxygen content necessary for normal growth and viability of juvenile and adult CRLFs and their food source.
- Reduction and/or modification of aquatic-based food sources for pre-metamorphs (*e.g.*, algae).

The effect determinations for indirect effects to the CRLF via direct effects to aquatic and terrestrial plants are used to determine whether modification to critical habitat may occur.

For aquatic plants, the aquatic plant LOC is not exceeded for glyphosate a.e. for both vascular and non-vascular plants; however, for formulations, the LOC is exceeded for non-vascular plants (aquatic applications). The LOC is not exceeded for either terrestrial applications (both vascular and non-vascular plants) or for aquatic applications (vascular plants).

For terrestrial plants, the terrestrial plant LOC for both monocots and dicots is exceeded following spray drift from aerial uses at application rates of 3.75 lbs a.e./A and above and from ground uses at a rate of 7.5 lbs a.e./A and above. Risks to emergent aquatic plants following spray drift were also assessed using the same model as the terrestrial plant community.

The effect determinations for both aquatic and terrestrial plants are "LAA" based on LOC exceedances for non-vascular aquatic plants following application of a formulation directly to water and for aquatic emergent plants and terrestrial plants following aerial application at rates of 3.75 lbs a.e./A and above and following ground applications at rates of 7.5 lbs a.e./A and above. As stated previously, glyphosate is used in every county in the state of California.

The remaining aquatic-phase PCE is "alteration of other chemical characteristics necessary for normal growth and viability of CRLFs and their food source." Other than impacts to algae as food items for tadpoles (discussed above), this PCE is assessed by considering direct and indirect effects to the aquatic-phase CRLF via acute and chronic freshwater fish and invertebrate toxicity endpoints as measures of effects.

For glyphosate, the acute and chronic RQs for direct effects to the aquatic-phase CRLF do not exceed either the acute or chronic LOC for freshwater fish and amphibians. Acute RQs from formulated products, both terrestrial and aquatic uses also do not exceed the acute LOC for freshwater fish and amphibians. The final effect determination is "no effect" for direct effects to the aquatic-phase CRLF.

For freshwater invertebrates, none of the acute or chronic RQs exceed either the acute or chronic aquatic LOC for either glyphosate a.e. or for formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low and the monitoring data are considerably lower than the modeled concentrations utilized in the risk assessment. Based on the weight-of-evidence, the final effect determination is "no effect" for aquatic invertebrates.

For freshwater fish as food items, as stated above, the final effect determination is "no effect".

5.2.4.2 Terrestrial-Phase PCEs

Two of the four assessment endpoints for the terrestrial-phase PCEs of designated critical habitat for the CRLF are related to potential effects to terrestrial plants:

- Elimination and/or disturbance of upland habitat; ability of habitat to support food source of CRLFs: Upland areas within 200 ft of the edge of the riparian vegetation or drip line surrounding aquatic and riparian habitat that are comprised of grasslands, woodlands, and/or wetland/riparian plant species that provides the CRLF shelter, forage, and predator avoidance.

- Elimination and/or disturbance of dispersal habitat: Upland or riparian dispersal habitat within designated units and between occupied locations within 0.7 mi of each other that allow for movement between sites including both natural and altered sites which do not contain barriers to dispersal.

For terrestrial plants, the risk from spray drift from aerial uses at application rates of 3.75 lbs a.e./A and above and ground uses at rates of 7.5 lbs a.e./A and above exceed the LOC of 1 for both monocots and dicots. The final effect determination for terrestrial plants is "LAA".

The third terrestrial-phase PCE is "reduction and/or modification of food sources for terrestrial phase juveniles and adults." To assess the impact of glyphosate on this PCE, acute and chronic toxicity endpoints for terrestrial invertebrates, mammals, and terrestrial-phase frogs are used as measures of effects.

For mammals, based on the weight-of-evidence, the final effects determination is LAA for indirect effects, reductions in prey base based on the potential risk following chronic exposure. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for use scenarios at application rates of 3.84 lbs a.e./A and above. This includes many crops, forestry, areas with impervious surfaces and rights of way. The RQs range from 0.16 to 1.66.

For terrestrial-phase amphibians, based on the weight-of-evidence, the final effect determination is LAA. This is based on the following statements. The concentration levels tested in the chronic avian studies were sufficiently low that at application rates of 7.5 lbs a.e./A and above, the terrestrial dietary EECs are greater than the highest concentration tested in the avian reproduction studies. This creates an uncertainty for direct effects following chronic exposure. This is supported by an open literature study on the effects of the formulation, Roundup on the epididymal region of drakes indicates that there may be some potential effects on the morphophysiology of the male duck reproductive system at dose levels as low as 5 mg/kg bw. The acute avian dose-based LOC is exceeded for one formulation at all application rates (1.1 to 5.5 lb formulation/A) listed on the label. When modeled using the T-HERPS model, the potential risk remains. The incident data, although categorized as possible, indicates that if the acute exposure is sufficiently high, there may be some avian (and thus, CRLF) mortality following acute exposure through drift.

For terrestrial invertebrates, based on the weight-of-evidence, the final effects determination is LAA. The effects determination is based on a potential exceedance of the LOC for listed terrestrial invertebrates at all application rates (small invertebrates),

for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A (small invertebrates) and for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above (large invertebrates). The probability of an individual effect and the percentage population effects are expected to be high at the higher application rates.

The fourth terrestrial-phase PCE is based on alteration of chemical characteristics necessary for normal growth and viability of juvenile and adult CRLFs and their food source.

For direct effects, as stated in the amphibian paragraph provided above, based on the weight-of-evidence, the final effect determination is "LAA" based on the uncertainty associated with chronic exposure and supporting evidence from an open literature study, exceedances of the acute avian LOC for a formulation with a discreet LD_{50} value and supporting incident data that there may be some avian (and thus, CRLF) mortality following acute exposure through drift.

For indirect effects, the final effect determination is "LAA" for mammals (potential risk following chronic exposure), terrestrial invertebrates (potential exceedance of the LOC for listed small invertebrates at all application rates, for non-listed small invertebrates at the higher application rates and for listed large invertebrates at the higher application rates. For amphibians, again as stated above, the final effect determination is "LAA".

6. Uncertainties

6.1 Exposure Assessment Uncertainties

6.1.1 Environmental Fate Data

Factors controlling the persistence, transformation, and transport of pesticides depend on the characteristics of the soil and microbial population. Studies that satisfy Agency environmental fate data requirements are conducted in limited systems (soil; water-sediment) and may not represent all of the potential use environments. Glyphosate is widely used in the United States and for multiple uses at the same site. The behavior of glyphosate based on data in a limited number of test systems extrapolated to multiple sites may overestimate or underestimate the exposure to glyphosate in specific sites and season. Environmental fate data used in estimating exposure concentrations do not specifically take into account the pH dependent dissociation and speciation of glyphosate in aquatic systems.

There are no environmental fate data available to describe the behavior of end use products in which glyphosate is formulated with a surfactant, leading to some uncertainty in the estimated exposures for these products.

6.1.2 Maximum Use Scenario

The screening-level risk assessment focuses on characterizing potential ecological risks resulting from a maximum use scenario, which is determined from labeled statements of maximum application rate and number of applications with the shortest time interval between applications. The frequency at which actual uses approach this maximum use scenario may be dependent on pest resistance, timing of applications, cultural practices, and market forces.

6.1.3 Aquatic Exposure Modeling of Glyphosate

The standard ecological water body scenario (EXAMS pond) used to calculate potential aquatic exposure to pesticides is intended to represent conservative estimates, and to avoid underestimations of the actual exposure. The standard scenario consists of application to a 10-hectare field bordering a 1-hectare, 2-meter deep (20,000 m^3) pond with no outlet. Exposure estimates generated using the EXAMS pond are intended to represent a wide variety of vulnerable water bodies that occur at the top of watersheds including prairie pot holes, playa lakes, wetlands, vernal pools, man-made and natural ponds, and intermittent and lower order streams. As a group, there are factors that make these water bodies more or less vulnerable than the EXAMS pond. Static water bodies that have larger ratios of pesticide-treated drainage area to water body volume would be expected to have higher peak EECs than the EXAMS pond. These water bodies will be either smaller in size or have larger drainage areas. Smaller water bodies have limited storage capacity and thus may overflow and carry pesticide in the discharge, whereas the EXAMS pond has no discharge. As watershed size increases beyond 10-hectares, it becomes increasingly unlikely that the entire watershed is planted with a single crop that is all treated simultaneously with the pesticide. Headwater streams can also have peak concentrations higher than the EXAMS pond, but they likely persist for only short periods of time and are then carried and dissipated downstream.

The Agency acknowledges that there are some unique aquatic habitats that are not accurately captured by this modeling scenario and modeling results may, therefore, under- or over-estimate exposure, depending on a number of variables. For example, aquatic-phase CRLFs may inhabit water bodies of different size and depth and/or are located adjacent to larger or smaller drainage areas than the EXAMS pond. The Agency does not currently have sufficient information regarding the hydrology of these aquatic habitats to develop a specific alternate scenario for the CRLF. CRLFs prefer habitat with perennial (present year-round) or near-perennial water and do not frequently inhabit vernal (temporary) pools because conditions in these habitats are generally not suitable (Hayes and Jennings 1988). Therefore, the EXAMS pond is assumed to be representative of exposure to aquatic-phase CRLFs. In addition, the Services agree that the existing EXAMS pond represents the best currently available approach for estimating aquatic exposure to pesticides (USFWS/NMFS 2004).

Aquatic exposure to glyphosate was assessed using a Tier I approach, which is designed as a coarse screen and estimates conservative pesticide concentrations in surface water

from a few basic chemical parameters and pesticide label use and application information. Tier 1 is used to screen chemicals to determine which ones potentially pose sufficient risk to warrant higher level modeling. Most aquatic EECs were generated using simple dilution calculations based on the mass of pesticide and the volume of the water body. These calculations do not account for any dissipation or degradation processes and so are likely to overestimate exposure. Exposure to glyphosate from terrestrial applications was estimated using the Tier I model GENEEC2.

For terrestrial uses, assumptions made about transport of the pesticide to the water body lead to some uncertainty in these estimates. Both the simple dilution calculation and the GENEEC2 modeling are based on the default assumption that 5% of applied pesticide is transported to the water body through spray drift. For formulations, quantitative exposure modeling for formulations is limited based on the expectation that the varying physical-chemical properties of individual components of pesticide formulations will result in progressively different formulation constituents in environmental media over time. As the proportions of formulation components in environmental media differ from the proportions in the tested formulation, the assumption that environmental residues are toxicologically equivalent to tested formulations cannot be supported beyond the time period immediately following product application. For this reason, spray drift of formulation directly to the water body is the only transport route considered for estimating formulation EECs.

To account for uncertainties associated with modeling, available monitoring data were compared to calculated estimates of peak EECs for the different uses. As discussed above, the NAWQA database includes data for glyphosate concentrations measured in surface waters at 3 sites in California, one receiving runoff from agricultural areas and two from mixed use areas. The specific use patterns (e.g. application rates and timing, crops) associated with the use areas are unknown, however, they are assumed to be representative of potential glyphosate terrestrial use areas. Glyphosate was detected most frequently at the agricultural site, where the highest measured concentration was 7.5 ug/L, an order of magnitude lower than the peak EEC for terrestrial applications of glyphosate estimated using Tier I modeling. Monitoring is not expected to capture peak concentrations due to limited sampling frequency. Monitoring only considers individual compounds, the active ingredient and its metabolites, and does not reflect exposure to formulated products. Additionally, there are no monitoring data available for direct aquatic applications of glyphosate, which are likely to have higher exposures than terrestrial applications.

6.1.4 Potential Groundwater Contributions to Surface Water Chemical Concentrations

Although the potential impact of discharging groundwater on CRLF populations is not explicitly delineated, it should be noted that groundwater could provide a source of pesticide to surface water bodies – especially low-order streams, headwaters, and groundwater-fed pools. This is particularly likely if the chemical is persistent and mobile. Soluble chemicals that are primarily subject to photolytic degradation will be

very likely to persist in groundwater, and can be transportable over long distances. Similarly, many chemicals degrade slowly under anaerobic conditions (common in aquifers) and are thus more persistent in groundwater. Much of this groundwater will eventually be discharged to the surface – often supporting stream flow in the absence of rainfall. Continuously flowing low-order streams in particular are sustained by groundwater discharge, which can constitute 100% of stream flow during baseflow (no runoff) conditions. Thus, it is important to keep in mind that pesticides in groundwater may have a major (detrimental) impact on surface water quality, and on CRLF habitats.

SciGrow may be used to determine likely 'high-end' groundwater vulnerability, with the assumption (based upon persistence in sub- and anoxic conditions, and mobility) that much of the compound entering the groundwater will be transported some distance and eventually discharged into surface water. Although concentrations in a receiving water body resulting from groundwater discharge cannot be explicitly quantified, it should be assumed that significant attenuation and retardation of the chemical will have occurred prior to discharge. Nevertheless, groundwater could still be a significant consistent source of chronic background concentrations in surface water, and may also add to surface runoff during storm events (as a result of enhanced groundwater discharge typically characterized by the 'tailing limb' of a storm hydrograph).

6.1.5 Usage Uncertainties

County-level usage data were obtained from California's Department of Pesticide Regulation Pesticide Use Reporting (CDPR PUR) database. Four years of data (2002 – 2005) were included in this analysis because statistical methodology for identifying outliers, in terms of area treated and pounds applied, was provided by CDPR for these years only. No methodology for removing outliers was provided by CDPR for 2001 and earlier pesticide data; therefore, this information was not included in the analysis because it may misrepresent actual usage patterns. CDPR PUR documentation indicates that errors in the data may include the following: a misplaced decimal; incorrect measures, area treated, or units; and reports of diluted pesticide concentrations. In addition, it is possible that the data may contain reports for pesticide uses that have been cancelled. The CPDR PUR data does not include home owner applied pesticides; therefore, residential uses are not likely to be reported. As with all pesticide usage data, there may be instances of misuse and misreporting. The Agency made use of the most current, verifiable information; in cases where there were discrepancies, the most conservative information was used.

6.1.6 Terrestrial Exposure Modeling of Glyphosate

The Agency relies on the work of Fletcher et al. (1994) for setting the assumed pesticide residues in wildlife dietary items. These residue assumptions are believed to reflect a realistic upper-bound residue estimate, although the degree to which this assumption reflects a specific percentile estimate is difficult to quantify. It is important to note that the field measurement efforts used to develop the Fletcher estimates of exposure involve highly varied sampling techniques. It is entirely possible that much of these data reflect

residues averaged over entire above ground plants in the case of grass and forage sampling.

It was assumed that ingestion of food items in the field occurs at rates commensurate with those in the laboratory. Although the screening assessment process adjusts dry-weight estimates of food intake to reflect the increased mass in fresh-weight wildlife food intake estimates, it does not allow for gross energy differences. Direct comparison of a laboratory dietary concentration- based effects threshold to a fresh-weight pesticide residue estimate would result in an underestimation of field exposure by food consumption by a factor of 1.25 – 2.5 for most food items.

Differences in assimilative efficiency between laboratory and wild diets suggest that current screening assessment methods do not account for a potentially important aspect of food requirements. Depending upon species and dietary matrix, bird assimilation of wild diet energy ranges from 23 – 80%, and mammal's assimilation ranges from 41 – 85% (U.S. Environmental Protection Agency, 1993). If it is assumed that laboratory chow is formulated to maximize assimilative efficiency (e.g., a value of 85%), a potential for underestimation of exposure may exist by assuming that consumption of food in the wild is comparable with consumption during laboratory testing. In the screening process, exposure may be underestimated because metabolic rates are not related to food consumption.

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

6.1.7 Spray Drift Modeling

Although there may be multiple glyphosate applications at a single site, it is unlikely that the same organism would be exposed to the maximum amount of spray drift from every application made. In order for an organism to receive the maximum concentration of glyphosate from multiple applications, each application of glyphosate would have to occur under identical atmospheric conditions (*e.g.*, same wind speed and – for plants – same wind direction) and (if it is an animal) the animal being exposed would have to be present directly downwind at the same distance after each application. Although there may be sites where the dominant wind direction is fairly consistent (at least during the relatively quiescent conditions that are most favorable for aerial spray applications), it is nevertheless highly unlikely that plants in any specific area would receive the maximum amount of spray drift repeatedly. It appears that in most areas (based upon available meteorological data) wind direction is temporally very changeable, even within the same day. Additionally, other factors, including variations in topography, cover, and meteorological conditions over the transport distance are not accounted for by the

AgDRIFT/AGDISP model (*i.e.*, it models spray drift from aerial and ground applications in a flat area with little to no ground cover and a steady, constant wind speed and direction). Therefore, in most cases, the drift estimates from AgDRIFT/AGDISP may overestimate exposure even from single applications, especially as the distance increases from the site of application, since the model does not account for potential obstructions (*e.g.*, large hills, berms, buildings, trees, *etc.*). Furthermore, conservative assumptions are made regarding the droplet size distributions being modeled ('ASAE Very Fine to Fine'), the application method (*e*.g., aerial), release heights and wind speeds. Alterations in any of these inputs would change the area of potential effect.

6.2 Effects Assessment Uncertainties

6.2.1 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. The acute toxicity data for fish are collected on juvenile fish between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes (e.g., first instar for daphnids, second instar for amphipods, stoneflies, mayflies, and third instar for midges).

Testing of juveniles may overestimate toxicity at older age classes for pesticide active ingredients that act directly without metabolic transformation because younger age classes may not have the enzymatic systems associated with detoxifying xenobiotics. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, this assessment uses the most sensitive life-stage information as measures of effect for surrogate aquatic animals, and is therefore, considered as protective of the CRLF.

6.2.2 Use of Surrogate Species Effects Data

Limited toxicity tests and open literature data on glyphosate are available for frogs or any other aquatic-phase amphibian; therefore, freshwater fish are used as surrogate species for aquatic-phase amphibians. Although limited data are available for glyphosate, the available open literature information on glyphosate toxicity to aquatic-phase amphibians shows that acute and chronic ecotoxicity endpoints for aquatic-phase amphibians are generally less sensitive than freshwater fish. Therefore, endpoints based on freshwater fish ecotoxicity data are assumed to be protective of potential direct effects to aquatic-phase amphibians including the CRLF, and extrapolation of the risk conclusions from the most sensitive tested species to the aquatic-phase CRLF is likely to overestimate the potential risks to those species. Efforts are made to select the organisms most likely to be affected by the type of compound and usage pattern; however, there is an inherent uncertainty in extrapolating across phyla. In addition, the Agency's LOCs are intentionally set very low, and conservative estimates are made in the screening level risk assessment to account for these uncertainties.

6.2.3 Sublethal Effects

When assessing acute risk, the screening risk assessment relies on the acute mortality endpoint as well as a suite of sublethal responses to the pesticide, as determined by the testing of species response to chronic exposure conditions and subsequent chronic risk assessment. Consideration of additional sublethal data in the effects determination t is exercised on a case-by-case basis and only after careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishing a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints. However, the full suite of sublethal effects from valid open literature studies is considered for the purposes of defining the action area.

Sublethal effects from exposure to glyphosate are presented throughout the Ecological Effects Section (Section 4.0). To the extent to which sublethal effects are not considered in this assessment, the potential direct and indirect effects of glyphosate on CRLF may be underestimated.

6.2.4 Location of Wildlife Species

For the terrestrial exposure analysis of this risk assessment, a generic bird or mammal was assumed to occupy either the treated field or adjacent areas receiving a treatment rate on the field. Actual habitat requirements of any particular terrestrial species were not considered, and it was assumed that species occupy, exclusively and permanently, the modeled treatment area. Spray drift model predictions suggest that this assumption leads to an overestimation of exposure to species that do not occupy the treated field exclusively and permanently.

6.2.5 Assessment of Risk to Terrestrial Species

Many of the ecological effects studies on terrestrial species did not show any effects at the highest dose/concentration tested. This included the acute toxicity studies on birds, mammals and invertebrates as well as the chronic avian study. For the acute toxicity studies, the dose/concentration levels were relatively high. With the exception of one scenario with terrestrial invertebrates, the terrestrial EECs were all lower than the highest dose/concentration tested in the acute studies; however, they are sufficiently high that there is an uncertainty in the acute risk to these taxonomic groups. The terrestrial EEC for chronic exposure is higher than the highest concentration tested in the chronic avian study. Due to the uncertainty of risk to avian species at concentration levels higher than those tested in the chronic study, this was considered to be a potential risk to this taxonomic group. These uncertainties may lead to an overestimation of risk to these taxomonic groups.

7. Risk Conclusions

In fulfilling its obligations under Section 7(a)(2) of the Endangered Species Act, the information presented in this endangered species risk assessment represents the best data

currently available to assess the potential risks of glyphosate to the CRLF and its designated critical habitat.

Based on the best available information, the Agency makes a Likely to Adversely Affect determination for the CRLF from the use of glyphosate. Additionally, the Agency has determined that there is the potential for modification of CRLF designated critical habitat from the use of the chemical.

This assessment indicates that direct effects to the terrestrial-phase CRLF eating broadleaf plants, small insects and small herbivorous mammals on a dietary-basis may be at risk following chronic exposure to glyphosate at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). In addition, for one particular formulation (Registration No. 524-424), medium and large-sized CRLF's eating small herbivorous mammals on a dose-basis may be at risk following acute exposure at an application rate of 5.5 lb formulation/A (industrial outdoor uses). At the lowest application rate of 1.1 lb formulation/A, there is potential risk to medium-sized CRLF's eating small herbivorous mammals on a dose-basis (ornamental lawns and turf).

Indirect effects to both the aquatic- and terrestrial-phase CRLF, based on reduction in prey base may occur with the following taxonomic groups: aquatic nonvascular plants with products specifically labelled for aquatic use; small insects with any use and large insects at application rates of 7.5 lb a.e./A and above; terrestrial phase amphibians following chronic exposure at application rates of 7.5 lb a.e./A and above; terrestrial phase amphibians following acute exposure to one particular formulation (Registration No. 524-424), at application rates of 1.1 lbs formulation/A and above (ornamental lawns and turf and industrial outdoor uses) and mammals following chronic exposure at application rates of 3.84 lbs a.e./A and above (i.e., many crops, forestry, rights of way and areas with impervious surfaces).

Indirect effects to both the aquatic- and terrestrial-phase CRLF, based on habitat effects may occur with aquatic non-vascular plants with products specifically labelled for aquatic use and with aquatic emergent plants and terrestrial plants (both monocots and dicots) following spray drift with aerial application at rates of 3.75 lbs/A and above (most crops, forestry, rangeland, residential, rights of way and turf) and with ground applications on areas with impervious surfaces at a rate of 7.95 lbs/A.

Buffers were estimated for specific uses associated with the risk to terrestrial plants. As stated previously, because the initial footprint and the action area encompass the entire state of California, for aggregate uses, the widest buffer for both terrestrial and aquatic uses would be applied and would effectively be the entire state. For similar reasons, the downstream analysis was not conducted. There is potentially no input of "glyphosate-clean" water to dilute existing concentrations of glyphosate downstream because it could be applied in the downstream waterbodies as well. In addition, no reference maps have been generated because the glyphosate uses overlap all of the frog habitat.

A summary of the risk conclusions and effects determinations for the CRLF and its critical habitat, given the uncertainties discussed in Section 6, is presented in Tables 7.1 and 7.2. Given the LAA determination for the CRLF and potential modification of designated critical habitat, a description of the baseline status and cumulative effects for the CRLF is provided in **Attachment 2**.

Table 7.1 Effects Determination Summary for Glyphosate Use and the CRLF

Assessment Endpoint	Effects Determination [1]	Basis for Determination
Survival, growth, and/or reproduction of CRLF individuals	LAA[1]	**Potential for Direct Effects**
		Aquatic-phase (Eggs, Larvae, and Adults): The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		Terrestrial-phase (Juveniles and Adults): The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. Initial area of concern and action area are the entire state of California. Glyphosate is used in all 58 counties in California with landscape maintenance and rights of way among the highest usages in the counties which may have some currently CRLF occupied areas.
		Potential for Indirect Effects
		Aquatic prey items, aquatic habitat, cover and/or primary productivity The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low, and the monitoring data are considerably lower than the modeled concentrations utilized in the risk assessment**.** For non-vascular plants, the LOC for aquatic plants is exceeded for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOC for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations.
		Terrestrial prey items, riparian habitat For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound

Table 7.1 Effects Determination Summary for Glyphosate Use and the CRLF		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
		RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way). The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. Initial area of concern and action area are the entire state of California.

[1] No effect (NE); May affect, but not likely to adversely affect (NLAA); May affect, likely to adversely affect (LAA)

Table 7.2 Effects Determination Summary for Glyphosate Use and CRLF Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
Modification of aquatic-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. For non-vascular plants, the LOC for aquatic plants is exceeded, only for formulations specified for aquatic uses. For vascular plants, the LOC for aquatic plants is not exceeded for either glyphosate, its salts or its formulations; however, for aquatic emergent plants, the terrestrial plant LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The acute and chronic LOCs for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations. The acute and chronic LOCs for freshwater invertebrates are not exceeded for glyphosate, its salts or formulations. In addition, the probit analysis indicates that the probability of an individual effect and the percentage effect to the freshwater invertebrate population prey base would be very low.

Table 7.2 Effects Determination Summary for Glyphosate Use and CRLF Critical Habitat Impact Analysis		
Assessment Endpoint	**Effects Determination [1]**	**Basis for Determination**
Modification of terrestrial-phase PCE	Habitat modification[1]	For terrestrial plants, the LOC is exceeded following spray drift with aerial applications at rates of 3.75 lbs/A and above and with ground applications at a rate of 7.95 lbs/A. The chronic LOC for avian species (surrogate for CRLF) is exceeded at application rates of 7.5 lb a.e./A and above (forestry, areas with impervious surfaces and rights of way). The acute LOC for one particular formulation is exceeded for medium and large- and for medium-sized CRLF's eating small herbivorous mammals on a dose-basis at application rates of 5.5 (highest rate: industrial outdoor uses) and 1.1 (lowest rate: ornamental lawns and turf) lb formulation/A, respectively. For the formulation, the probability of an individual effect at the RQs for the highest and lowest application rates are 1 in 9.32 and 1 in 1.25E+05, respectively. For terrestrial invertebrates, the upper bound RQs for small insects exceed the LOC for listed terrestrial invertebrates for all uses and for non-listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. The upper bound RQs for large insects exceed the LOC for listed terrestrial invertebrates at application rates of 7.5 lbs a.e./A and above. At the highest upper bound RQ (<1.4 at 7.95 lbs a.e./A with uses on forestry and areas with impervious surfaces), the chance of an individual effect is <1 in 1.34 with a <75% percentage effect to the terrestrial invertebrate prey base. At the lowest upper bound RQ (<0.01 with 0.387 lbs a.e./A on rangeland), the chance of an individual effect is <8.86E+18 with a <1.13E-17 percentage effect to the terrestrial invertebrate prey base. The chronic RQs exceed the chronic LOC for small mammals on a dose-basis for application rates of 3.84 lbs/A and above (i.e., most crops, forestry, areas with impervious surfaces and rights of way).

[1] Habitat Modification or No effect (NE)

Based on the conclusions of this assessment, a formal consultation with the U. S. Fish and Wildlife Service under Section 7 of the Endangered Species Act should be initiated to determine whether there are reasonable and prudent alternatives and/or measures to reduce and/or eliminate potential incidental take.

When evaluating the significance of this risk assessment's direct/indirect and adverse habitat modification effects determinations, it is important to note that pesticide exposures and predicted risks to the species and its resources (i.e., food and habitat) are not expected to be uniform across the action area. In fact, given the assumptions of drift and downstream transport (i.e., attenuation with distance), pesticide exposure and associated risks to the species and its resources are expected to decrease with increasing distance away from the treated field or site of application. Evaluation of the implication of this non-uniform distribution of risk to the species would require information and assessment techniques that are not currently available. Examples of such information and methodology required for this type of analysis would include the following:

- Enhanced information on the density and distribution of CRLF life stages within specific recovery units and/or designated critical habitat within the action area. This information would allow for quantitative extrapolation of the present risk assessment's predictions of individual effects to the proportion of the population extant within geographical areas where those effects are predicted. Furthermore, such population information would allow for a more comprehensive evaluation of the significance of potential resource impairment to individuals of the species.
- Quantitative information on prey base requirements for individual aquatic- and terrestrial-phase frogs. While existing information provides a preliminary picture of the types of food sources utilized by the frog, it does not establish minimal requirements to sustain healthy individuals at varying life stages. Such information could be used to establish biologically relevant thresholds of effects on the prey base, and ultimately establish geographical limits to those effects. This information could be used together with the density data discussed above to characterize the likelihood of adverse effects to individuals.
- Information on population responses of prey base organisms to the pesticide. Currently, methodologies are limited to predicting exposures and likely levels of direct mortality, growth or reproductive impairment immediately following exposure to the pesticide. The degree to which repeated exposure events and the inherent demographic characteristics of the prey population play into the extent to which prey resources may recover is not predictable. An enhanced understanding of long-term prey responses to pesticide exposure would allow for a more refined determination of the magnitude and duration of resource impairment, and together with the information described above, a more complete prediction of effects to individual frogs and potential modification to critical habitat.

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California Environmental Protection Agency
Office of Environmental Health Hazard Assessment (OEHHA)

Glyphosate: Public Health Goals for Chemicals in Drinking Water

June 2007

PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

GLYPHOSATE

June 2007

Governor of the State of California
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Public Health Goal for
GLYPHOSATE
in Drinking Water

Prepared by

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June 2007

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PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DHS shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DHS must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR GLYPHOSATE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the scientific literature on glyphosate and evaluated risk assessment methods that have been developed since the publication of the original Public Health Goal (PHG) for glyphosate in 1997. The Office has reduced the PHG for glyphosate in drinking water from 1,000 to 900 parts per billion (ppb), based on an updated exposure calculation for adult females, on whom the PHG value is based.

OEHHA chose a developmental study in rabbits as the key study in the development of the updated PHG for glyphosate. At the highest gavage dose, 350 mg/kg-day, diarrhea, nasal discharge, and early mortality were observed in the exposed rabbits. Developmental toxicity was not observed at any dose tested. The next lower dose of 175 mg/kg-day was identified as the No Observed Adverse Effect Level (NOAEL). An acceptable daily dose (ADD) of glyphosate of 0.175 mg/kg-day was derived from this by dividing by an uncertainty factor of 1,000 (100 for inter- and intra-species variation and another factor of 10 to account for the severity of the endpoint (mortality) and the short exposure duration of the rabbit study). The updated PHG of 0.9 mg/L (900 ppb) was developed using a body weight per liter of water consumed of 25.2 kg-day/L, and a relative source contribution of 20 percent. The 25.2 kg-day/L value represents the upper 95 percent confidence limit for relative water consumption by pregnant women (OEHHA, 2000). The relative source contribution is a default value commonly used for chemicals for which drinking water is assumed to be a minor source.

Glyphosate is a non-selective systemic herbicide used in agriculture, rights-of-way and aquatic systems. Exposure to glyphosate may occur from its normal use due to spray drift, residues in food crops, and from runoff into drinking water sources. Following acute exposure, glyphosate has low systemic toxicity to mice and rats. In humans, irritation of the oral mucous membrane and gastrointestinal tract is the most frequently reported effect in suicide attempts with glyphosate-surfactant formulations. In most of the short- and long-term toxicity studies in animals, there were no treatment-related gross or cellular changes except reduced body weights, increased liver weights, and ocular lesions at relatively high doses. Three carcinogenicity studies have been conducted, two in rats and one in mice, and all are considered to be negative. *In vitro* and *in vivo* genotoxicity tests are generally negative. There are a few reports of increased sister chromatid exchange in human and bovine lymphocytes at high concentrations *in vitro*, which could be secondary to oxidative stress, and effects on mouse bone marrow after very large intraperitoneal doses. Based on the weight of evidence, glyphosate is judged unlikely to pose a cancer hazard to humans.

OEHHA's review of the glyphosate toxicity literature includes many new scientific studies, plus comments received from the public. Our evaluation has concluded that a

PHG of 900 ppb provides adequate protection against adverse effects of glyphosate in drinking water for the general population and potential sensitive subpopulations such as pregnant women and their fetuses, infants, and the elderly.

INTRODUCTION

Glyphosate, N-(phosphonomethyl) glycine, is used as a non-selective post-emergence herbicide for controlling weeds in agriculture (cropped and non-cropped), forestry, rights-of-way and aquatic systems. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase activity and blocks aromatic amino acid synthesis. This enzyme is found in plants but not in mammals, thereby providing a selective toxicity to plants. In affected plants, this causes reduced protein synthesis, cessation of growth, and leads to cellular disruption and death. Glyphosate has nonspecific metal-chelating properties; it inhibits enzymes that require transition metal cations for activity, such as 3-deoxy-2-oxo-D-arabino-heptulosonate-7-phosphate synthase and 5-dehydroquinate synthase (NTP, 1992).

Glyphosate was first introduced in 1974 and is sold under various trade names such as Roundup branded herbicides, Rodeo®, and Accord®. The major product is a family of herbicides sold under the trade name of Roundup, which consists of the isopropylamine salt of N-(phosphonomethyl) glycine and a surfactant. The predominant surfactant used is a polyethoxylated tallow amine (POEA), which is a mixture of polyethoxylated long-chain alkylamines (Williams *et al*., 2000). Roundup branded herbicides are sprayed as a liquid with ground and aerial equipment. According to U.S. EPA (2004), glyphosate was the second most commonly used pesticide in both the agricultural and non-agricultural (home, garden, and commercial) market sectors. In the agricultural market sector, it was estimated that 34 to 38 million pounds and 67 to 73 million pounds of glyphosate were used in 1997 and 1999, respectively. In the non-agricultural market sector, it was estimated that the annual usage was approximately 7 million pounds of glyphosate during that period. In 2003, approximately 12 million pounds of glyphosate, isopropylamine salt were sold in California. In the same year, approximately 5.6 million pounds were reported used in California. This would cover primarily agricultural uses.

The California Department of Health Services (DHS, 1989) conducted a risk assessment on glyphosate and set the Proposed Maximum Contaminant Level (PMCL) and MCL for drinking water at 0.7 mg/L (700 ppb). This was based on systemic toxicity in a three-generation rat reproduction study with a NOAEL of 10 mg/kg-day (Bio/Dynamics, Inc, 1981b) and an uncertainty factor of 100. The California MCL was established at that level in 1990.

According to the Integrated Risk Information System (IRIS) (U.S. EPA, 2007), the U.S. EPA chose the same rat study, NOAEL, and uncertainty factor in developing a reference dose (RfD) of 0.1 mg/kg-day (in 1990). Applying default exposure assumptions and a relative source contribution (RSC) of 20 percent, U.S. EPA developed a MCL of 0.7 mg/L (U.S. EPA, 1992a). However, a subsequent two-generation rat developmental study at much higher doses (Monsanto, 1990b) did not confirm the findings of this study.

Despite the availability of some new studies, the oral RfD listed in IRIS has not been updated since 1990.

Another RfD of 2 mg/kg-day is listed in the Federal Register (Fed Reg, 1997) for use in the development of the pesticide tolerance for glyphosate in crops. This RfD is based on adverse health effects observed in pregnant rabbits exposed during gestation (21 days) by gavage (IRDC, 1980b). At the highest dose, 350 mg/kg-day, diarrhea, nasal discharge, and early mortality were observed in the exposed rabbits. Developmental toxicity was not observed at any dose tested. The next lower dose of 175 mg/kg-day was identified as the NOAEL. U.S. EPA derived the RfD of 2 mg/kg-day by applying an uncertainty factor of 100.

In 1997, OEHHA evaluated the glyphosate toxicity literature and developed a PHG of 1,000 ppb for glyphosate in drinking water (OEHHA, 1997). The PHG was based on the same rabbit teratology study that was used by U.S. EPA in deriving the RfD of 2 mg/kg-day. OEHHA used an uncertainty factor of 1,000, an assumed body weight of 60 kg for an adult female, a water consumption rate of 2 L/day, and a relative source contribution of 20 percent.

Several health effects studies and review papers on glyphosate have been published over the past several years. This document provides a brief summary of toxicity studies of glyphosate in the context of the updated review of chemical contaminants in drinking water that is required under Health and Safety Code 116365, including the amendments under AB 2342 (2004) for special consideration of infants and children.

CHEMICAL PROFILE

The structure of glyphosate, N-(phosphonomethyl) glycine, is shown in Figure 1; its properties are summarized in Table 1. Glyphosate is usually formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine or trimethylsulfonium. Surfactants and inert ingredients are often added to formulations of glyphosate such as Roundup branded herbicides and Vision®. Common surfactants are polyoxyethylene amine, ortho X-77, Li-700, R-11 and Widespread. Other additives that may be found in formulations are sulfuric and phosphoric acids. The amount of glyphosate in these products varies over a wide range. The percentage by weight can be as low as less than one percent in ready to use commercial products to over 40 percent in some concentrates (WHO, 1994). As the subject of this evaluation is glyphosate, and there are many possible compositions of commercial products, most of the data and discussion presented in this analysis are on glyphosate rather than the formulated products. In drinking water, the glyphosate anion is likely to be associated with alkali metal cations such as sodium ion (Montgomery, 1993). Toxicity results for commercial products are included only when they provide additional insights to the health hazards associated with the oral exposure to the active ingredient, glyphosate.

Figure 1. The structure of glyphosate [N-(phosphonomethyl) glycine]

Table 1. Physical and Chemical Properties of Glyphosate

Name	Glyphosate (N-(phosphonomethyl)-glycine)
Trade names	Roundup branded herbicides, Rodeo®, Accord®
CAS No.	1071-83-6
Physical state	White crystalline solid
Melting point	230 ^{o}C (decomposes)
Molecular weight	169.07
Density	1.74 g/mL
Solubility in water	12 g/L at 25^{0}C
Solubility in organic solvents	Insoluble
Vapor pressure	7.50x10^{-6} mm Hg at 25^{o} C
Henry's Law constant	1.39x10^{-10} atm-m^{3}/mol.
Octanol-water partition coefficient (Log K_{ow})	-2.8, -1.6
pKa values	2.32, 5.86, 10.86
pH (1% solution in water)	2.5

(Adapted from Edmund, 1988; Montgomery, 1993; WHO, 1994.)

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Soil

Glyphosate may reach soil in its normal use as a liquid spray, through spillage or accidental discharge. Once in soils, it is strongly adsorbed onto the soil forming insoluble complexes with metal ions. Glyphosate is readily degraded by soil microbes to aminomethyl phosphonic acid (AMPA), which is then degraded to inorganic constituents, including carbon dioxide and phosphate. Based on field experiment data, the dissipation

half-life of glyphosate from soil can range from 3 to 174 days (WHO, 1994), depending on soil and climatic conditions.

Precipitation, soil composition, presence and absence of a soil constricting layer and drainage type may influence the leaching of glyphosate from soil. Field and laboratory studies indicate that glyphosate generally does not move vertically in the soil below the topmost six-inch soil layer (U.S. EPA, 1993).

Air

There are no data available on ambient air concentrations of glyphosate. Air concentrations during silvicultural spraying were mostly below 1.3 $\mu g/m^3$; the highest value observed was 15.7 $\mu g/m^3$ (Jauhiainen *et al*., 1991). Due to the low vapor pressure of the chemical, volatilization of glyphosate from a sprayed area is not expected to be significant. Inhalation of spray droplets by agricultural workers and residents living near agricultural fields can be an important exposure pathway.

Water

Glyphosate may enter water via runoff, from overspray, or from spray drift. In water, it adsorbs strongly to sediment and particulate matter in the water column. It may also form insoluble complexes with metal ions and precipitates. In water, glyphosate does not degrade readily. Under laboratory conditions, no appreciable degradation of glyphosate was observed in dechlorinated tap water via chemical, microbiological or photolytic processes 78 days, with or without aeration (Anton *et al.*, 1993). Sediment adsorption and biodegradation represent the major dissipation processes in aquatic systems (Goldsborough and Brown, 1989). Laboratory experiments showed that the rate of biodegradation varied, depending on the experimental conditions, e.g., availability of oxygen, temperature, and type of sediment. The time needed for 50 percent degradation of glyphosate in a test system with water and sediment was estimated to be less than 14 days under aerobic and 14-22 days under anaerobic conditions (WHO, 1994).

The half-lives of glyphosate in three forest ponds in Manitoba, Canada that were aerially sprayed in August were approximately 1.5 to 2 days; glyphosate was not detected in any sample by day 38 (Goldsborough and Brown, 1989). However in two field studies (Feng *et al*., 1990 and Monsanto, 1990a, as cited in WHO, 1994), it was noted that under certain conditions, glyphosate and its degradation product, AMPA, could persist in the pond sediment for up to one year.

The off-target movement of glyphosate had been studied (Smith *et al.*, 1996) in Newfoundland, Canada. A 2 percent solution of Roundup was sprayed evenly at the rate of about 11.4 to 13 L/hectare to a site called Massey Drive that was located on a fractured lime stonebed. Drinking water wells from the sprayed site were sampled at 1, 2 and 4 weeks after the first spray and at 1, 2, 4, 13 and 32 weeks after the second spray. Glyphosate was detected in well water at the Massey Drive site at levels ranging from 0.0072 to 0.045 mg/L. Levels peaked two weeks post-spray at 0.025 mg/L in well water and then dropped off to 0.004 mg/L by the fourth week of sampling. After the second

treatment, the concentration in the well increased to a maximum of 0.045 mg/L at seven weeks post-spray and again dropped off. This study showed that though glyphosate is known to adsorb strongly to soils, this factor alone did not prevent off-target movement of glyphosate on a limestone bed where the topsoil was replaced with gravel, and thus the potential for off-target movement of chemical was increased.

Food

Glyphosate is not absorbed by a plant's root system because of its strong adsorption to the soil. However, it is easily absorbed by leaves from spray residues and is translocated throughout the plants and fruits. Glyphosate is not metabolized to any significant degree in plant tissues (Ghassemi *et al*., 1982 as cited in NTP, 1992). Therefore, glyphosate concentration may increase in plants immediately after spray. Ingestion of sprayed food material or products from animals fed treated vegetation may lead to glyphosate exposure. Glyphosate residues in cattle, pig, and poultry meat, eggs, and milk were found to be negligible after the animals were fed a diet containing 100 mg/kg glyphosate and AMPA (WHO, 1994).

Bioconcentration factors are low in laboratory tests with invertebrates and fish. In one study, a bioconcentration factor of 0.5 was estimated in bluegill sunfish exposed to 11 to 13 mg/L for 35 days. Maximum glyphosate concentrations in the whole fish, viscera and fillet were 13, 7.6, and 4.8 mg/kg, respectively (ABC Inc., 1989 as cited in WHO, 1994).

In its dietary risk assessment based on a worst-case scenario, U.S. EPA (1993) concluded that the chronic dietary risk from food use is minimal. The calculated theoretical maximum residue contribution for the U.S. population is 0.025 mg/kg-day. The exposure for the most highly exposed subgroup, non-nursing infants less than one-year-old, is 0.058 mg/kg-day. The major dietary contribution is from wheat products. Though the U.S. EPA dietary risk assessment methods have changed since that time, the overall conclusions regarding dietary risk probably would not change.

Biomonitoring

A biomonitoring survey of 48 farmers and their family members who had potential exposure to glyphosate was reported by Acquavella *et al*. (2004). Composite urine samples (24-hr) of the farmer, the spouse and their children were collected the day before, the day of, and for three days after glyphosate application. It was reported that 60 percent of farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. For spouses, 4 percent had detectable levels in their urine on the day of application. Their maximum urine concentration was 3 ppb. For children, 12 percent had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application.

METABOLISM AND PHARMACOKINETICS

The absorption of glyphosate from oral administration in various species is about 30 to 36 percent. In a single dose (5.6 or 56 mg/kg) study in F344 rats (NTP, 1992), 30 percent of the oral dose was absorbed. In a comparable study, after a single oral dose of 10 or 1,000 mg/kg body weight, 30 to 36 percent absorption was reported based on percentage excretion in the urine. The remaining total body burden was about 1 percent, which was widely distributed in the body but mainly associated with bone. Only a very small percentage (less than 0.2 percent) of the administered dose was expired as carbon dioxide. The results are summarized in Table 2 (Monsanto, 1988 as cited by WHO, 1994). The dermal absorption from a diluted Roundup herbicide in Rhesus monkeys was about 3.7-5.5 percent after 12 hours of exposure (Wester *et al.*, 1991).

Glyphosate is poorly metabolized in rats and most of the dose was excreted unchanged as the parent compound. AMPA is the only metabolite found in feces and accounts for 0.2 percent to 0.3 percent of a 10 mg/kg administered dose (Brewster *et al.*, 1991).

Table 2. Concentrations of C^{14} label (as mg Glyphosate-Equivalents/kg Fresh Weight) in Selected Rat Tissues 7 Days after a Single Oral Dose

	Dose: 10 mg/kg		Dose: 1,000 mg/kg	
	Male	**Female**	**Male**	**Female**
Blood	0.0045	0.0027	0.33	0.17
Liver	0.030	0.014	1.9	1.3
Kidney	0.022	0.013	1.9	1.4
Spleen	0.012	0.0073	2.6	3.0
Lung	0.015	0.012	1.5	1.1
Thyroid	0.00080	0.00036	1.5	1.2
Nasal mucosa	0.0050	0.023	1.7	1.8
Stomach	0.0080	0.0037	2.4	2.4
Small intestines	0.022	0.018	1.9	1.6
Colon	0.034	0.016	11.0	9.2
Bone	0.55	0.31	30.6	19.7
Bone marrow	0.029	0.0064	4.1	12.5

(Monsanto, 1988, as cited in WHO, 1994.)

After a single oral dose of glyphosate (10 or 1,000 mg/kg) to male and female rats, fecal elimination was 62-70 percent (at both doses) and excretion in urine was 14-18 percent (at the high dose) or 22-29 percent (at the low dose); less than 0.2 percent of the dose was expired as carbon dioxide (Monsanto 1988 as cited in WHO, 1994). The elimination data suggest a two-compartment model. At the 10 mg/kg dose level, the half-life for the α

phase was 5.9 to 6.2 hours and for the β phase was 79 to 106 hours. At 1,000 mg/kg, the half-life for the α phase was 5.3 to 6.4 hours and for the β phase was 181 (male rats) to 337 hours (female rates). Pretreatment with unlabelled compound for 14 days at the low dose level had no effect on whole body elimination rate.

In the National Toxicology Program (NTP) study, a single gavage dose of ^{14}C-labelled glyphosate (5.6 or 56 mg/kg) was given to male F344/N rats. Approximately 50 percent of the radioactivity at both dose levels was eliminated in the feces in the first 24 hours, and urinary elimination of radioactivity was essentially complete by 12 hours. More than 90 percent of the radioactivity was eliminated within 72 hours (NTP, 1992). When glyphosate was administered by intravenous injection at 5.6 mg/kg, the blood radioactivity vs. time plot fitted a two-compartment model with an α phase of about 0.5 hour and a β phase of 13 hours.

In lactating goats, excretion in milk was shown to occur to a minor extent. Concentration of glyphosate in whole milk was equal to or less than 0.1 ppm at a concentration of 120 ppm in diet (WHO, 1994).

TOXICOLOGY

Toxicological Effects in Animals

Acute Effects

The acute lethal dose (LD_{50}) of glyphosate in various species by different routes is given in Table 3. Glyphosate has very low toxicity by the oral and dermal routes, partly due to its limited absorption. It is significantly more toxic by the intraperitoneal (ip) route. The reported toxic effects following acute exposure were hyperemia, severe stress, accelerated breathing and occasional asphyxial convulsion.

Table 3. Acute Toxicity of Glyphosate in Experimental Animals

Species	Administration mode	LD_{50} (mg/kg)
Rat	oral	4,873
Rat	ip	235
Mouse	oral	1,568
Mouse	ip	130
Rabbit	oral	3,800
Goat	oral	3,500
Rat	dermal	>2,000
Rabbit	dermal	>5,000

(Adapted from NTP, 1992; WHO, 1994.)

Most studies reviewed by WHO (1994) reported that the LD_{50} of glyphosate is at or above 5,000 mg/kg. In a study by Knapek *et al.* (1986 as cited in WHO, 1994), a commercial product containing glyphosate showed a LD_{50} of 2,047 mg/kg. Several acute toxicity studies using Roundup branded herbicides indicated that its LD_{50} is at or above 5,000 mg/kg, and the LD_{50s} of other products such as Sting® and Legend® are approximately 2,000 mg/kg (WHO, 1994).

Dermal and Ocular Effects

Glyphosate technical and Shackle®, at various concentrations, were tested for eye irritation in rabbits. Slight irritation was reported in some animals, and the irritation disappeared after a day or more (Monsanto, 1971, 1975, and 1979a; Branch *et al*., 1983). Glyphosate was not found to be a strong dermal irritant. Several irritation studies using rabbit intact or abraded skin showed glyphosate produced a relatively low response (Monsanto, 1979b and 1979c). When a formulated glyphosate was tested at a concentration five-fold higher than the normal field application level, severe local skin reaction, reduced food consumption, body weight loss, mortality, and testicular effects were observed (Heydens, 1988).

Subchronic Effects

Glyphosate (purity 98.7 percent) was administered in the diets of CD-1 mice for 90 days at levels of 5,000, 10,000 or 50,000 ppm (calculated to be 940, 1,890, and 9,710 mg/kg-day in males and 1,530, 2,730, and 14,860 mg/kg-day in females). Liver weights were increased at 10,000 and 50,000 ppm and growth retardation and increased organ weights of brain, heart and kidney were observed at 50,000 ppm (Monsanto, 1979d as cited by WHO, 1994). The authors concluded that the NOAEL was 10,000 ppm.

In a 90-day study, Sprague-Dawley rats were administered glyphosate at 1,000, 5,000 or 20,000 ppm in the diet (calculated to be 63, 317, and 1,267 mg/kg-day in males and 84, 404, and 1,623 mg/kg-day in females). No toxic effects were observed. Hematology, blood chemistry, and organ weights were not affected by the treatment. Limited histopathology revealed no adverse effect in any tissue that was examined. The NOAEL from this study was 20,000 ppm (1,267 mg/kg-day) (Monsanto, 1987 as cited by WHO, 1994).

Glyphosate was administered in the diets of 10 F344N rats or $B6C3F_1$ mice per sex per dose for 13 weeks at concentrations of 0, 3,125, 6,250, 12,500, 25,000 or 50,000 ppm. Ten additional rats per sex were included for evaluation of hematology and clinical pathology parameters (NTP, 1992). In the rats, reduced weight gain was observed in males in the 25,000 (males only) and 50,000 ppm groups (males and females). The treatment had no effect on survival of both sexes. The final body weight of the males in the highest dose group was about 18 percent less than controls. In female rats, only a slight (5 percent) reduction in body weight was observed at the highest dose level. In males, there were slight increases in relative weights of liver at $\geq$ 3,125 ppm, kidney and testes at $\geq$ 25,000 ppm, and a decrease in thymus weight at 50,000 ppm. In females, changes in organ weights were minor and could not be related definitely to treatment. Of

the hematological parameters, there was a mild increase in hematocrit and red blood cell (RBC) count at ≥ 12,500 ppm, hemoglobin at ≥ 25,000 ppm, and platelets at 50,000 ppm. In female rats, significant increases were observed in lymphocytes at ≥ 25,000 ppm and platelet counts at ≥ 3,125 ppm, white blood cells (WBC) at ≥ 12,500 ppm, mean corpuscular hemoglobin (MCH) at 50,000 ppm, and mean corpuscular volume (MCV) at 50,000 ppm.

The changes in clinical chemistry parameters included an increase in alkaline phosphatase at ≥ 6,250 ppm in male and at ≥ 12,500 ppm in female rats. Alanine aminotransferase activity was also increased in both sexes. NTP (1992) noted that these findings likely reflect hepatocellular leakage or single cell necrosis and cholestasis. Increases in absolute and relative liver weights in male rats also indicate the effect of glyphosate on the liver. A significant decrease (20 percent) was observed in sperm density in the 25,000 and 50,000 ppm dose groups. The only histopathological changes found were cytoplasmic alterations in the parotid and submandibular salivary glands of male and female rats. These lesions consisted of basophilic changes and hypertrophy of acinar cells. The magnitude of the effect was dose-dependent in both sexes. Because the effects on the salivary glands were observed at all dose levels, no NOAEL was identified.

In mice, the treatment had no effect on survival of either sex. Body weight gains of male and female mice were depressed at the two highest doses. Increased organ weights of heart, kidney, liver, thymus and testes were not dose-dependent and were not considered compound-related. No effects were observed on sperm motility. Pathological changes in salivary glands were similar to rats but were not observed at the lowest level of 3,125 ppm in the diet (calculated to be 507 mg/kg-day in male and 753 mg/kg-day in female mice). Therefore, the NOAEL for glyphosate in mice appears to be 507 mg/kg-day. The salivary gland lesions were similar to those induced by exposure to high subcutaneous doses of the β-adrenergic agonist isoproterenol and could be partially ameliorated with the β-adrenergic antagonist propanolol. These data suggest that glyphosate may induce the salivary gland lesions by acting as a weak adrenergic agonist (NTP, 1992).

Glyphosate (96 percent) was administered orally by capsule at 0, 20, 100 or 500 mg/kg-day to six beagle dogs per sex per dose for 52 weeks (Monsanto, 1985). No adverse effects occurred with respect to clinical signs, body weight, ophthalmoscopy, hematology, blood chemistry, gross pathology, and histopathology. Changes in pituitary weights (absolute and relative) in the males dosed at 100 or 500 mg/kg were noted. The authors suggested that because there were no concomitant histological changes in pituitaries and similar findings were not observed in other animal studies, the toxicological significance of the change in pituitary weights is questionable; they concluded the NOAEL to be the highest dose tested of 500 mg/kg-day. In its evaluation of the toxicity of glyphosate, California Department of Pesticide Regulation concurred with this interpretation.

In a dermal study, glyphosate at levels of 100, 1,000 or 5,000 mg/kg-day was applied to shaven intact or abraded skin of rabbits for six hours/day, five days/week for three weeks. No effect on survival and growth occurred. At the high dose, a slight erythema and edema was observed in intact and abraded skin. No evidence of systemic toxicity was found (IRDC, 1982 as cited by WHO, 1994).

Chronic Effects and Carcinogenicity Studies

Rat

Glyphosate (98.7 percent) was administered in diet to Sprague-Dawley rats (50 per sex per group) for 24 months at approximately 0, 3.1, 10.3 or 31.5 mg/kg-day for male and 0, 3.4, 11.2 or 34 mg/kg-day for female rats (Bio/Dynamics, Inc., 1981a; Monsanto, 1984). Survival, hematology, blood chemistry, urinalysis, and organ weights were not affected by the treatment. The systemic NOAEL for this study was estimated to be 31.5 to 34 mg/kg-day.

C-cell carcinoma of the thyroid was increased in the 34 mg/kg-day female group (1/47 in the control and 6/47 in the high-dose group) (Monsanto, 1984). However, the authors argued that the finding might not be treatment related because the incidence of hyperplasia and adenoma of the thyroid was greater in the control females than in the high-dose females. Due to the difficulties in differentiating c-cell adenoma from carcinoma, Monsanto argued that one should not compare the incidence of animals bearing only C-cell carcinoma, but should instead compare the combined incidence of animals bearing either C-cell adenoma or carcinoma. The incidence of females with either a thyroid C-cell adenoma or carcinoma is similar for the control and high-dose groups (6/47 and 9/47, respectively). Furthermore, there is no dose-response relationship in terms of females bearing thyroid C-cell adenoma or carcinoma (6/47, 3/49, 8/50, 9/47 for the control, low-, mid-, and high-dose groups, respectively).

A statistically significant increase in interstitial cell tumors of the testes was found in the high-dose males, compared to concurrent controls (incidences: 0/50, 3/50, 1/50, and 6/50; historical control range: 3-7 percent) (Bio/Dynamics, Inc., 1981a). However, this tumor is known to be age-related and primarily occurs in older rats. It has been pointed out that survival of control males was lower than that of high-dose males; the mean survival time of control males (660 days) was shorter than that of the high-dose males (732 days). Also, the significance of this result has been questioned because a similar effect was not observed in a more recent two-year rat study at much higher doses (see the study below).

Glyphosate (purity 96.5 percent) was administered to Sprague-Dawley rats (60 per sex per group) for 24 months at concentrations of 0, 2,000, 8,000 or 20,000 ppm in diet (calculated to be 0, 100, 410, and 1,060 mg/kg-day) (Monsanto, 1990c). The highest dose was considered close to the maximum tolerated dose. An additional 10 rats per sex per group were included for one-year interim sacrifice. No change in survival or appearance was noted in the treated animals. Statistically significant reduction in body weight gain was observed in the high dose female rats. There was a significant increase in the incidence of basophilic degeneration of the posterior subcapsular lens capsule fibers in the eye of male rats in the highest dose group; however, the finding was within the historical control range. No changes were observed in hematology and blood chemistry. Liver weight was also increased in male rats of the highest dose group. No other statistically significant changes in organ weights occurred in a dose-related manner.

There was a statistically significant increased incidence of inflammation of the gastric squamous mucosa in the medium- and high-dose females (0/59, 3/60, 9/60, and 6/59 for the control, low-, mid-, and high- dose groups, respectively; historical range: 0-13.3

percent). Though a similar increase was also observed in males, the increase was not statistically significant (2/58, 3/58, 5/59, and 7/59 for the control, low-, mid-, and high-dose groups, respectively). The lesions were not considered neoplastic by Monsanto (1990c). Because there was no dose-related trend across the female groups and no significant difference among the males, it is questionable if the finding was treatment-related.

There was a statistically significant increase in the incidence of pancreatic islet cell adenomas in the low- and high-dose males (incidences: 1/58, 8/57, 5/60, and 7/59; historical control range: 1.8-8.5 percent). The incidence in the control group was below the historical control range, and the trend test for this tumor was negative. Furthermore, there was no evidence of dose-related pancreatic damage or preneoplastic lesions. One pancreatic islet cell carcinoma was found in a control male, but none was found in the dosed males. No significant increase in this lesion was observed in females (5/60, 1/60, 4/60, and 0/59 for the control, low-, mid-, and high-dose groups, respectively) (Monsanto, 1990c). A modest incidence of a relatively uncommon tumor type (adrenal cortical carcinoma) was found only in the highest dosed females (3/50, none in other groups of either sex). Though the trend test is positive, the increased incidence in the highest-dosed female could be by chance. The biological significance of this finding is unknown.

The NOAEL for this study was estimated to be 8,000 ppm (equal to 410 mg/kg-day) for the reduction in female body weight gain, cataractous lens changes in males, and increased liver weights in males at the highest dose (20,000 ppm).

Mouse

Glyphosate (purity 99.7 percent) was administered for 24 months in the diet of 50 CD-1 mice per sex per dose at concentrations of 0, 1,000, 5,000 or 30,000 ppm (calculated to be 0, 157, 814 and 4,841 mg/kg-day for males and 0, 190, 955 and 5,874 mg/kg-day for females) (Bio/Dynamics 1983). There was a slight decrease in the mean body weights of male mice in the highest dose group.

At the highest dose, a number of adverse liver and kidney effects were reported: central lobular hepatocyte hypertrophy in males, central lobular hepatocyte necrosis in males, chronic interstitial nephritis in males, and proximal tubule epithelial basophilia and hypertrophy in females. In addition, increased incidences of epithelial hyperplasia (thickening) in the urinary bladder were observed in male mice in the mid and highest dose groups (incidences: 3, 3, 10, and 8 for the control, low, mid, and high exposures, respectively)(Bio/Dynamics Inc., 1983; DPR, 1992). The increased epithelial thickening was described as minimal to mild. The report suggested that although the incidence was increased in mid and high dose males, the observed changes might not be related to the treatment.

Bronchiolar-alveolar lung tumors, hepatic tumors, and tumors of the lymphoreticular system were responsible for the majority of tumors observed in the study. No clear dose-response relationships were noted for these tumors.

Renal tubule adenoma and carcinoma incidence was increased in the high-dose male group (1, 0, 1, and 3 for the control, low, mid, and high dose, respectively). After reviewing the data, the FIFRA Scientific Advisory Panel noted that age-adjusted tumor incidence data did not demonstrate a statistically significant increase based on concurrent controls; nevertheless the incidence in the highest dosed males was statistically significant compared to historical controls (DPR, 1992).

Genetic Toxicity

Glyphosate was mostly negative in *in vivo* and *in vitro* test systems evaluating gene mutation, chromosomal aberration and DNA damage. By the weight-of-evidence, glyphosate is considered to be neither genotoxic nor clastogenic.

Though most of the tests show glyphosate is not genotoxic, a number of positive results have been reported in the literature. Bolognesi *et al*. (1997) first reported that glyphosate increased sister chromatid exchange in human lymphocytes *in vitro*. This finding was supported by two other *in vitro* studies reported by Lioi *et al*. Lioi *et al*. (1998a) showed that glyphosate increased chromosomal aberration and sister chromatid exchange in human lymphocytes above 1.4 mg/L; similarly, they also reported that glyphosate increased chromosomal aberration and sister chromatid exchange in bovine lymphocytes above 2.9 mg/L (Lioi *et al*., 1998b). Lioi *et al*. found glyphosate at these levels caused increased oxidative stress as well as reduced glutathione level in the lymphocytes, and these events might have contributed to the observed genotoxicity of the compound.

Bolognesi *et al*. (1997) administered glyphosate by intraperitoneal injection (at 2 x 150 mg/kg) to three male mice and found the chemical increased micronuclei in bone marrow cells. Negative results have been reported by NTP (1992) and Rank *et al*. (1993). The discrepancies may be explained by the different exposure routes and the difference in dosage. Bolognesi *et al*. (1997) found that glyphosate at 300 mg/kg by intraperitoneal injection increased DNA damage in mice liver and kidney tissues. Furthermore, they found this treatment also increased oxidative damage in the liver but not in the kidney. It should be noted that the dose used in the studies reported by Bolognesi *et al*. was very high, as the estimated intraperitoneal LD_{50} of glyphosate in mouse is only 130 mg/kg (see Table 3).

Teratogenicity

Glyphosate (purity 98.7 percent) was administered by gavage at levels of 0, 300, 1,000 or 3,500 mg/kg-day to female COBS CD rats on days 6 to 19 of gestation. In the highest dose group, a statistically significant decrease in viable fetuses and mean fetal body weight were noted. The highest dose was also toxic to the dam, because it reduced mean maternal body weight gain and caused early death in several animals. The maternal and developmental toxicity NOAELs were 1,000 mg/kg-day (IRDC, 1980a).

Glyphosate technical (98.7 percent) was administered by gavage to 16 female Dutch Belted rabbits per dose at 0, 75, 175 or 350 mg/kg-day on days 6 to 27 of gestation (IRDC, 1980b). The control group received the vehicle only, 0.5 percent aqueous

Methocel®, on a comparable regimen. Cesarean sections were performed on all surviving females on gestation day 28.

No treatment-related abnormal clinical signs were observed in rabbits dosed at 75 mg/kg-day. A slight increase in the incidence of soft stools and diarrhea was noted in the 175 mg/kg-day group and a definite increase in these signs and nasal discharge were noted in the 350 mg/kg-day group, compared to the controls. The mean maternal body weight gain for each dosed group was comparable to that of the control group. Early mortality was reported in the highest dose group (0, 1, 2, and 10 for the control, low, mid, and high doses, respectively). Causes of death were determined for five of the rabbits dying prior to the scheduled sacrifice; they were pneumonia, respiratory disease, enteritis or gastroenteritis. Causes of death for the other eight rabbits could not be determined at necropsy. Two rabbits in the control group and one each in the 175 and 350 mg/kg-day groups aborted and were sacrificed.

The researchers found no biologically meaningful differences in mean number of viable fetuses, early or late resorptions, total implantations, corpora lutea, fetal body weights, the fetal sex distribution, or the number of fetuses or litters with malformations in any of the treatment groups compared to the control group. The number of fetuses and litters with developmental and genetic variations were also comparable for all groups. A slight decrease was noted in mean fetal body weight of all treated groups compared to the controls. However, mean fetal body weights for all groups were comparable to historical control mean fetal body weight values (IRDC, 1980b).

The maternal NOAEL in this study was 175 mg/kg-day. At the highest dose (350 mg/kg-day), there was a significant increase in early mortality (10/16 in the highest dose group versus 0/16 in the control; Fisher exact test, $p = 1.24 \times 10^{-4}$).

Daruich *et al*. (2001) exposed pregnant rats (eight/group) to glyphosate in drinking water at 0, 0.5 or 1 percent w/v throughout the gestation period. On gestation day 21, fetuses were removed and weighed. Maternal and fetal livers, hearts, and brains were also isolated and processed for enzymatic activity analyses. They reported that rats exposed to glyphosate had decreased water and food ingestion. In the high dose group, there was a significant decrease of maternal body and liver weights compared to the controls. However, there were no differences in fetal body weights. Exposure to glyphosate appeared to affect many enzymes in various organs, although a dose-response relationship was not always observed. For comparison purposes, Daruich *et al*. studied the effect of low water and low food intake in another group of pregnant rats and found there were no significant differences in the enzymatic activities, compared to the control group. They therefore attributed the observed changes in the enzyme activities to the effects of glyphosate.

Dallegrave *et al*. (2003) studied the teratogenic effects of Roundup (consisting of 360 g/L glyphosate and 18 percent (w/v) polyoxyethyleneamine) to Wistar rats. Sixty pregnant rats were divided into 4 groups. The control group received distilled water and the treatment groups received 500, 750, or 1,000 mg/kg-day glyphosate diluted in water. The dosing regimen was based on the NOAEL of 1,000 mg/kg-day for developmental toxicity in rats reported by Williams *et al*. (2000). The rats were treated by gavage from days 6 to

15 of pregnancy, defined as the critical period for the embryonic structural development in rats.

Dallegrave *et al*. (2003) found that Roundup was more toxic than glyphosate. At 1,000 mg/kg, 50 percent of the dams died between day 7 and 14 of pregnancy. In the study reported by IRDC (1980a), no significant fatality was noted in pregnant rats treated with 3,500 mg/kg-day on days 6 to 19 of gestation. Among the dams that survived the treatment, the authors found no significant differences in total weight gain and relative weight of the organs. The number of fetuses, corpora lutea, implantation sites and embryo resorption was similar for all groups.

Concerning the fetal variables, Dallegrave *et al*. (2003) found no significant difference among the groups in terms of weight, male:female sex ratio, and external malformation rate. However, they reported that the total percentage of skeletal alterations was significantly increased ($P<0.001$, χ^2-test) in all the groups exposed to Roundup, compared with control, with a clear dose-response relationship. The percentage of altered fetuses was 15.4, 33.1, 42.0, and 57.3 for the control and the 500, 750, and 1,000 mg/kg-day groups, respectively. The most frequent skeletal alterations observed were incomplete skull ossification and enlarged fontanel. The occurrence of multiple alterations was also significantly higher in the treated groups compared with the control, but did not show a dose-response relationship. Because Roundup and not glyphosate was the test material in this study, it is possible that the surfactant, polyoxyethyleneamine, in the commercial formulation might have contributed to the observed teratogenicity.

Reproductive Toxicity

Glyphosate (purity 98.7 percent) was administered to CD rats at doses of 0, 3, 10 or 30 mg/kg-day for three successive generations (Bio/Dynamics Inc, 1981b). The diet was prepared weekly during various growth periods and adjusted to achieve the desired dose levels. Groups of 12 males and 24 females F_0 rats were administered test diets for 60 days. Treatment continued through mating, gestation and lactation for two successive litters (F_{2a} and F_{2b}). Groups of 12 males and 24 females were retained at weaning from the second litters of each dose level as parental animals for the succeeding generation.

Early mortalities appeared unrelated to dose and were not considered to be treatment-related. Adult body weights and food consumption during growth, rest, gestation or lactation were comparable between all treated and control groups for all generations. For the entire study, no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment. The mean liver to body weight ratios of the F_{2b} parental females for all treated groups were significantly lower than the control values. Slightly reduced liver to brain weight ratios also were noted for all treated groups. These differences did not show a dose-response relationship and similar effects were not observed in treated parents from previous generations and no microscopic lesions attributed to treatment were observed in hepatic tissues.

The report concluded that gross necropsy and histopathologic evaluations did not reveal any evidence of effects related to treatment. However, it has been noted that there was an

increased incidence of unilateral renal tubular dilation in the male pups of the F_{3b} generation at the highest dose (Bio/Dynamics Inc, 1981b).

In a two-generation study, glyphosate was administered to CD rats at 0, 2,000, 10,000 or 30,000 ppm in the diet (calculated to be 0, 150, 720 and 2,200 mg/kg-day for the F_0 animals) for 11 weeks before they were mated to produce the F_1 generation. Litters were culled to 8 pups on lactation day 4 and weaned on lactation day 21. At the time of weaning, 30 F_1 rats/sex/group were randomly selected to continue on the study as parental F_1 animals. Following an approximate 14-week period, these animals were mated twice to produce F_{2a} and F_{2b} generations (Monsanto, 1990b).

The F_0 and F_1 male and female adults had reduced body weights (8 to 11 percent) in the highest dose group. Mating, pregnancy, and fertility indices were not affected by the treatment in both F_0 and F_1 animals. On lactation day 0, the average litter size of high-dose F_0 dams was approximately 2 pups less than controls, and a smaller difference (approximately 1 pup/litter) was noted after the first F_1 mating. However, these differences were not statistically significant and there was no increase in the number of dead pups/litter. No treatment-related decrease in litter size was observed in the F_{2b} generation (Monsanto, 1990b).

Postnatal pup survival was not changed by the administration of glyphosate in all three groups (F_1, F_{2a} and F_{2b}). Body weights of some high dose offspring were 4 to 11 percent below controls on lactation day 14. This effect was more pronounced on lactation day 21, as body weights were reduced 11 to 19 percent in all offspring groups. Smaller reduction in body weight (5.6 to 6.6 percent) was noted in some mid-dose offspring on lactation day 21. However, significant body weight decreases were not observed in these animals before or after lactation day 21. The authors of the report did not consider the body weight decreases in mid-dose pups to be treatment-related as they were small, transient, and did not occur consistently in both sexes from all litters (Monsanto, 1990b).

There were no gross or microscopic pathology changes in parents or offspring attributed to the treatment. In a previous developmental toxicity study, 10 pups/sex/generation were examined, and focal renal tubular dilation was noted in the high dose (30 mg/kg-day) male offspring from the last generation. In this study, the high dose level was 30,000 ppm (approximately 2,200 mg/kg-day), and several more offspring were examined (1/sex/litter). No treatment-related renal effect was found, indicating that the previous finding may not be related to glyphosate exposure.

Based on the reduced body weights in adults and pups observed in the high dose group, the NOAEL in this study was estimated as 10,000 ppm in the diet (720 mg/kg-day) (Monsanto, 1990b).

Yousef *et al*. (1995) studied the effects of glyphosate on semen characteristics in rabbits. Glyphosate was given orally in gelatin capsules to four male New Zealand white rabbits per dose at levels of 0, 1/100 LD_{50}, or 1/10 LD_{50} daily for six weeks. A preliminary six-week evaluation period was followed by a six-week treatment period, followed by a six-week recovery period without pesticide administration. The animals were weighed and semen collected weekly throughout the 18-week period. Semen volume, fructose level in semen, semen osmolarity, sperm concentration and live, dead and abnormal spermatozoa were evaluated. The authors concluded that glyphosate treatment reduced body weight,

ejaculate volume and sperm concentration and increased abnormal and dead sperm at both dose levels. The adverse effects continued into the recovery period. Actual dose or LD_{50} values were not given in the paper, and dose-response relationship cannot be characterized.

In an *in vitro* system, Walsh *et al*. (2000) showed that Roundup decreased steroidogenesis in mouse Leydig tumor cells and has the potential to impact the production of testosterone. However, the researchers also found that glyphosate alone did not alter steroid production in the test system. They postulated that other components of the Roundup formulation are required to disrupt steroidogenesis.

Toxicological Effects in Humans

Case Studies and Human Clinical Studies

A number of studies have reported clinical observations in patients who ingested relatively large quantities of glyphosate surfactant mixtures. Some of these cases were suicide attempts and others were accidents. The importance of these results to environmental exposure is limited because the exposures were many times higher than what is likely to be encountered in the environment and the toxicity of glyphosate might have been increased by the presence of surfactants (Sorensen and Gregersen, 1999; Dallegrave *et al*., 2003).

Talbot *et al.* (1991) reported a number of cases of acute intoxication (suicide attempts) with herbicides containing glyphosate. The reported acute symptoms were: sore throat, dysphagia, gastrointestinal hemorrhage, and erosion of the gastrointestinal tract. Other less commonly affected organs were lung, liver, kidney and the central nervous system. The estimated amount of Roundup (41 percent glyphosate) ingested by non-survivors was 184 +/- 70 mL (range 85 to 200 mL). Most of the deaths occurred within a few hours of the herbicide ingestion. In another study, Tominack *et al.* (1991) estimated a dose of 120 +/-112 mL in survivors and 263 +/-100 mL for non-survivors of suicide attempts. The most common reported symptoms in this study were irritation of mucous membrane and gastrointestinal tract. Minor reported effects were pulmonary dysfunction, metabolic acidosis, hypotension, leukocytosis and fever. The high concentrations of both glyphosate and its constituent surfactant in the formulated product in the suicide cases are not anticipated in drinking water.

Hung *et al*. (1997) studied 53 patients with known ingestion of a glyphosate-surfactant pesticide (Roundup) and found the occurrence and severity of laryngeal injury may be an important factor in determining the degree of morbidity and mortality. They suggested that the surfactant (POEA) rather than glyphosate was the likely cause of the observed acute toxicity. It is also possible that POEA and glyphosate potentiate each other's toxicity. In a similar study, Chang *et al*. (1999) reported that the severity of esophageal injuries in patients exposed to glyphosate-surfactants was associated with increased white blood cell count, length of hospital stay, and the occurrence of serious complications. They suggested the severity of esophageal injuries might be used as a prognostic factor in giving treatments.

Lin *et al*. (1999) reported a case of glyphosate-induced cardiogenic shock in a young man who drank approximately 150 mL of glyphosate with surfactant. It is not clear what was the mechanism of this health effect.

Sorensen and Gregersen (1999) reported two cases of lethal intoxication with the herbicide glyphosate-trimesium (Touchdown). They reported a 6-year-old boy and a 34-year-old woman died within minutes after oral ingestion of the pesticide. The post-mortem examination revealed pulmonary edema, cerebral edema, and dilated right atrium and ventricles of the heart, in addition to some of the symptoms described above. The authors speculated that the surfactant, trimethylsulfonium, in the Touchdown might facilitate the absorption after oral ingestion. Round-Up was identified as the probable toxic agent in the suicide of a California woman in 2005 (DPR, 2007a).

Barbosa and Leite (2001) reported that a 54-year old man accidentally exposed to glyphosate developed disseminated skin lesions 6 hours after the accident. One month later, the subject developed a symmetrical Parkinsonian syndrome. The researchers acknowledged that it is not possible to exclude the coincidence of the illness with exposure to glyphosate, and the magnetic resonance imaging findings were not compatible with Parkinson's disease.

In two dermal irritation studies, diluted and undiluted Roundup solutions were applied to intact or abraded skin sites of volunteers. Using the undiluted solution, Maibach (1986, as cited in WHO, 1994) found erythema in 1/24 subjects for the intact skin sites and erythema in 10/24 subjects for the abraded skin sites. The researcher also noted that 4/24 subjects showed an equivocal reaction. However, some glyphosate products are in toxicity category I and II for primary eye irritation and dermal irritation, based on animal testing of the formulations. Applicator exposures to glyphosate formulations have resulted in many reports of minor skin and eye irritation (U.S. EPA 1993; Bradberry *et al.*, 2004). Glyphosate is among the more common pesticides named in pesticide illness reports in California (DPR, 2007b).

Ecological and epidemiological studies

Goldstein *et al*. (2002) reviewed illnesses reports related to glyphosate exposure for the years 1982-1997. Using the data in the California Environmental Protection Agency Pesticide Illness Surveillance Program, they found most of the cases involved topical irritation of the eye, skin, upper airway or combinations of these sites. They noted 187 cases out of a total of 815 reported illnesses also included systemic symptoms, such as nausea, vomiting, diarrhea, headache, and fever. According to Goldstein *et al*, 140 cases were classified as "possibly" related to exposure and the remaining 47 cases as having probably or definite relationship to exposure. Of the 47 cases, Goldstein *et al*. found only 22 cases as probably or definitely related to glyphosate exposure alone.

Hardell *et al*. (2002) studied the association between exposure to pesticides and non-Hodgkin's lymphoma or hairy cell leukemia in a case-control study. They matched each of 563 Swedish patients diagnosed during 1987-1990 with two or four controls obtained from the general population, and evaluated previous pesticide use over many years with a questionnaire. They reported a significant association for glyphosate (odds ratio of 3.04,

95 percent confidence interval 1.08-8.52). The data set is weakened by the fact that there were only 8 glyphosate-exposed cases, as well as the potential for recall bias in this type of study.

Arbuckle *et al*. (2001) studied the association of pesticide exposure with spontaneous abortion in 2,110 farm couples in Ontario, Canada. Women (44 years old or younger) were asked to recall all their pregnancies, including spontaneous abortions. The study involved a total of 3,936 pregnancies and 395 spontaneous abortions. The researchers obtained pesticide exposure information from the farm operator and the couple to construct a history of monthly agricultural and residential pesticide use. Among the many pesticides investigated, Arbuckle *et al*. found that preconception exposure (3 months before and up to the month of conception) to glyphosate increased the risk of both early (<12 weeks) and late (12-19 weeks) spontaneous abortions (crude odd ratio = 1.4 (95 percent confidence interval 1.0-2.1). The researchers cautioned that the data should be interpreted with care because of several limitations. Dose information was not available and misclassification of exposure is possible. Due to the different ways pesticides were handled and used, there could be significant variability in the degree of exposure among the study population. Also, due to the nature of the study, recall bias and interaction between two or more pesticides might have affected the results.

Savitz *et al*. (1997) used the Ontario Farm Family Health Study data to investigate the relationship between male farm activities and reproductive outcomes such as miscarriage, preterm delivery, and small-for-gestational-age births. The combination of engaging in pesticide activities and reported use of specific chemicals produced some elevated risk estimates. For instance, crop herbicide activity combined with glyphosate yielded an odds ratio of 2.4 (after adjustment for a number of characteristics of the mother). However, the lack of reliable exposure information, the potential of recall bias, and the small number of exposed cases (5) make the interpretation difficult.

A similar study was reported by Garry *et al*. (2002). The researchers conducted a study in 1997-1998 of 695 families and 1,532 children in Minnesota that used pesticides for farming. The subjects were interviewed by phone and by written questionnaire. Parent-reported reproductive health information was confirmed through birth certificate and medical records examination. The researchers investigated the association between pesticide usage and birth defects identified in the first year of life and later. Inclusion of children diagnosed with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Garry *et al*. reported a tentative association between attention-deficit disorder/attention-deficit hyperactivity disorder and use of glyphosate (an odds ratio of 3.6, 95 percent confidence interval 1.3-9.6), as well as an increased odds ratio (2.48, 95 percent confidence interval 1.2-5.1) for adverse neurologic and neurobehavioral developmental effects among children born to applicators of the fumigant phosphine. However, small number of subjects, exposures to multiple chemicals, difficulties in diagnosis, and the possibility of recall bias limit the interpretation of this study. The researchers also noted that there is little evidence of neurotoxicity of glyphosate other than by intentional ingestion.

De Doos *et al*. (2005) reported a study on cancer incidence among glyphosate applicators in the U.S. They evaluated data in the Agricultural Health Study, a prospective cohort

study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrollment (1993-1997). Incident cancers were identified for the time period from the date of enrollment until 31 December 2001. Among private and commercial applicators, 75.5 percent reported having ever used glyphosate. The authors found glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes that were studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the future. However, potential bias in subject selection, small number of cases, known association of multiple myeloma with farming occupation, and the possibility of some unknown confounders decrease the confidence in the result.

DOSE-RESPONSE ASSESSMENT

Carcinogenic Effects

In 1985, glyphosate was first classified as a Group C carcinogen (possible human carcinogen) based on an inadequate rat carcinogenicity study (high dose less than the maximum tolerated dose) and an equivocal renal tumor response in a mouse carcinogenicity study. U.S. EPA re-examined the mouse renal tumor slides and changed the glyphosate classification to Group D (not classifiable as to human carcinogenicity) in 1986. However, U.S. EPA required the registrant to repeat the rat study because of the equivocal cancer toxicity data. Following review of the new rat study, U.S. EPA's peer review committee classified glyphosate as a Group E chemical (evidence of noncarcinogenicity) because the tumors observed (pancreatic islet and thyroid C cell adenomas in rats and renal epithelial cell hyperplasia in mice) were not considered to be compound-related and the studies of glyphosate genotoxicity were negative (Fed Reg, 1997). In its 2004 review of the toxicity of glyphosate, WHO (2004) found the chemical has no genotoxic potential and there is no evidence of carcinogenicity in rats or mice. Therefore, no dose-response assessment was conducted for glyphosate carcinogenicity in developing the PHG.

Noncarcinogenic Effects

In the absence of adequate human data, a reference dose (RfD) is generally calculated by U.S. EPA from the most sensitive endpoint in a long-term mammalian toxicology study. An RfD, as defined by the U.S. EPA, is an estimate of a daily exposure to the human population that is likely to be without appreciable effect. It is calculated by dividing a NOAEL by an uncertainty factor (UF). A factor of 100 is used as the default, representing one factor of 10 to account for the extrapolation of animal data to humans and another factor of 10 to account for human variability in susceptibility to toxic chemicals.

The U.S. EPA RfD of 0.1 mg/kg was based on the three-generation rat reproduction study (Bio/Dynamics Inc., 1981b) with a NOAEL of 10 mg/kg and an UF of 100. The

NOAEL was based on renal tubular dilation in F_{3b} pups at the next higher dose of 30 mg/kg. This RfD is the basis for U.S. EPA's drinking water equivalent level (U.S. EPA, 1992a) and the current Maximum Contaminant Level Goal (MCLG) and MCL (U.S. EPA, 1996) of 700 ppb. In an earlier California risk assessment, the Department of Health Services (DHS) used the same RfD and critical study in calculating a proposed California MCL (PMCL) (DHS, 1989).

In a more recent two-generation rat reproduction study (Monsanto, 1990b), no histopathological effects on kidneys of F_{2b} pups were observed at a much higher dose level (30,000 ppm in diet). The NOAEL from this study was 10,000 ppm (approximately 720 mg/kg-day) based on decreased body weights and soft stool in the next higher dose group. Therefore, the results from this study suggest that the renal changes in the three-generation rat reproduction study were not compound-related. In addition, other toxicity studies do not support that the renal effects are compound-related.

U.S. EPA's most recently-developed RfD of 2 mg/kg (Fed Reg, 1997) is based on a maternal NOAEL of 175 mg/kg and an UF of 100 in a rabbit study (IRDC, 1980b). The NOAEL is based on maternal mortality at the next higher dose. A recent review of glyphosate considered the rabbit teratology study with a NOAEL of 175 mg/kg-day as the appropriate basis for toxicological evaluation in humans (WHO, 1994). The RfD of 2 mg/kg-day used by the U.S. EPA Office of Pesticide Program is also based on this study.

The OEHHA evaluation has also concluded that the rabbit teratology study of IRDC (1980b) provides the most appropriate endpoint for our risk assessment for glyphosate in drinking water. The maternal NOAEL in this study was 175 mg/kg-day. At the highest dose (350 mg/kg-day), there was treatment-related diarrhea, nasal discharge and early mortality. No teratological effects or other significant toxicity was observed in offspring.

CALCULATION OF PHG

For estimation of a health-protective concentration of glyphosate in drinking water, an acceptable daily dose of the chemical from all sources will first be calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the critical toxic dose from human or animal studies to the estimation of a lifetime acceptable daily dose (ADD) that is unlikely to result in any toxic effects. For this purpose, the following equation will be used:

$$\text{ADD} = \frac{\text{NOAEL/LOAEL in mg/kg-day}}{\text{UF}}$$

where,

ADD = an estimate of the maximum daily dose which can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL = no-observed-adverse-effect level or lowest-observed-adverse-effect level in the critical study;

UF = uncertainty factor.

For glyphosate, the no-observed-adverse-effect-level of 175 mg/kg-day for diarrhea and increased maternal mortality from the IRDC (1980b) rabbit teratology study is used. The combined uncertainty factor is 1,000, which includes 10-fold for inter-species variation, 10-fold for human variability and 10-fold for the severity of the endpoint (mortality) and the short exposure duration. Thus,

$$\text{ADD} = \frac{175 \text{ mg/kg-day}}{1{,}000} = 0.175 \text{ mg/kg-day}$$

Calculation of a public health-protective concentration (C, in mg/L) for glyphosate in drinking water uses the following equation for noncarcinogenic endpoints:

$$C = \text{ADD mg/kg-day} \times \text{BW/WC} \times \text{RSC}$$

where,

BW/WC = the ratio of body weight (kg) and tap water consumption rate (L/day) for the 95th percentile of the pregnant woman population, estimated to be 25.2 kg-day/L (OEHHA, 2000); and

RSC = relative source contribution (usually 20 to 80 percent (0.20 to 0.80), and the lower default value of 0.2 in this case;

Therefore,

$$C = 0.175 \text{ mg/kg-day} \times 25.2 \text{ kg-day/L} \times 0.2$$
$$= 0.88 \text{ mg/L} = 900 \text{ ppb (rounded)}$$

Based on the results of this calculation, OEHHA has derived a public health goal of 900 ppb for glyphosate in drinking water. This PHG is slightly lower than the value published by our office in 1997 of 1,000 ppb, and slightly higher than the U.S. EPA MCL of 700 ppb. The value is judged to be protective of potential sensitive subpopulations, including pregnant women and their fetuses, infants and children, and the elderly.

RISK CHARACTERIZATION

Glyphosate is relatively low in toxicity. In most of the short-term and long-term toxicity studies, reduced body weight, increased liver weights, ocular lesion, and cytoplasmic

changes in the parotid and submandibular salivary glands were observed. These effects were observed at ≥ 350 mg/kg-day dose levels. Glyphosate is not considered to be a mutagen; currently, it is identified as a Group E chemical (evidence of no carcinogenic effects for humans) by U.S. EPA (Fed Reg, 1997). Glyphosate is not a teratogen or a reproductive toxicant, but early maternal death was observed at 350 mg/kg-day in the rabbit teratology study on which the PHG is based. The increased mortality in female rabbits may be due to species-specific sensitivity to glyphosate and/or an increase in sensitivity during pregnancy. Mortality was not observed at much higher dose levels in chronic studies in rats and mice.

The other endpoint of concern is reduced sperm concentration as observed in the subchronic study of Yousef *et al.* (1994). In this study, reduced sperm concentrations were observed at both of the levels tested (1/100 LD_{50} or 1/10 LD_{50}) and therefore no NOAEL was identified. This study had only four rabbits per dose group and the LD_{50} value on which the doses were based and the actual doses administered were not specified. Due to these limitations, the study was not selected for the development of the PHG. Significant reduction in the sperm concentration (20 percent) was also identified in the NTP (1992) study at the high doses of 1,678 and 3,393 mg/kg-day in rats. This toxic effect in the male reproductive system warrants further study.

There are no human data on which to develop a PHG for glyphosate. The human epidemiological studies do not substantiate any effects of population exposures to glyphosate in its use as an herbicide. The PHG for glyphosate is based on diarrhea and increased mortality observed in pregnant rabbits in a teratology study, with a NOAEL of 175 mg/kg-day. In estimating a PHG from animals for application to humans there is an inherent assumption that the data obtained in animals are relevant to humans. An UF of 100 is used to account for inter- and intra-species variation. An additional UF of 10 is added because of the use of a severe endpoint (mortality) from a short-term exposure study (teratology). It should be noted that toxicity tests have been conducted in young and developing laboratory animals and no extra sensitivity, relative to adults, has been observed. No other more susceptible subgroups have been identified in laboratory or epidemiological studies.

In derivation of the PHG, the upper 95th confidence limit for ratio of body weight to drinking water consumption rate of a pregnant female (OEHHA, 2000) was used in the calculation because the critical study involves adverse health effects observed in pregnant females. Relative source contribution was assumed to be 20 percent because glyphosate-containing herbicides are commonly used in residential, commercial, and agricultural settings. Thus it is expected that drinking water will be a relatively minor proportion of total exposure to glyphosate. The RSC value we used is identical to that used by U.S. EPA in deriving the glyphosate MCLG, and is also consistent with current U.S. EPA policy recommendations (U.S. EPA, 2000).

OTHER REGULATORY STANDARDS

The federal MCL of glyphosate in drinking water is 700 ppb (U.S. EPA, 1992a). This value has not been updated to make it consistent with the U.S. EPA's revised RfD (Fed

Reg, 1997). The states of California, Arizona, and Maine all have a drinking water regulatory level of 700 ppb (HSDB, 2005), based on the federal level.

The U.S. EPA has completed a reregistration eligibility document for glyphosate isopropyl amine use as an herbicide (U.S. EPA, 1993). The allowable tolerances of glyphosate and its metabolites in or on produce range from 0.2 ppm to 200 ppm (HSDB, 2005).

WHO (2005) reviewed the toxicological information on AMPA, a major biodegradation product of glyphosate, and derived a health-based drinking water value of 0.9 mg/L or 900 ppb.

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World Health Organization (WHO)

Glyphosate and AMPA in Drinking-water

June 2005

WHO/SDE/WSH/03.04/97
English only
(updated June 2005 to include additional sentence
in section 3.2 and new reference (Kjaer et al., 2004)

Glyphosate and AMPA in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

WHO information products on water, sanitation, hygiene and health can be freely downloaded at:
http://www.who.int/water_sanitation_health/

Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared/updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants examined in drinking-water.

For each chemical contaminant or substance considered, a lead institution prepared a health criteria document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the requested health criteria documents.

Under the responsibility of the coordinators for a group of chemicals considered in the guidelines, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors before the documents were submitted for final evaluation by the experts meetings. A "final task force" meeting reviewed the health risk assessments and public and peer review comments and, where appropriate, decided upon guideline values. During preparation of the third edition of the GDWQ, it was decided to include a public review via the world wide web in the process of development of the health criteria documents.

During the preparation of health criteria documents and at experts meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the joint FAO/WHO Meetings on Pesticide Residues and the joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO internet site and in the current edition of the GDWQ.

Acknowledgements

The first draft of Glyphosate and AMPA in Drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality*, was prepared by Dr P. Toft, Canada, to whom special thanks are due.

The work of the following working group coordinators was crucial in the development of this document and others in the third edition:

Mr J.K. Fawell, United Kingdom (*Organic and inorganic constituents*)
Dr E. Ohanian, Environmental Protection Agency, USA (*Disinfectants and disinfection by-products*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Dr P. Toft, Canada (*Pesticides*)
Prof. Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Treatment achievability*)

The contribution of peer reviewers is greatly appreciated. The draft text was posted on the world wide web for comments from the public. The revised text and the comments were discussed at the Final Task Force Meeting for the third edition of the GDWQ, held on 31 March to 4 April 2003, at which time the present version was finalized. The input of those who provided comments and of participants in the meeting is gratefully reflected in the final text.

The WHO coordinators were as follows:

Dr J. Bartram, Coordinator, Water Sanitation and Health Programme, WHO Headquarters, and formerly WHO European Centre for Environmental Health
Mr P. Callan, Water Sanitation and Health Programme, WHO Headquarters
Mr H. Hashizume, Water Sanitation and Health Programme, WHO Headquarters

Ms C. Vickers provided a liaison with the International Chemical Safety Programme, WHO Headquarters.

Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

ADI	acceptable daily intake
AMPA	aminomethylphosphonic acid
CAS	Chemical Abstracts Service
FAO	Food and Agriculture Organization of the United Nations
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LD_{50}	median lethal dose
NOAEL	no-observed-adverse-effect level
USA	United States of America
WHO	World Health Organization

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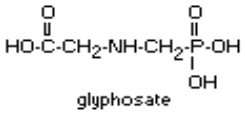
1. GENERAL DESCRIPTION

1.1 Identity

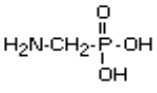
	Glyphosate	*AMPA*
CAS No.:	1071-83-6	1066-51-9
Molecular formula:	$C_3H_8NO_5P$	CH_6NO_3P

The IUPAC name for glyphosate is *N*-(phosphonomethyl)glycine. Glyphosate is a weak organic acid; it consists of a glycine moiety and a phosphonomethyl moiety.

The primary degradation product of glyphosate in plants, soil and water is aminomethylphosphonic acid (AMPA), whose chemical structure is very similar to that of glyphosate (see below).



glyphosate



AMPA

1.2 Physicochemical properties of glyphosate (IPCS, 1994)

Property	*Value*
Vapour pressure	$<10^{-5}$ Pa at 25 °C (negligible)
Melting point	185 °C (decomposes at 199 °C)
Log *n*-octanol/water partition coefficient	-2.8
Water solubility	10.1 g/litre at 20 °C
Specific gravity	1.70 g/cm^3

1.3 Major uses

Glyphosate is a broad-spectrum post-emergence herbicide. It has a high activity when applied to foliage, and it is used worldwide in both agriculture and forestry. Glyphosate is also used for aquatic weed control (IPCS, 1994). AMPA has no commercial use.

1.4 Environmental fate

Glyphosate is strongly bound to soil particles and is not taken up by the roots of plants. It is metabolized very little by plants, the major metabolite being AMPA. Glyphosate readily translocates from treated foliage to other parts of the plant. Residues from treated weeds passing into the soil are not taken up by other plants (FAO/WHO, 1986).

Microbial biodegradation of glyphosate occurs in soil, aquatic sediment and water. The main route of biodegradation of glyphosate appears to be by splitting the C–N bond to produce AMPA, the principal microbial metabolite; AMPA is also

biologically degradable, with liberation of carbon dioxide. Degradation occurs more rapidly in aerobic than in anaerobic conditions. Half-lives for biodegradation in soil vary widely and range between a few days and several months; in water, half-lives between 12 h and 7 weeks have been measured (CCME, 1989).

Glyphosate is chemically stable in water and is not subject to photochemical degradation (FAO/WHO, 1986). The low mobility of glyphosate in soil indicates a minimal potential for the contamination of groundwater. Glyphosate can, however, enter surface and subsurface waters by direct use near aquatic environments or by runoff or leaching from terrestrial applications. This has been substantiated by reports that indicate the presence of glyphosate residues in water from direct overspray in forestry operations, from runoff and from irrigation canal discharges. Furthermore, the possibility of aquatic contamination from drift during agricultural or silvicultural applications also exists. Depending upon the suspended solids loading and the microbial activity of flowing water, glyphosate may be transported several kilometres downstream from the site of aquatic application (CCME, 1989).

Glyphosate is not expected to bioaccumulate in food in view of its high water solubility and its ionic character. Although residues of glyphosate were found in fish, crustaceans and molluscs after exposure to water containing glyphosate, residues declined to about 50–90% of the accumulated levels when these aquatic organisms were subsequently exposed to water free from glyphosate for 14–28 days (FAO/WHO, 1986).

2. ANALYTICAL METHODS

Various analytical methods for the determination of glyphosate have been described, including thin-layer chromatography, high-performance liquid chromatography and gas chromatography–mass spectrometry. The limits of determination were 0.02–50 µg/litre in water, 0.05–1 mg/kg in soil, 0.01–0.05 mg/kg in plants and about 0.3 µg/m^3 in air. The limit of determination of AMPA in water is reported to be 1.2 µg/litre (IPCS, 1994).

3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

3.1 Air

Concentrations in air are available only from studies on exposures of workers involved in application of the herbicide. Air concentrations during silvicultural spraying were mostly below 1.3 µg/m^3; the highest value observed was 15.7 µg/m^3. The highest estimated exposure (dermal and inhalation) of about 8000 µg/h, as reported in a study with spray applicators, corrected for incomplete absorption, equals about 40 µg/kg of body weight per day (8-h working day for a 60-kg adult) (IPCS, 1994).

3.2 Water

In a survey conducted in 1988–1989 in the Netherlands, surface water contained 0.5–1 µg of glyphosate per litre and 6 µg of the metabolite AMPA per litre (IPCS, 1994). In Canada, glyphosate residues as high as 5153 µg/litre were measured after direct aerial application over lakes, ponds or streams. Glyphosate concentrations in water declined to a few µg/litre or to non-detectable levels hours or days post-treatment, depending on the extent of vegetation present. The concentration of AMPA in water without substantial vegetation was about 3 µg/litre (CCME, 1989). In the USA, pond water contained 90–1700 µg of glyphosate per litre and 2–35 µg of AMPA per litre, whereas stream water contained 35–1237 and <1.0–10 µg of glyphosate and AMPA, respectively, per litre (IPCS, 1994). Intensive monitoring studies over a number of years in Denmark have identified glyphosate and AMPA in the root zone and in groundwater at monitoring sites; however, the concentrations in groundwater were less than 0.1 µg/litre (Kjaer et al., 2004).

3.3 Food

No information was available on direct measurements of glyphosate in foodstuffs (as part of food surveillance) or total diets. The only information available comes from residue levels resulting from supervised trials. In pre-planting use of glyphosate, residues of glyphosate and its metabolite were not detected (<0.05 mg/kg) in cereal grains at harvest. Pre-harvest application of glyphosate to cereals and pulses resulted in mean residue levels ranging from 0.2 to 4.8 mg/kg, when the glyphosate was used according to good agricultural practice. Industrial processing of wheat to flour resulted in a decrease in glyphosate level from 1.6 to 0.16 mg/kg (FAO/WHO, 1986).

Fish exposed to water containing 10 mg of glyphosate per litre for 14 days contained 0.2–0.7 mg of glyphosate per kg. Residues were reduced when fish were exposed to glyphosate-free water. In controlled feeding studies, mean residues of glyphosate found in muscle tissues of pigs, poultry and cattle were <0.05 mg/kg. Livers of these animals contained up to 0.12 mg/kg, whereas residues in cattle milk were not detectable (FAO/WHO, 1986).

3.4 Estimated total exposure and relative contribution of drinking-water

Use of glyphosate as a herbicide may result in the presence of residues in air, drinking-water, crops and animal tissues destined for human consumption. Main routes of exposure to glyphosate are expected to be inhalation and dermal exposure in the occupational setting and consumption of water and food for the general population. Because of its sorption to particulate matter and its microbial degradation in the aquatic environment (CCME, 1989), the major source of exposure to glyphosate is expected to be food.

4. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

The results of oral studies with [^{14}C]glyphosate in rats, rabbits and goats indicate that absorption from the gastrointestinal tract is incomplete and amounts to approximately 30% of the dose or less.

On day 7 after administration of a single oral dose of [^{14}C]glyphosate to rats, the isotope was widely distributed throughout the body, with the highest concentration found in the bones.

Biotransformation of glyphosate occurs to a very low degree only. In rats, it was shown that almost all of the ^{14}C in urine and faeces, after a single oral administration of [^{14}C]glyphosate, was present as unchanged parent compound. Elimination through exhaled air is very low. AMPA was the only metabolite, accounting for only 0.2–0.3% of the applied dose of [^{14}C]glyphosate (IPCS, 1994).

In a study of the metabolic fate of AMPA in rats, AMPA was only moderately absorbed (approximately 20%); excretion was almost exclusively via the urine, with less than 0.1% of the dose expired as carbon dioxide (FAO/WHO, 1987).

5. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

5.1 Acute exposure

Glyphosate and its formulations have very low acute toxicity by the oral and dermal administration routes. Median oral lethal doses (LD_{50}s) of glyphosate range from 1950 to >5000 mg/kg of body weight for mice, rats and goats (IPCS, 1994). Glyphosate has been classified by WHO (1996) as unlikely to present an acute hazard in normal use.

5.2 Short-term exposure

In a 13-week feeding study, groups of 15 male and 15 female Charles River CD-1 mice were fed technical glyphosate (purity 98.7%) in their diet at dose levels of 0, 0.5, 1.0 or 5.0%. No effect on appearance or survival was observed. Growth retardation and increased weights of brain, heart and kidneys were observed at 5.0%. Liver weights were increased at 1.0% and 5.0%. Limited histopathology showed no adverse effects. The authors of the study concluded that the NOAEL was 1.0% glyphosate in the diet, equal to 1890 mg/kg of body weight per day (Bio/Dynamics Inc., 1979; FAO/WHO, 1987; IPCS, 1994).

In a 13-week feeding study, Sprague-Dawley rats received 0.1, 0.5 or 2% technical glyphosate in their diet. No effects on appearance, survival or growth were observed. Haematology, blood biochemistry and urinalysis, carried out at test end only, were also unaffected. Organ weights determined for liver, kidneys and testes were not affected. Limited histopathology showed no adverse effect in any tissue. The NOAEL

in this study was 2% glyphosate in the diet (the highest dose tested), equal to 1267 mg/kg of body weight per day (Monsanto, 1987).

Two further 13-week studies in rodents were conducted. Both mice ($B6C3F_1$) and rats (F-344/N) were administered glyphosate (purity approximately 99%) in feed at levels of 0, 3125, 6250, 12 500, 25 000 or 50 000 mg/kg (NTP, 1992). In mice, reduced weight gains were observed at 50 000 mg/kg of diet in both sexes. Dose-dependent lesions in the parotid gland were observed at 6250 mg/kg of feed and higher but were not seen at the lowest dose level tested. The NOAEL in this study was 3125 mg/kg of feed, equal to 507 mg/kg of body weight per day (NTP, 1992).

In rats, reduced weight gains were observed in males at 25 000 mg/kg of feed and in both sexes at 50 000 mg/kg of feed. Clinical chemistry showed increased alkaline phosphatase and alanine aminotransferase at 6250 mg/kg of feed in males and at 12 500 mg/kg of feed in females. Decreases in sperm count were observed in males at 25 000 and 50 000 mg/kg of feed. Cytoplasmic alterations of the parotid and submandibular salivary glands, consisting of basophilic changes and hypertrophy of acinar cells, were observed. Effects on the salivary glands were observed at the lowest dose tested (3125 mg/kg of feed, equal to 205 mg/kg of body weight per day for males and 213 mg/kg of body weight per day for females). Thus, a NOAEL could not be identified in this study (NTP, 1992).

Groups of six male and six female beagle dogs were administered technical glyphosate (96.1% pure) in gelatin capsules at dose levels of 0, 20, 100 or 500 mg/kg of body weight per day for 52 weeks. No effects were observed with respect to clinical signs, body weight, feed consumption, ophthalmoscopy, haematology, urinalysis, gross pathology and histopathology. The NOAEL in this study was 500 mg/kg of body weight per day, the highest dose tested (FAO/WHO, 1987; IPCS, 1994).

5.3 Long-term exposure and carcinogenicity

In a combined chronic toxicity and carcinogenicity study, groups of Charles River CD-1 mice (50 per sex per group) were fed technical glyphosate in the diet for 24 months at levels of 0, 0.1, 0.5 or 3.0%. No effect on survival or appearance was noted. Body weights were decreased in the males of the high-dose group. Haematology and organ weights showed no effects. Histopathology in liver revealed an increased incidence of central lobular hepatocyte hypertrophy and hepatocyte necrosis among high-dose males. Hyperplasia of the urinary bladder was increased in frequency in mid- and high-dose males (incidences: 3/49, 3/50, 10/50, and 8/50), but not in treated females. There were no statistically significant increases in the frequency of neoplastic lesions. The NOAEL in this study was 0.5% glyphosate, equal to 814 mg/kg of body weight per day (Bio/Dynamics Inc., 1983).

Groups of Charles River Sprague-Dawley rats (50 per sex per dose) were fed technical glyphosate in their diets at dose levels of about 0, 3, 10 or 32 mg/kg of body weight per day for 26 months. Survival, appearance, haematology, blood

biochemistry, urinalysis and organ weights were not changed. Slight growth retardation during part of the study was noted in the high-dose males. The incidence of interstitial cell tumours in testes showed a statistically significant increase (incidences: 0/50, 3/50, 1/50 and 6/50; historical control range: 3–7%) (Bio/Dynamics Inc., 1981a). This finding, in itself constituting evidence of a carcinogenic effect in rats, should be judged in light of the absence of an effect at much higher dose levels in the more recent 2-year study in rats (see below). This is also valid for the slight growth retardation. The NOAEL was 32 mg/kg of body weight per day, the highest dose tested (Bio/Dynamics Inc., 1981a).

In the recent 2-year study, groups of Charles River Sprague-Dawley rats (60 per sex per dose) were fed technical glyphosate in their diets at dose levels of about 0, 100, 410 or 1060 mg/kg of body weight per day for 24 months. There was no effect on survival or appearance. Growth was retarded in the high-dose females. Haematology and blood biochemistry showed no effects. In the high-dose males, the urine specific gravity and urine pH were increased. A statistically significant increased incidence of degenerative lens changes was found among the high-dose males; however, this finding was within the historical control range. Liver weights were increased in the high-dose males only. Increased incidence of inflammation of the gastric squamous mucosa was observed in the mid- and high-dose groups (incidences in males: 2/58, 3/58, 5/59 and 7/59; females: 0/59, 3/60, 9/60 and 6/59; historical range: 0–13.3%). The incidence of pancreatic islet cell adenomas was increased (statistically significant) among low- and high-dose animals. However, these effects were within the historical control range. No pancreatic carcinomas were found. The NOAEL in this study was 410 mg/kg of body weight per day (Monsanto, 1990a).

5.4 Reproductive and developmental toxicity

Groups of female Charles River CD-1 rats were administered technical glyphosate by gavage at dose levels of 0, 300, 1000 or 3500 mg/kg of body weight per day on days 6–19 of gestation. At 3500 mg/kg of body weight per day, the following effects were observed: increased incidence of soft stools, diarrhoea, breathing rattles, red nasal discharge, reduced activity, increased mortality (6/25 dams dying before the end of the treatment period), growth retardation, increased incidence of early resorptions, decreases in total number of implantations and the number of viable fetuses, and increased number of fetuses with reduced ossification of sternebrae. At the lower dose levels, these effects were absent. The NOAEL in this study was 1000 mg/kg of body weight per day (IRDC, 1980a).

Groups of 16 female Dutch belted rabbits received technical glyphosate by gavage in 0.5% Methocel at dose levels of 0, 75, 175 or 350 mg/kg of body weight per day on days 6–27 of gestation. The control group received the vehicle only. The incidence of diarrhoea and soft stools was increased in the high-dose group and also, to a slight degree, in the mid-dose group. The incidence of nasal discharge was increased in the high-dose group only. In the mid- and high-dose groups, 2 and 10 dams, respectively, died during the study from unknown causes. The IPCS Task Group concluded that the NOAEL was 175 mg/kg of body weight per day (IRDC, 1980a; IPCS, 1994).

In a three-generation study, groups of Sprague-Dawley rats were given glyphosate (98.7% pure) in the diet at doses of 0, 3, 10 or 30 mg/kg of body weight per day for 60 days. The only effect noted was an increased incidence of unilateral renal tubular dilation in the F_{3b} male pups of the high-dose group (incidence not determined in mid-dose group; earlier litters not examined). The NOAEL in this study was 30 mg/kg of body weight per day, the highest dose tested (Bio/Dynamics Inc., 1981b; IPCS, 1994).

In a more recent two-generation feeding study, Sprague-Dawley rats received glyphosate at doses of 0, 100, 500 or 1500 mg/kg of body weight per day. Soft stools and decreased body weights in parent animals and slightly decreased litter size and pup weights were seen in the high-dose group. Decreased body weights of parents and pups were seen to a slight degree in the mid-dose group. No histological effect on kidneys was present in the F_{2b} male pups (15 and 23 pups examined in control and high-dose groups, respectively; first generation and F_{2a} pups not examined). The NOAEL in this study was 500 mg/kg of body weight per day (Monsanto, 1990b; IPCS, 1994).

In its evaluation of these latter two reproductive toxicity studies, the IPCS Task Group noted that the number of pups submitted to histopathological examination in both studies was limited. These limitations made it difficult to evaluate the renal effect seen in pups at 30 mg/kg of body weight per day in the Bio/Dynamics Inc. (1981b) study (IPCS, 1994).

5.5 Mutagenicity and related end-points

Glyphosate was consistently without mutagenic effect in a range of genotoxicity assays *in vitro* and *in vivo* (IPCS, 1994).

5.6 Toxicity of AMPA[1]

AMPA is slightly hazardous to rats given a single oral dose, with an LD_{50} of 8300 mg/kg of body weight (WHO, 1996).

In a 90-day study of toxicity, rats received AMPA in the diet at 0, 400, 1200 or 4800 mg/kg of body weight per day. A significant, dose-related decrease in body weight gain was seen in males at the two highest doses and in females at the highest dose. The two highest doses also resulted in significantly increased lactate dehydrogenase activity, whereas aspartate aminotransferase activity and cholesterol levels were significantly increased only at the highest dose. Urinalysis showed a significant decrease in urinary pH and increased amounts of calcium oxalate crystals in the urine of animals at the highest dose.

Dose-related irritation of the mucosal and submucosal layers of the urinary tract, corresponding to hyperplasia of the urinary bladder, was seen in rats at 1200 and 4800

[1] This section was taken from FAO/WHO (1998).

mg/kg of body weight per day, the effect being more marked in males than in females. In addition, epithelial hyperplasia in the renal pelvis was observed at the highest dose. The NOAEL was 400 mg/kg of body weight per day.

In a 90-day study of toxicity in dogs receiving AMPA at 0, 10, 30, 100 or 300 mg/kg of body weight per day in gelatin capsules, no statistically significant treatment-related changes were observed. The NOAEL was thus the highest dose, 300 mg/kg of body weight per day. It should be noted that in a 1-month range-finding study with groups of only two male and two female dogs, changes in some haematological parameters (e.g., decreased haemoglobin, packed cell volume and erythrocyte counts) were seen in animals at 300 or 1000 mg/kg of body weight per day. These effects were not reproduced in the 90-day study.

No indication of genotoxic activity was seen in studies of gene mutation in bacteria, of DNA repair in bacteria and mammalian cells *in vitro* or of micronucleus formation *in vivo*. No assays for gene mutation were performed in mammalian cells *in vitro*, but the structural similarity of AMPA to glyphosate and the lack of genotoxicity of glyphosate, including in an assay for gene mutation in mammalian cells *in vitro*, indicate that such an assay with AMPA would be redundant.

In a study of developmental toxicity, rats received AMPA at 0, 150, 400 or 1000 mg/kg of body weight per day in corn oil by gavage. Dose-related increases in the incidences of soft stools, mucoid faeces and hair loss were seen in dams at the two higher doses. Dams at the highest dose also had short periods of decreased body weight gain and food consumption. Fetal body weight was decreased at 1000 mg/kg of body weight per day. No teratogenic effects were observed. Dams at 150 mg/kg of body weight per day also had an increased incidence of soft stools; however, in the absence of any associated effects, such as hair loss or mucoid faeces, the Meeting considered this dose to be the NOAEL for maternal toxicity. The NOAEL for developmental toxicity was 400 mg/kg of body weight per day.

AMPA did not induce dermal or ocular irritation in rabbits.

No long-term study of the toxicity or carcinogenicity of AMPA has been carried out, but in the more recent of two such studies with technical-grade glyphosate in rats at dietary levels of 0.2, 0.8 or 2%, the AMPA content of the test compound was given, namely 0.68%. At the highest dose of 2% glyphosate in the diet, females showed decreased body weight gain and males showed an increased incidence of degenerative lenticular changes. The NOAEL for technical-grade glyphosate was 0.8% in the diet, corresponding to 400 mg/kg of body weight per day for glyphosate and 2.7 mg/kg of body weight per day for AMPA. No increase in tumour incidence was seen in this study.

No multigeneration study of the reproductive toxicity of AMPA has been reported, but in a recent two-generation study in rats with technical-grade glyphosate at dietary levels of 0.2, 1 or 3%, the test compound contained 0.61% AMPA. At the highest dose, soft stools, decreased parental body weights, slightly decreased litter sizes and

decreased pup weights were observed. The NOAEL was 1% in the diet, corresponding to 740 mg of glyphosate per kg of body weight per day and 4.5 mg of AMPA per kg of body weight per day.

6. EFFECTS ON HUMANS

Several cases of (mostly intentional) intoxications with technical glyphosate herbicide formulation have been reported. A typical symptom is erosion of the gastrointestinal tract. No compound-related effects were observed in a test group of five applicators prior to and after exposure for 1 week. No controlled studies have been conducted in humans.

7. CONCLUSIONS

Glyphosate and AMPA have very similar chemical structures. Studies of the metabolism of glyphosate in experimental animals indicate that essentially none is biotransformed into AMPA. The 1997 JMPR Meeting (FAO/WHO, 1998) compared the toxicity profile of AMPA with that of glyphosate and concluded that the major targets of the toxicity of AMPA had been investigated. The results showed little toxicity. JMPR concluded that the two compounds have similar toxicological profiles and considered that a full database on AMPA is unnecessary. AMPA was considered to be of no greater toxicological concern than its parent compound.

JMPR established a group ADI for AMPA alone or in combination with glyphosate of 0.3 mg/kg of body weight, based upon a NOAEL of 32 mg/kg of body weight per day, the highest dose tested, identified in a 26-month study of toxicity in rats fed technical-grade glyphosate and using an uncertainty factor of 100. A health-based value of 0.9 mg/litre can be derived based on the ADI of 0.3 mg/kg of body weight, assuming a 60-kg adult consuming 2 litres of drinking-water per day, and allocating 10% of the ADI to drinking-water.

Because of their low toxicity, the health-based value derived for AMPA alone or in combination with glyphosate is orders of magnitude higher than concentrations of glyphosate or AMPA normally found in drinking-water. Under usual conditions, therefore, the presence of glyphosate and AMPA in drinking-water does not represent a hazard to human health. For this reason, the establishment of a numerical guideline value for glyphosate and AMPA is not deemed necessary.

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Monsanto (1987) *90 day study of glyphosate administered in feed to Sprague-Dawley rats.* Unpublished report No. MSL 7575, prepared and submitted to WHO by Monsanto Ltd., Monsanto Environmental Health Laboratory, St. Louis, MO (Project No. ML-86-351/EHL 86128).

Monsanto (1990a) *Chronic study of glyphosate administered in feed to albino rats*. Unpublished report prepared and submitted to WHO by Monsanto Ltd., Monsanto Environmental Health Laboratory, St. Louis, MO (Project No. MSL-10495).

Monsanto (1990b) *Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats.* Unpublished report prepared and submitted to WHO by Monsanto Ltd., Monsanto Environmental Health Laboratory, St. Louis, MO (Project No. MSL-10387).

NTP (1992) *NTP technical report on toxicity studies of glyphosate (CAS No. 1071-83-6).* Research Triangle Park, NC, National Toxicology Program (Toxicity Report Series No. 16).

WHO (1996) *The WHO recommended classification of pesticides by hazard and guidelines to classification 1996–1997*. Geneva, World Health Organization, International Programme on Chemical Safety (WHO/PCS/96.3).

Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

Glyphosate and AMPA in Drinking-water

2004

WHO/SDE/WSH/03.04/97
English only

Glyphosate and AMPA in Drinking-water

Summary statement
Extract from Chapter 12 - Chemical fact sheets of
WHO *Guidelines for Drinking-water Quality, 3rd edition,2004.*

WHO information products on water, sanitation, hygiene and health can be freely downloaded at:
http://www.who.int/water_sanitation_health/

12.65 Glyphosate and AMPA

Glyphosate (CAS No. 1071-83-6) is a broad-spectrum herbicide used in both agriculture and forestry and for aquatic weed control. Microbial biodegradation of glyphosate occurs in soil, aquatic sediment and water, the major metabolite being aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9). Glyphosate is chemically stable in water and is not subject to photochemical degradation. The low mobility of glyphosate in soil indicates minimal potential for the contamination of groundwater. Glyphosate can, however, enter surface and subsurface waters after direct use near aquatic environments or by runoff or leaching from terrestrial applications.

Glyphosate and AMPA have similar toxicological profiles, and both are considered to exhibit low toxicity. A health-based value of 0.9 mg/litre can be derived based on the group ADI for AMPA alone or in combination with glyphosate of 0.3 mg/kg of body weight, based upon a NOAEL of 32 mg/kg of body weight per day, the highest dose tested, identified in a 26-month study of toxicity in rats fed technical-grade glyphosate and using an uncertainty factor of 100.

Because of their low toxicity, the health-based value derived for AMPA alone or in combination with glyphosate is orders of magnitude higher than concentrations of glyphosate or AMPA normally found in drinking-water. Under usual conditions, therefore, the presence of glyphosate and AMPA in drinking-water does not represent a hazard to human health. For this reason, the establishment of a guideline value for glyphosate and AMPA is not deemed necessary.

History of guideline development

The 1958 and 1963 WHO *International Standards for Drinking-water* did not refer to glyphosate, but the 1971 International Standards suggested that pesticide residues that may occur in community water supplies make only a minimal contribution to the total daily intake of pesticides for the population served. Glyphosate was not evaluated in the first two editions of the *Guidelines for Drinking-water Quality*, published in 1984 and 1993. In the addendum to these Guidelines, published in 1998, a health-based value of 5 mg/litre was derived for glyphosate using the ADI derived in the EHC monograph for glyphosate published in 1994. However, the health-based value is orders of magnitude higher than the concentrations normally found in drinking-water. Under usual conditions, therefore, the presence of glyphosate in drinking-water does not represent a hazard to human health, and it was not deemed necessary to establish a guideline value for glyphosate. It was noted that most AMPA, the major metabolite of glyphosate, found in water comes from sources other than glyphosate degradation.

Assessment date

The risk assessment was conducted in 2003.

Principal references

FAO/WHO (1998) *Pesticide residues in food – 1997 evaluations. Part II – Toxicological and environmental*.Geneva, World Health Organization, Joint FAO/WHO Meeting on Pesticide Residues (WHO/PCS/98.6).

IPCS (1994) *Glyphosate.* Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 159).

WHO (2003) Glyphosate and AMPA in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality.Geneva, World Health Organization (WHO/SDE/WSH/03.04/97).

United Nations Food and Agriculture Organization (FAO)

FAO Specifications and Evaluations for Plant Protection Products

2000

FAO SPECIFICATIONS AND EVALUATIONS

FOR PLANT PROTECTION PRODUCTS

GLYPHOSATE

N-(phosphonomethyl)glycine

2000/2001

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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GLYPHOSATE

Disclaimer[1]

FAO specifications are developed with the basic objective of ensuring that pesticides complying with them are satisfactory for the purpose for which they are intended so that they may serve as an international point of reference. The specifications do not constitute an endorsement or warranty of the use of a particular pesticide for a particular purpose. Neither do they constitute a warranty that pesticides complying with these specifications are suitable for the control of any given pest, or for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular application must be decided at the national or provincial level.

Furthermore, the preparation and use of pesticides complying with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable thereto. FAO shall not be liable for any injury, loss, damage or prejudice of any kind that may be suffered as a result of the preparation, transportation, sale or use of pesticides complying with these specifications.

Additionally, FAO wishes to alert users of specifications to the fact that improper field mixing and/or application of pesticides can result in either a lowering or complete loss of efficacy. This holds true even where the pesticide complies with the specification. Accordingly, FAO can accept no responsibility for the consequences of improper field mixing and/or application.

FAO is not responsible for ensuring that any product claimed to comply with FAO specifications actually does so.

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INTRODUCTION

FAO establishes and publishes specifications* for technical material and related formulations of plant protection products with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

Since 1999 the development of FAO specifications follows the **New Procedure**, described in the 5th edition of the "Manual on the development and use of FAO specifications for plant protection products"(FAO Plant Production and Protection Page No. 149). This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by FAO and the Experts of the "FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent."
FAO Specifications now only apply to products for which the technical materials have been evaluated. Consequently from the year 2000 onwards the publication of FAO specifications under the **New Procedure** has changed. Every specification consists now of two parts namely the specifications and the evaluation report(s):

Part One: The Specification of the technical material and the related formulations of the plant protection product in accordance with chapter 4, 5 and 6 of the 5th edition of the "Manual on the development and use of FAO specifications for plant protection products".

Part Two: The Evaluation Report(s) of the plant protection product reflecting the evaluation of the data package carried out by FAO and the Panel of Experts. The data are to be provided by the manufacturer(s) according to the requirements of Appendix A, annex 1 or 2 of the "Manual on the development and use of FAO specifications for plant protection products" and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

FAO Specifications under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other methods of synthesis. FAO has the possibility to extend the scope of the specifications to similar products, but only when the Panel of Experts has been satisfied that the additional products are equivalent to those which formed the basis of the reference specification.

* Footnote: The publications are available on Internet at
http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmps/en/

PART ONE

SPECIFICATIONS

GLYPHOSATE

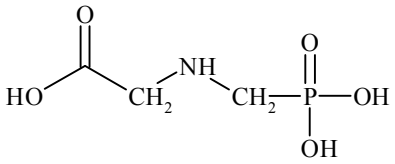
FAO SPECIFICATIONS AND EVALUATIONS FOR
PLANT PROTECTION PRODUCTS

GLYPHOSATE

INFORMATION

COMMON NAME : Glyphosate (ISO)

Structural formula



EMPIRICAL FORMULA: $C_3H_8NO_5P$

RMM: 169

CAS REGISTRY NUMBER: 1071-83-6

CIPAC CODE NUMBER: 284

CHEMICAL NAMES: *N*-(phosphonomethyl)glycine (IUPAC and CA)

GLYPHOSATE ACID TECHNICAL
284/TC (2000/2001)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000 + 2001). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation reports (284/2000 + 2001) as PART TWO forms an integral part of this publication.

.1 DESCRIPTION

The material shall consist of glyphosate (acid), together with related manufacturing impurities. It shall be a white dry powder, free from visible extraneous matter and added modifying agents.

.2 ACTIVE INGREDIENT

.2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, p.2132),

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

.2.2 Glyphosate acid (284/TC/(M)/3, CIPAC 1C, p.2132) (AOAC 983.10, 1990)

The glyphosate acid content shall be declared (not less than 950 g/kg) and, when determined, the mean measured content shall not be lower than the declared minimum content.

.3 RELEVANT IMPURITIES

.3.1 Formaldehyde (Note 1)

Maximum 1.3 g/kg of the glyphosate acid content found under .2.2.

.3.2 *N*-Nitrosoglyphosate (Note 2)

Maximum 1 mg/kg

.3.3 Insolubles in 1 M NaOH (MT 71)

Maximum: 0.2 g/kg

Note 1 The analytical method for determination of Formaldehyde is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 2 The analytical method for determination of N-Nitrosoglyphosate is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

GLYPHOSATE ACID TECHNICAL CONCENTRATES
284/TK (2000/2001)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturers whose names are listed in the evaluation reports (284/2000 + 2001). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation reports (284/2000 + 2001) as PART TWO forms an integral part of this publication.

.1 DESCRIPTION

The material shall consist of glyphosate (acid) together with related manufacturing impurities. It shall be a white to greyish wet cake, free from visible extraneous matter and added modifying agents.

.2 ACTIVE INGREDIENT

2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, p.2132),

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

.2.2 Glyphosate acid (284/TC/(M)/3, CIPAC 1C, p.2132) (AOAC 983.10, 1990)

The glyphosate acid content shall be declared (not less than 950 g/kg on a dry basis) and, when determined, the mean measured content obtained shall not differ from that declared by more than ± 20 g/kg.

.3 IMPURITIES

.3.1 Formaldehyde (Note 1)

Maximum 1.3 g/kg of the glyphosate acid content found under .2.2.

.3.2 *N*-Nitrosoglyphosate (Note 2)

Maximum 1 mg/kg

.3.3 Loss on drying (MT 17.3, Sample weight: 10 g; temperature: 105°C, time: 3 hours.).

The loss on drying shall be declared and, when measured the average loss shall be not more than 200 g/kg.

.3.4 Insolubles in 1 M NaOH (MT 71)

Maximum: 0.2 g/kg, dry weight basis

Note 1 The analytical method for determination of Formaldehyde is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 2 The analytical method for determination of N-Nitrosoglyphosate is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

GLYPHOSATE ISOPROPYLAMINE SALT TECHNICAL CONCENTRATES 284 /TK (2000)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (284/2000). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation report (284/2000) as PART TWO forms an integral part of this publication.

.1 DESCRIPTION

The material shall consist of glyphosate (acid), complying with the requirements of FAO specification 284/TC, together with related manufacturing impurities in the form of the isopropylamine salt, and shall be a solution in water, free from visible extraneous matter and added modifying agents except for the diluent.

.2 ACTIVE INGREDIENT

2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, p.2132)

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

.2.2 Glyphosate acid (284/TC/(M)/3, CIPAC 1C, p.2132) (AOAC 983.10, 1990)

The glyphosate acid content shall be declared, (g/l or g/kg at 20 ± 2°C) and, when determined, the mean measured content shall not differ from that declared by more than the appropriate FAO proposed tolerance as given below:

Declared content in g/kg or g/l at 20±2°C	Tolerance
above 250 up to 500	5 % of the declared content
above 500	25 g/kg or g/l

.3 IMPURITIES

.3.1 Formaldehyde (Note 1)

Maximum 1.3 g/kg of the glyphosate acid content found under .2.2.

.3.2 *N*-Nitrosoglyphosate (Note 2)

Maximum: 1 mg/kg

.3.3 Insolubles in Water (MT 10.2)

Maximum: 0.1 g/kg, dry weight basis

.4 PHYSICAL PROPERTIES

.4.1 pH range (MT 75) (Note 3)

pH 4.5 to pH 6.8

Note 1 The analytical method for determination of Formaldehyde is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 2 The analytical method for determination of N-Nitrosoglyphosate is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

GLYPHOSATE SOLUBLE CONCENTRATES
284/SL (2000)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (284/2000). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation report (284/2000) as PART TWO forms an integral part of this publication.

.1 DESCRIPTION

The material shall consist of a solution of technical glyphosate, complying with the requirements of FAO specification 284/TC in the form of a soluble salt, dissolved in water, together with any necessary formulants.
It shall be in the form of a clear or opalescent liquid, free from suspended matter and sediment, to be applied as a true solution of the glyphosate salt in water.

.2 ACTIVE INGREDIENT

2.1 Identity tests (284/TC/(M)/2, CIPAC 1C, p.2132)

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

.2.2 Glyphosate (284/SL/(M)/3, CIPAC 1C, p.2134) (AOAC 983.10, 1990)

The glyphosate acid content shall be declared for each specific soluble concentrate (g/kg or g/l at 20 2 °C, Note 2) and, when determined, the content measured shall not differ from that declared by more than the following amounts:

Declared content in g/kg or g/l	Tolerance
up to 25	15 % of the declared content
25 to 100	10 % of the declared content
100 to 250	6 % of the declared content
250 to 500	5 % of the declared content
above 500	25 g/kg or g/l
in each range the upper limit is included	

.3 IMPURITIES

.3.1 Formaldehyde (Note 2)

Maximum 1.3 g/kg of the glyphosate acid content found under .2.2.

.3.2 *N*-Nitrosoglyphosate (Note 3)

Maximum 1 mg/kg

.4 **PHYSICAL PROPERTIES** (Note 4)

.4.1 Solution stability (MT 41)

After the stability test at 54°C (.5.2), the product, after dilution with CIPAC Standard Water D and standing for 18 h. at 30 2°C (Note 5), shall give a clear or opalescent solution, free from more than a trace of sediment or, particles produced shall pass through a 45 µm test sieve.

.4.2 Persistent foam (MT 47.2)

Maximum 60 ml after 1 minute.

.5 **STORAGE STABILITY**

.5.1 Stability at 0°C (MT 39.3)

After storage at 0 ± 2°C for 7 days, the volume of solid and/or liquid which separates shall be not more than 0.3 ml.

.5.2 Stability at elevated temperature (MT 46.3)

After storage at 54 ± 2°C for 14 days, the average determined glyphosate content must not be lower than 95 % relative to the determined content found before storage and the product shall continue to comply with .3.3.1, 3.3.2 and .4.1.

Note 1 Where the buyer requires both g/kg and g/l at 20oC then, in case of dispute, the analytical results shall be calculated as g/kg.

Note 2 The analytical method for determination of Formaldehyde is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 3 The analytical method for determination of N-Nitrosoglyphosate is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 4 In the case of isopropylamine salt containing formulations and depending on the climatical conditions the pH of the formulation has to be taken into account

because the equilibrium glyphosate acid-glyphosate monoispropylamine salt-diisopropylamine salt and properties of the formulants added will determine the stability towards crystallisation of glyphosate acid

Note 5 Unless another temperature is specified.

GLYPHOSATE WATER SOLUBLE GRANULES
284/SG (2000)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (284/2000). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for the products of other manufacturers. The evaluation report (284/2000) as PART TWO forms an integral part of this publication.

.1 DESCRIPTION

The material shall consist of granules containing technical glyphosate, complying with the requirements of FAO specification 284/TC, in the form a suitable salt, together with suitable carriers and formulants.
It shall be homogeneous, free from visible extraneous matter and/or hard lumps, free flowing, and essentially non-dusty. The glyphosate salt shall be soluble in water (Note 1). Insoluble carriers and formulants shall not interfere with compliance with .4.2.

.2 ACTIVE INGREDIENT

.2.1 Identity test (284/SG/(M)/2, CIPAC H, p.182), (Note 2)

The active ingredient shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

.2.2 Glyphosate (284/SG/(M)/3, CIPAC H, p.182)

The glyphosate acid or salt content shall be declared (g/kg) and, when determined, the content obtained shall not differ from that declared by more than the following amounts:

Declared content in g/kg	Tolerance
100 to 250	6 % of the declared content
250 to 500	5 % of the declared content
above 500	25 g/kg
in each range the upper limit is included	

.3 IMPURITIES

.3.1 Formaldehyde (Note 2)

Maximum 1.3 g/kg of the glyphosate acid content found under .2.2.

.3.2 *N*-Nitrosoglyphosate (Note 3)

Maximum 1 mg/kg

.4 PHYSICAL PROPERTIES

.4.1 Degree of dissolution and solution stability (MT 179)

Residue of formulation retained on a 75 µm test sieve after dissolution in CIPAC Water D at 30 2°C (Note 4).
Maximum: 2 % after 5 minutes.
Maximum: 0.05 % after 18 hours.

.4.2 Persistent foam (MT 47.2)

Maximum: 40 ml after 1 minute.

.4.3 Dustiness (MT 171)

Essentially non-dusty with a maximum of 15 mg (0.05 % wt) collected dust applying the gravimetric method.

.4.4 Flowability (MT 172)

98 % to pass a 5 mm test sieve after 20 drops of the sieve.

.5 STORAGE STABILITY

.5.1 Stability at elevated temperatures (MT 46.3)

After storage at 54 ± 2°C for 14 days, the average determined glyphosate content shall not be lower than 95 % relative to the determined content found before storage and the product shall continue to comply with .3.1, .3.2, .4.1, 4.3 and 4.4 as required.

Note 1 Glyphosate acid as the sodium- or ammonium salt.

Note 2 The analytical method for determination of Formaldehyde is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 3 The analytical method for determination of N-Nitrosoglyphosate is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here.

Note 4 Unless another temperature is specified.

PART TWO

EVALUATION REPORT(S)

GLYPHOSATE

FAO SPECIFICATIONS AND EVALUATIONS FOR PLANT PROTECTION PRODUCTS

GLYPHOSATE

EVALUATION REPORT 284/2000

EXPLANATION

Glyphosate was scheduled as an existing FAO specification to be reviewed in 1999 under the procedure introduced by FAO in 1998 (FAO Panel, 1998).

The current FAO specifications for glyphosate acid technical concentrates (FAO Specification 284/TK/S, 1991) and glyphosate soluble concentrates (FAO Specification 284/SL/S , 1991) were published in 1992 (AGP:CP/301) with a correction in 1994 (AGP:CP/311).

Glyphosate was evaluated for the first time by JMPR for toxicology and residues in 1986, for residues again in 1988 and 1994, and for toxicology and residues in 1997.

The new draft specifications were submitted 1999 by Monsanto and Cheminova jointly. Data were provided by both companies.

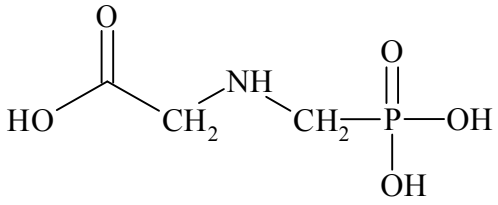
USES

Glyphosate is a non-selective contact herbicide with a broad spectrum of applications in agriculture, horticulture viticulture, forestry orchards, plantation crops, amenities, home gardening and greenhouses for the control of annual and perennial grasses and broad-leaved weeds. Furthermore it is used for weed control on aquatic areas, industrial areas, railroad tracks and on other non-cultivated areas. Besides the weed control it is used for root sucker control, for reseeding of grassland and to facilitate harvest. In addition there are uses in transgenic crops which are tolerant to glyphosate (rape, maize, soybeans, in sugar and fodder beets, cotton).

IDENTITY

ISO common name : Glyphosate
Chemical name
IUPAC: *N*-(phosphonomethyl)glycine
CA: *N*-(phosphonomethyl)glycine
CAS No: 1071-83-6
EINECS No: 213-997-4
CIPAC No: 284
Synonyms: MON 0573
CP 67573

Structural formula:



Molecular formula: $C_3H_8NO_5P$
Molecular weight: 169
Identity test: HPLC method (284/TC/(M)/3, CIPAC 1C, p.2132), retention time.
Spectrophotometric method:
Reaction of glyphosate under acidic conditions to form *N*-nitroso-glyphosate. UV determination at 243 nm.

PHYSICAL AND CHEMICAL PROPERTIES OF PURE ACTIVE INGREDIENT

Vapour pressure:	1.3 10^{-5} Pa at 25°C Method: EEC A4 Substance purity: 986 g/kg
Melting point:	189.5°C ± 0.5°C Method: OECD 102 Substance purity: 999 g/kg
Temperature of decomposition:	199°C ± 1°C Method: OECD 102 Substance purity: 999 g/kg
Solubility in water:	10.5 g/l at 20°C Method OECD 105 Substance purity: 995 g/kg
Octanol/water partition coefficient:	log K_{ow} = < -3.2 at 25°C equivalent K_{ow} = < 6 10^{-4} (same K_{ow} was found at pH 5, 7 and 9) Method OECD 107 Substance purity: 974 g/kg

Hydrolysis:	glyphosate can be considered hydrolytically stable at pH 3, 6 and 9 at 5 or 35°C (half-life >> 30 days). ^{14}C-glyphosate can be considered hydrolytically stable at pH 5, 7 and 9 at 25°C (half-life >> 30 days). Method US EPA similar to OECD 111. Substance purity: 974 g/kg
Photolysis	No change noted after 24 hours exposure to sunlight. Method: US EPA FIFRA subdivision D- no 63-13.

CHEMICAL COMPOSITION AND PROPERTIES OF THE TECHNICAL MATERIAL (TC and TK)

All necessary information on the manufacturing process and the impurity profile including batch analysis was presented by both of the data submitters in the proposal.

Methods of manufacture –

A summary of the commercially confidential manufacturing process was provided to the Meeting from both of the companies. The Meeting was also provided with information on the nature of the impurities at or exceeding 1 g/kg and their maximum limits in technical material.

Purity (content of active ingredient): glyphosate content in technical material, not less than 950 g/kg.

The impurity profile submitted by Monsanto was different from that provided to the German authorities before with regard to the maximum limits of the specified impurities, but no new impurities were specified. The impurity profile of Cheminova was in line with the information submitted to the German authorities. The impurity profiles have been compared by the German authorities and were regarded to be equivalent with regard to toxicological and ecotoxicological properties.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on impurities present at or above 1 g/kg, from both companies. The mean mass balances of the batches were 994.5 (Monsanto) and 1045 g/kg (Cheminova).

HAZARD SUMMARY

Evaluations referred to: JPMR 1986/97
ICPS Environmental Health Criteria 159
Agriculture Canada, Discussion Document 1991

Hazard classification. normal use: WHO: Unlikely to present acute hazard in

Table 1. Acute toxicity of glyphosate acid technical material

Species	Test	Test result
Rat	Oral LD_{50}	> 5000 mg/kg
Rat	Dermal LD_{50}	> 5000 mg/kg
Rabbit	Skin irritancy	essentially non-irritating
Rabbit	Eye irritancy	moderate/severe irritation
Guinea Pig	Skin sensitization	not a dermal sensitizer

Table 2. Summary of NOAELs for studies on short term toxicity, long term toxicity and carcinogenicity (EHC 159, 1994*)

Species	Test compound	Dose levels mg kg^{-1} diet unless otherwise stated	Effects, dose level (mg/kg diet)	NOAEL [mg/kg diet] mg kg^{-1} b.w. d^{-1}
Short-term studies				
Mouse	Technical glyphosate	5000, 10000, 50000	decreased growth and increased weights in brain, heart, kidneys (50000)	[10000] 1890 m, 2730 f
Mouse	Technical glyphosate	3125, 6250, 12500, 25000, 50000	reduced weight gain (50 000), lesions of salivary glands ($\geq$ 6250)	[3125] 507
Rat	Technical glyphosate	1000, 5000, 20000	no adverse effects	[20000]** 1267**m 1623**f
Rat	Technical glyphosate	200 to 12500	no adverse effects	[12500] NG**
Rat	Technical glyphosate	3125, 6250, 12500, 25000, 50000	increased AP and ALAT ($\geq$6250), increased haematocrit and red cell parameters ($\geq$12 500), increased bile acids, decreased sperm counts ($\geq$25 000), histological alterations in salivary glands ($\geq$3 125), reduced weight gain ($\geq$25 000)	[< 3125] < 205 m < 213 f
Dogs	Technical glyphosate	20, 100, 500 mg kg^{-1} bw	no adverse effects	500**

Cattle	Roundup	400, 500, 630, 790 mg kg^{-1} bw	decreased feed intake (≥630 mg kg^{-1} bw d^{-1}), diarrhoea (≥500), increased blood parameters (790)	400
Long-term studies				
Mouse	technical glyphosate	1000, 5000, 30000	decreased growth (30 000), increased incidence of hepatocyte hypertrophy and necrosis (30 000), increased incidence of urinary bladder epithelial hyperplasia (30 000)	[5000] 814
Rat	technical glyphosate	2000, 8000, 20000	decreased growth (20 000), increased liver weights (20 000), increased incidences of degenerative lens changes (20 000) and of gastric inflammation (8000 and 20 000)	[8000] 410
Rat	technical glyphosate	60, 200, 600	slightly decreased growth (600)	a

* note taken of corrigenda on the IPCS web site; m = males; f = females;
** Highest dose tested; NG, not given;
[a] The slight effect at 600 mg/kg diet (32 mg/kg bw) is considered marginal in the light of the absence of an effect on growth at higher dose levels (2000 and 8000 mg/kg diet) in a more recent 2-year study in rats.

Table 3. Summary of teratogenicity and reproduction studies on glyphosate (EHC 159)

Species	Test compound	Dose levels	Effects, dose level	NOAEL[a] mg kg^{-1} b.w. d^{-1}
Rat	technical glyphosate	300, 1000, 3500 mg kg^{-1} diet d^{-1} gestation days 6-19	mortality, clinical signs and decreased growth in dams, early resorptions, decreased numbers of implantations and visible fetuses, decreased ossification of fetal sternebrae (all at 3500 only); no fetal malformations	1000
Rabbit	technical glyphosate	75, 175, 350 mg/kg body weight, gestation days 6-27	diarrhoea and soft stools (350, slight at 175), nasal discharge (350)	175
Rat	technical glyphosate	3, 10, 30 mg/kg body	increased incidence of renal tubular dilation in	< 30[b]

		weight given in diet, 3 generations	F_{3b} male pups (30)	
Rat	technical glyphosate	2000, 10 000, 30 000 mg/kg diet, 2 generations	soft stools of parents (30 000), decreased litter size (30 000), decreased body weights of parents and pups (30 000 and 10 000)	100[b] [2000 mg/kg diet]

[a] Based on all observed effects (both in dams and offspring)

[b] There is some discrepancy in the results, and in the NOAELs, of the two reproduction studies carried out with technical glyphosate; the renal effects in the 3-generation study were not reproduced in the more recent 2-generation study with higher dose levels.

Table 4. Genotoxicity testing, in Vitro Mutagenicity studies (Monsanto)

Test system	Target cells	Results
Bacterial mutation assay with and without metabolic activation	Salmonella typhimurium TA98, TA100, TA1535 TA 1538; *B. subtilis; E. coli*	negative
Mammalian cell gene mutation assay with and without metabolic activation	Chinese Hamster ovary	negative
Mammalian cell cytogenetic Assay	Human Lymphocytes (chromosomal aberrations)	negative
Rat hepatocyte culture unscheduled DNA synthesis assay	Rat hepatocytes UDS	negative

Table 5. In vivo Mutagenicity studies (Monsanto)

Test system	Target cells	Results
Mouse bone marrow Micronucleus assay	Mouse bone marrow	negative

Acute toxicity

Glyphosate acid and its salts exhibited a low acute toxicity in laboratory animals by the oral and dermal route with LD_{50} values greater than 5000 mg/kg bw
Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant, at least to intact skin. In contrast, undiluted glyphosate acid was found to be strongly irritant to rabbit eyes. There was markedly less eye irritation observed with the salts.
Sensitization was not observed with either glyphosate acid or the salts.

Short-term toxicity

Subacute and subchronic oral toxicity studies also show a low toxicity of glyphosate. Repeated dermal exposure of rabbits and rats to glyphosate did not result in any systemic effects. Dermal irritation was not observed.

Mutagenicity / carcinogenicity

Glyphosate was examined for mutagenicity in a wide range of test systems covering all relevant endpoints in vitro as well as in vivo.
From this large database, it can be concluded that the active ingredient does not exhibit a mutagenic risk to humans. It should be also taken into consideration that there is no evidence of carcinogenic effects in humans, although glyphosate products have been in world-wide use for many years.

Reproduction toxicity

Multigeneration studies in rats did not indicate a specific hazard of glyphosate for reproduction.
Glyphosate is not teratogenic. The NOEL for developmental effects was 1000 mg/kg bw/day in rats and 175 mg/kg bw/day in rabbits.

Metabolites

The metabolite AMPA was investigated for acute and subchronic effects, mutagenicity and teratogenicity. These studies have shown that AMPA has a lower toxicity than the parent compound and is devoid of a mutagenic or teratogenic potential.

Ecotoxicology

Table 6. Acute and chronic toxicity of Glyphosate to aquatic organisms

Species	Test duration/type	EC_{50}/LC_{50}	Assessment
Daphnia magna (with aeration)	48–hr EC_{50}	37 mg/L	Slightly toxic
Daphnia magna (Without aeration)	48–hr EC_{50}	24 mg/L	Slightly toxic
Daphnia magna	48-hr EC_{50}	13 mg/L	Slightly toxic
Gammarus pseudolimnaeus (Flow-through water)	48–hr EC_{50}	42 mg/L	Slightly toxic
Carp	96–hr EC_{50}	19.mg/L	Slightly toxic
Bluegill Sunfish (Static water)	96–hr LC_{50}	34.0 mg/L	Slightly toxic
Bluegill Sunfish (Flow-through water)	96–hr LC_{50}	5.8 mg/L	Moderately toxic
Rainbow trout (Static water)	96–hr LC_{50}	15-26 mg/L	Slightly toxic
Rainbow trout (Flow-through water)	96–hr LC_{50}	8.2 mg/L	Moderately toxic
Channel Catfish	96–hr LC_{50}	39 mg/L	Slightly toxic
Fathead minnow	96–hr LC_{50}	23 mg/L	Moderately toxic
Coho Salmon	96–hr LC_{50}	22mg/L	Slightly toxic
Chinook Salmon	96–hr LC_{50}	20 mg/L	Slightly toxic
Pink Salmon	96–hr LC_{50}	14-33mg/L	Slightly toxic

Table 7. Acute and chronic toxicity of Glyphosate to birds

Bird Species	Toxicity (mg a.i./kg)
Bobwhite quail acute and short term	8-day LC_{50} > 4640 mg/kg Non-toxic 14-day LD_{50} > 3851 mg/kg Non toxic
Bobwhite quail Reproduction	NOEC >1000 mg/kg diet
Mallard duck acute and short term	LC_{50} > 4640 mg/kg Non toxic
Mallard duck Reproduction	NOEC >1000 mg/kg diet
Chicken	LD_{50} >2500 mg/kg Non-toxic

Table 8. Toxicity* to bees

Exposure Route	Toxicity Response
Oral LD_{50}	> 100 µg/bee (Non-toxic)
Dermal LD_{50}	> 100 µg/bee (Non-toxic)

* determined with formulated product

On the basis of toxicity data and application rates for the active substance glyphosate, the risks for birds, mammals, aquatic organisms, bees, earthworms and micro-organisms in soil in observance of corresponding risk management measures are regarded as slight.

FORMULATIONS

Glyphosate liquid formulations (GIFAP code SL) and glyphosate water soluble granules (GIFAP code SG).
Registered and sold in most countries of the world.

METHODS OF ANALYSIS AND TESTING

- **Chemical analytical methods for active ingredient (including identity tests):**

  AOAC-CIPAC method 284/TC/(M)/3, CIPAC 1C, p.2132, and AOAC 983.10, 1990.
  AOAC-CIPAC method 284/SG/(M)/3, CIPAC H, p. 182, and AOAC Official Method 996.12, 1997.

  The principle is HPLC using an anion exchange column, UV detection at 195 nm and quantification by external standardisation.

  Identity Tests

  - AOAC-CIPAC method 284/TC/(M)/2, CIPAC 1C, p.2132, retention time.
  - AOAC-CIPAC method 284/SG/(M)/2, CIPAC H, p.182 for SG's, retention time.
  - Record the UV scan of the main peak of the chromatogram and compare with an UV scan of the calibration solution.
  - Spectrophotometric method. Reaction of glyphosate with sodium nitrite under acidic conditions to form *N*-nitroso-glyphosate. UV determination at 243 nm.
  - **-**

- **Method(s) for determination of relevant impurities in the technical material**

  Formaldehyde is determined by a reversed phase HPLC column, off-line derivatization with Hatzsch reagent and UV-VIS detection at 412 nm. This method has been validated from 10 - 300 ppm. (Monsanto Method No AQC 678-86).

  N-Nitroso-*N*-phosphonomethylglycine (NNG) is determined by strong anion exchange HPLC with UV-visible detection. Samples are dissolved in water and reacted with hydrobromic acid to form a nitrosyl cation; the nitrosyl cation reacts with *N*-(1-naphthyl)ethylenediamine and sulfanilamide to form a purple azo dye that is detected at 550 nm. Because nitrite ion will react with glyphosate to form NNG, all glassware and equipment must be rinsed with sulfamic acid. This method has been validated to 200 ppb in glyphosate technical and 100 ppb in formulated products (Monsanto method no AQC 684-86).

- Physical testing methods: See the specifications.

- **PHYSICAL PROPERTIES**

  The proposers declared that glyphosate produced and commercialised by Monsanto and Cheminova complies with the FAO specifications (2000).

The clause for specifying the pH range in the case of glyphosate isopropylamine salt concentrates (284.105/TK) and glyphosate soluble concentrates (284/SL) was introduced because, depending on the climatic conditions, the equilibrium glyphosate acid - glyphosate monoispropylamine salt - diisopropylamine salt will determine the potential crystallisation of glyphosate acid, which has lower water solubility than its salts.
The clause specifying the flowability of soluble granules was changed from 100% to 98% because it was too stringent. Such granules sometimes have the tendency to form loose aggregates, which may remain on the sieve but readily disappear during dissolution in water.

CONTAINERS AND PACKAGING

No special requirements have been reported for containers and packaging but metal containers should not be used unless lined with suitable material to resist the products if they are acidic.

EXPRESSION OF ACTIVE INGREDIENT (Sections 4.2.5 and 4.2.7 of the Manual)

The active ingredient content is expressed as glyphosate (acid) in g/kg or g/l (for liquid formulations at 20°C).

APPRAISAL

The current FAO specifications for glyphosate acid technical concentrates (FAO Specification 284/TK/S, 1991) and glyphosate soluble concentrates (FAO Specification 284/SL/S, 1991) were based on data submitted from Monsanto and were published 1992 (AGP:CP/301) with a correction 1994 (AGP:CP/311). The proposers for the revised specification are Monsanto Agricultural Company and Cheminova Agro A/S.

Glyphosate acid is a colourless crystalline solid without odour. It melts at 189.5 °C. The acid is of medium water solubility (10 g/l), the salts are highly soluble in water.
It is formulated as water soluble concentrates and water soluble granules, in both of which it is used as a salt (isopropylamine salt, ammonium salt or sodium salt).
Glyphosate is stable to hydrolysis in the range of pH 5 to pH 9 and relatively stable to photodegradation.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on impurities present at or above 1 g/kg, from both of the companies.
Two impurities were identified (formaldehyde and *N*-nitroso-*N*-phosphonomethyl-glycine, NNG) as relevant and maximum limits are specified.
The same absolute limit of 1 mg/kg for NNG has been set for the TC, TK's and the formulations because this impurity may be formed during the synthesis of glyphosate acid, as well as during the subsequent steps of acid neutralisation (formation of the salt) and during the final steps of formulation.

During the synthesis, the presence of nitrites in the process water, or the presence of $[NO]_x$ in the air or oxygen, used in the oxidation process, are the main causes of the formation of *N*-nitrosoglyphosate (NNG).
During the step of acid to salt conversion, the presence of free nitrites in the water being used, might increase the level of *N*-nitrosoglyphosate.
Finally, the formulation or granulation steps, again might cause an increase in the NNG level due to the presence of free nitrites in the water used. Here also again, the $[NO]_x$ present in the air, e.g. hot air being used to dry the granules, might cause increase of NNG.

For formaldehyde the limit was set to 1.3 g/kg on a glyphosate acid basis, according to the rules of FAO as published in the Manual. This limit corresponds closely to the limit in the US OSHA regulations which was set on "as is" basis and not on an acid basis.

The differences in the impurity profiles of the two sources had been assessed by the German authorities and were regarded to be of no relevance with regard to toxicological or ecotoxicological properties. This assessment included all toxicological and ecotoxicological studies available to the German authorities. Taking the more detailed Monsanto impurity profile as the reference profile the Cheminova profile is equivalent to the Monsanto impurity profile according to the criteria given in the Manual.

Glyphosate is of low acute toxicity and shows no adverse effects with regard to carcinogenicity, mutagenicity, teratogenicity or reproduction toxicity.

The proposal for an ADI of 0.3 mg/kg bw for glyphosate based on long term studies in rats is in line with the value published by WHO based on the JMPR evaluation of 1986.

Glyphosate is of low risk to birds, mammals, aquatic organisms, bees, earthworms and micro-organisms in soil.

The proposers declared that glyphosate produced and commercialized by Monsanto and Cheminova comply with the FAO specifications (1999)

RECOMMENDATIONS

The draft specifications for glyphosate acid technical, glyphosate acid technical concentrates, glyphosate isopropylamine salt technical concentrates, glyphosate soluble concentrates and glyphosate water soluble granules, proposed jointly by Monsanto and Cheminova were regarded as acceptable by the Meeting. As the Cheminova impurity profile is covered by the Monsanto impurity profile is the Meeting recommended that the Monsanto profile should be the reference profile.

REFERENCES

- Manual on Development and Use of FAO Specifications for Plant Protection Products, January 1999, Rome.

- FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent. Group of Experts on Pesticide Specifications, 3rd Session. 5 - 8 October 1998, Rome.

- IPCS Environmental Health Criteria 159, WHO 1994, Geneva.

- IPCS, The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 1998-1999, WHO 1999, Geneva.

- CIPAC Handbook 1C, 1985

- CIPAC Handbook F, 1995

- CIPAC Handbook H, 1998

GLYPHOSATE

EVALUATION REPORT 284/2001

Explanation

The data for glyphosate were evaluated in support of existing FAO specifications 284/TC, 284/TK, 284/SL, 284/SG (2000). The supporting data were provided by Syngenta to extend the scope of the existing specification to their product.

Uses

See Evaluation Report for glyphosate (2000).

Identity

ISO common name: Glyphosate

Chemical name:

IUPAC: *N*-(phosphonomethyl)-glycine

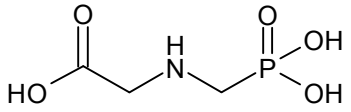
CA: *N*-(phosphonomethyl)-glycine

CAS No: 1071-83-6

CIPAC No: 284

Synonyms: none

Structural formula:



Molecular formula: $C_3H_8NO_5P$

Relative molecular mass: 169.1

Identity tests: see FAO Specification 284/TC (2000)

Physico-chemical properties of pure glyphosate

See FAO Specification 284/TC (2000)

Chemical composition and properties of glyphosate technical materials

See FAO Specification 284/TC (2000) and confidential information to this report.

Hazard summary

See Evaluation Report for glyphosate (2000).

It was recognised that the acute dermal toxicity given (< 2000 mg/kg bw) by Syngenta was higher than stated in the Evaluation Report for glyphosate (2000) (< 5000 mg/kg bw).

Justification submitted by Syngenta:

The guideline used in the acute dermal study [CTL/P/4464] was OECD 402 as specified in 91/414/EEC. In accordance with this guideline the limit dose of 2000 mg/kg was applied following a range finding test to set the dose. A limit dose at this level is, from a technical perspective, appropriate as this is approaching the maximum quantity that can be applied with reasonable confidence that the totality of the dose applied will remain in contact with the rat skin for the duration of the exposure. Applications of amounts greater than 2000 mg/kg are less likely to result in the total dose achieving and/or maintaining contact with the rat skin during the exposure period.

Hence a dermal topical application of 5000 mg/kg leading to an acute dermal MLD50 value of >5000 mg/kg does not signify a lower intrinsic acute dermal toxicity than an MLD50 of >2000 mg/kg resulting from a study using a limit dose of only 2000 mg/kg. The difference in endpoints being simply a reflection of limit dose set used in the individual studies.

It is therefore reasonable to consider that acute dermal MLD50 values in the rat of >2000 and >5000 mg glyphosate acid/kg, where the variance is only a reflection of the differing limit doses of the individual studies, indicate an equivalent profile of the acute dermal toxicity.

This justification was accepted by WHO.

Formulations

Not submitted by Syngenta

Methods of analysis and testing

Analytical method for the active ingredient (including identity tests): see FAO Specification 284/TC (2000).

Fully validated analytical methods for the impurities were provided by Syngenta.

Physical properties

See FAO Specification 284/TC (2000)

Containers and packaging

See FAO Specification 284/TC (2000)

Expression of the active ingredient

See FAO Specification 284/TC (2000)

Appraisal

The data submitted by Syngenta were in accordance with the requirements of the FAO Manual (5th edition) and supported the draft specification. The deviations from reference data set were justified by the proposer and regarded as acceptable by the evaluator.

The Meeting was provided with commercially confidential information on the manufacturing process and batch analysis data on all impurities present at or above 1 g/kg.

The manufacturing process and the impurity profile of Syngenta were different from those submitted with the reference specification. The deviations from reference data set were >50% or 3 g/kg in the case of R025029 and R290510 impurities. However, these differences do not lead to differences in toxicological assessment, as evidenced by the data submitted by the proposer for acute oral, dermal, inhalation, skin and eye irritation and sensitization. The Syngenta product is therefore considered to be equivalent to the products upon which the reference profile is based.

Recommendations

The draft specification for technical glyphosate proposed by Syngenta was accepted by the Meeting. The proposer had requested a specification for this material as a TC but the Syngenta product is considered to be equivalent to the existing TK specification. The difference between TC acid and TK acid is the water content only and therefore the extension of the TK specification is recommended. From the production Syngenta isolates the TK acid as a wet paste with a minimum content of 760 g/kg glyphosate. This is within the reference specification for the TK.

References

See Evaluation Report for glyphosate (2000).

World Health Organization International Programme on Chemical Safety (IPCS)

Environmental Health Criteria for Glyphosate

1994

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

ENVIRONMENTAL HEALTH CRITERIA 159

GLYPHOSATE

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organisation, or the World Health Organization.

First draft prepared by Dr H. Mensink and
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and the World Health Organization

World Health Orgnization
Geneva, 1994

The International Programme on Chemical Safety (IPCS) is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. The main objective of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment. Supporting activities include the development of epidemiological, experimental laboratory, and risk-assessment methods that could produce internationally comparable results, and the development of manpower in the field of toxicology. Other activities carried out by the IPCS include the development of know-how for coping with chemical accidents, coordination of laboratory testing and epidemiological studies, and promotion of research on the mechanisms of the biological action of chemicals.

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ENVIRONMENTAL HEALTH CRITERIA FOR GLYPHOSATE

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Dr M. Gilbert, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland *(Secretary)*

_____

[a] Invited but unable to attend.

NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are kindly requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (Telephone No. 9799111).

* * *

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ENVIRONMENTAL HEALTH CRITERIA FOR GLYPHOSATE

A Task Group on Environmental Health Criteria for Glyphosate met at the Institute of Terrestrial Ecology, Monks Wood, United Kingdom, from 23 to 27 August 1993. Dr S. Dobson welcomed the participants on behalf of the host institution, and Dr M. Gilbert opened the Meeting on behalf of the three cooperating organizations of the IPCS (UNEP/ILO/WHO). The Task Group reviewed and revised the draft monograph and made an evaluation of the risks for human health and the environment from exposure to glyphosate.

The first draft of this monograph was prepared by Dr H. Mensink and Dr P. Janssen, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Dr M. Gilbert was responsible for the overall scientific content of the monograph and for the organization of the meeting, and Dr P.G. Jenkins, IPCS, for the technical editing of the monograph.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

ABBREVIATIONS

a.i.	active ingredient
ALAT	alanine aminotransferase
AMPA	aminomethylphosphonic acid
AP	alkaline phosphatase
CHO	Chinese hamster ovary
CNS	central nervous system
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IPA	isopropylamine
MATC	maximum acceptable toxicant concentration
NOAEL	no-observed-adverse-effect level

NOEC no-observed-effect concentration

1. SUMMARY

1.1 Identity, physical and chemical properties, and analytical methods

Glyphosate is a weak organic acid consisting of a glycine and a phosphonomethyl moiety. The empirical formula is $C_3H_8NO_5P$. Glyphosate is usually formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine or trimethylsulfonium. The purity of technical grade glyphosate is generally above 90%. Technical grade glyphosate is an odourless white crystalline powder with a specific gravity of 1.704, a very low vapour pressure, and a high solubility in water. The octanol-water partition coefficient (log K_{ow}) is -2.8. Glyphosate is amphoteric and may exist as different ionic species, dependent on the actual pH.

Determination of glyphosate is in general laborious, complex, and costly. Derivatization with fluorogenic substances is the most common method and may be applied pre- or post-column. Determination is usually carried out with high performance liquid chromatography or gas liquid chromatography. Limits of determination for glyphosate in water, plants, soil and human urine, are 0.02-3.2 µg/litre, 0.01-0.3 mg/kg, 0.05-1 mg/kg and 0.1 mg/litre, respectively.

1.2 Sources of human and environmental exposure

Glyphosate is a post-emergent, systemic and non-selective herbicide that is used in both agricultural and non-agricultural areas all over the world. Glyphosate is applied to many crops and in various commercial formulations. The major formulation is Roundup in which glyphosate is formulated as the isopropylamine salt. Recommended application rates do not exceed 5.8 kg a.i./ha and are dependent on the type of use. Environmental exposure may occur because of deposition due to drift and accidental releases.

1.3 Environmental transport, distribution and transformation

The most important processes of dissipation that may be involved after application of glyphosate are complexation in water with ions, e.g., Ca^{2+} and Mg^{2+}, sorption to sediment, suspended particles in water, and soil, photodegradation in water, uptake by plants, and biodegradation.

Glyphosate dissipates from the water with DT_{50} values (dissipation) ranging from a few days to more than 91 days. Sediment or suspended particles are shown to be the major sink.

The adsorption coefficients ($K_{s/l}$) of glyphosate in laboratory experiments vary between 8 and 377 dm^3/kg for various soils and clay minerals. No data on the sorption of aminomethylphosphonic acid (AMPA), the major metabolite, under laboratory conditions are available.

R_f values of glyphosate do not exceed 0.2 in soil thin-layer chromatography experiments. Between less than 0.1% and 11% of the applied activity is recovered in the eluate of soil columns under leaching conditions simulating an extremely high rainfall. From field experiments it appears that AMPA is not likely to leach.

Glyphosate dissipates in field experiments from the soil with DT_{50} values between 3 and 174 days, mainly depending on edaphic and climatic conditions. Up to 1.8% of the applied dose dissipated from the soil due to run-off in some field experiments.

Under laboratory conditions, up to 45% of the applied activity may be absorbed by treated leaves, and this is followed by a substantial translocation.

Hydrolysis of glyphosate in sterile buffers is very slow with DT_{50} values >> 35 days. Photodegradation in water under natural conditions occurs with DT_{50} values $\leq$ 28 days. No substantial photodegradation in soil was recorded in a study lasting 31 days.

The time needed for 50% biodegradation of glyphosate in the whole system of a test with water and sediment is $\geq$ 14 days under aerobic conditions and 14-22 days under anaerobic conditions in the laboratory. The time needed for 50% biodegradation of glyphosate in the soil is 2-3 days under aerobic conditions.

The major metabolite in soil and water is AMPA. Maximum amounts of AMPA in soils are approximately 20% of the applied activity under aerobic conditions and 0.5% under anaerobic conditions. Maximum amounts of AMPA in sediments are 25% under both aerobic and anaerobic conditions.

Bioconcentration factors are low in laboratory tests with invertebrates and fish. Bluegill sunfish in a flow-through test showed a depuration half-life of 35 days, after being exposed for 35 days. AMPA is recovered in bluegill sunfish up to 21 days after continuous exposure to glyphosate. Glyphosate has not been detected in fish living in directly sprayed water in field experiments. In one experiment, AMPA was detectable in carp up to 90 days after application. No biomagnification of glyphosate in litter by herbivorous and omnivorous small mammals in a forest brush ecosystem was indicated in a field experiment. Concentrations of up to 5 mg a.i./kg were measured in deermice immediately after spraying in this experiment.

A range of bacterial strains can degrade glyphosate. Bacteria capable of using the compound as sole phosphorus, sole carbon or sole nitrogen source have been identified. Growth is slow compared to growth on inorganic sources of P, C and N. There is evidence from the field that bacterial populations adapted to metabolise glyphosate. The presence of inorganic phosphate inhibits degradation of glyphosate with some, but not all, bacteria. Biodegradation of glyphosate may involve co-metabolism with other energy sources.

1.4 Environmental levels and human exposure

Data on the occurrence of glyphosate in environmental biota and abiota as part of regular monitoring programmes are very scarce. Data from field experiments in which common agricultural practice is simulated are used to indicate maximum environmental concentrations: < 1-1700 µg/litre surface water, 0.07-40 mg/kg dry weight soil, < 0.05-19 mg/kg dry weight sediment, 261-1300 mg/kg foliage, 5 mg/kg the viscera of deermice, 1.6-19 mg/kg wild berries, and 45 mg/kg lichens. The corresponding maximum concentrations of AMPA are: < 1-35 µg/litre (surface water), 0.1-9 mg/kg dry weight (soil), < 0.05-1.8 mg/kg dry weight (sediment), 1.7-< 9 mg/kg (foliage), 0.02-0.1 mg/kg (wild berries), and 2.1 mg/kg (lichens). The above-mentioned concentrations of glyphosate are generally found immediately after application. The concentration in lichens was found 270 days after application.

Measurements of daily human intake of glyphosate via food and drinking-water (total diet studies) are not available. The few data on occupational exposure indicate that exposure levels for workers applying glyphosate as the herbicide formulation Roundup are low.

1.5 Kinetics and metabolism in laboratory animals and humans

Technical glyphosate is only partially absorbed from the gastrointestinal tract. In studies with ^{14}C-labelled glyphosate, absorption percentages of 30-36% were found in several species. Dermal absorption is low. From the herbicide formulation Roundup, $\leq$ 5.5% of the glyphosate present is absorbed through the skin (contact time about 24 h). In body tissues, the highest concentrations, approximately 1% of the oral dose, are found in bone. Following a single oral dose, 62-69% is eliminated in the faeces without absorption. Of the absorbed glyphosate, 14-29% is excreted in urine and 0.2% or less in expired air. Biliary excretion following intravenous application was only 5-8%. In lactating goats, excretion in milk was shown to occur to a minor extent only (concentration $\leq$ 0.1 mg/kg whole milk at a dose level of 120 mg/kg diet). Biotransformation of glyphosate occurs to a very low degree only. The only metabolite, AMPA, accounts for 0.3% of the dose or less; the rest is unchanged glyphosate. Whole body clearance (99% of an oral dose) occurs in approximately 168 h.

1.6 Effects on laboratory mammals, and *in vitro* test systems

In experimental animals, technical glyphosate has very low acute toxicity by the oral and dermal administration routes; it is markedly more toxic by the intraperitoneal route than by other routes. Short-term feeding studies have been conducted in several species, but few effects were seen in most of these tests. In one 13-week study in mice with technical glyphosate, increased weights of several organs and growth retardation were observed at 50 000 mg/kg diet. In a 13-week study in rats no effect occurred (technical glyphosate dose levels up to 20 000 mg/kg diet). In another 13-week study, lesions of the salivary glands were found in rats and mice. In mice, the NOAEL was 3125 mg/kg diet; in rats, it was < 3125 mg/kg diet. These findings were not present in any other short-term or long-term studies conducted in different strains and species. The salivary lesions suggest that glyphosate may be acting as a weak adrenergic agonist.

Long-term toxicity was studied in mice and rats. Few effects were observed and, in almost all cases, at relatively high dose levels only. In mice, technical glyphosate produced growth retardation, hepatocyte hypertrophy or necrosis and urinary bladder epithelial hyperplasia at 30 000 mg/kg. In rats, the same test compound produced decreased growth, increased liver weights, degenerative lens changes and gastric inflammation at 20 000 mg/kg diet.

The available studies do not indicate that technical glyphosate is mutagenic, carcinogenic or teratogenic. Two multigeneration studies were carried out in rats. The main effects of technical glyphosate were decreased body weights of parent animals and pups and decreased litter size at 30 000 mg/kg diet. In one reproduction study, an increase in the incidence of unilateral renal tubular dilation in F_{3b} male pups at 30 mg/kg body weight was reported. The absence of a renal effect in pups at a higher dose level in the other reproduction study indicates that the reproducibility of this lesion is uncertain.

1.7 Effects on humans

The available controlled studies are limited to three irritation/sensitization studies in human volunteers, the results of which indicated no effect. Several cases of (mostly intentional) intoxications with technical glyphosate herbicide formulation Roundup have been reported. In a study on health effects in workers applying Roundup herbicide formulation, no adverse effects were found. Available data on occupational exposure for workers applying Roundup indicate exposure levels far below the NOAELs from the relevant animal experiments.

1.8 Effects on other organisms in the laboratory and field

Technical grade glyphosate is moderately to slightly toxic to aquatic microorganisms, with EC_{50} (3-4 days) values of 1.2-7.8 mg/litre, and 7-day NOEC values of 0.3-34 mg/litre. Formulations of glyphosate are slightly to highly toxic to aquatic microorganisms with 3-day EC_{50} values of 1.0 to > 55 mg product per litre. Cyanophyta (blue-green algae) are more sensitive to Roundup than true algae. Physiological processes that are affected include the greening process, respiration, photosynthesis, and the synthesis of aromatic amino acids.

Soil bacteria in culture have shown effects of glyphosate on nitrogen fixation, denitrification and nitrification. However, field studies after application of formulations have not shown significant effects. Closely related species of bacteria have been shown capable of degrading glyphosate.

Mycelial growth of ectomycorrhizal fungi in pure cultures is inhibited at concentrations of $\geq$ 29 µg Roundup/litre. Sensitive genera are *Cenococcum, Hebeloma and Laccaria*.

Glyphosate is slightly toxic to aquatic macrophytes with a 14-day NOEC value of 9 mg/litre, when dissolved in water. Roundup is also slightly toxic with 14-day NOEC values of 2.4-56 mg Roundup/litre, when dissolved in water. No data on acute toxicity are available. Phytotoxicity is much higher when sprayed deposits are not washed off.

Technical grade glyphosate is slightly to very slightly toxic to aquatic invertebrates with 2- to 4-day LC_{50} or EC_{50} values of $\geq$ 55 mg/litre, and a 21-day NOEC value of 100 mg/litre. Formulations of glyphosate are moderately to very slightly toxic to aquatic invertebrates with 2-day EC_{50} values of 5.3-5600 mg product/litre and 21-day MATC values of 1.4-4.9 mg product per litre. The higher toxicity of Roundup is mainly due to the presence of surfactants.

Technical grade glyphosate is moderately to very slightly toxic to fish, with 4-day LC_{50} values of 10 to > 1000 mg/litre, a 21-day NOEC value of 52 mg/litre, and an MATC value of > 26 mg/litre. Formulations of glyphosate are also moderately to very slightly toxic to fish with 4-day LC_{50} values of 2.4 to > 1000 mg product per litre, and 21-day NOEC values of 0.8-2.4 mg product/litre. The most sensitive species is the carp, when exposed to the formulation Sting. No treatment-related effects of Roundup on fish have been found under field conditions, with the exception of stress immediately after application of a recommended rate and avoidance of concentrations of $\geq$ 40 mg Roundup/litre.

Nodulation of sub-clover inoculated with Rhizobium is inhibited in a dose-related way in soil-free systems with nutrient solutions at concentrations of $\geq$ 2 mg a.i./litre. Seed germination of various forest species is not affected by glyphosate at the recommended application rates. The root length of red pine seedlings is decreased under laboratory conditions in a dose-related way at application rates of $\geq$ 0.54 kg a.i./ha. This decrease was not confirmed in a comparable field experiment.

Technical grade glyphosate and Roundup are slightly toxic to bees when applied either orally or topically. The 2-day LD_{50} values are $\geq$ 100 µg (a.i. or product) per bee. The oral 2-day LD_{50} of Sting to bees is > 100 µg/bee. Roundup and Roundup D-pak are slightly toxic to earthworms with 14-day NOEC values of 500 and 158 mg product per kg dry weight, respectively. No adverse effects of Roundup were found on the fecundity and fertility of green

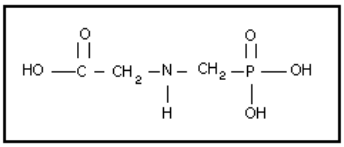
lacewings, and there were no effects of Sting on the food uptake and mortality of the beetle *Poecilus*.

Technical grade glyphosate is slightly toxic to birds, with an LD_{50} of >3851 mg/kg body weight, an 8-day LC_{50} of >4640 mg/kg feed, and 112- to 119-day NOEC values of $\geq$ 1000 mg/kg feed. Roundup and an unknown formulation are also slightly toxic to birds, with an LD_{50} of > 2686 mg product/kg body weight and an 8-day LC_{50} of > 5620 mg product/kg feed. Generally no treatment-related effects of technical grade glyphosate or Roundup on mammals are found under laboratory conditions, except at very high application rates. Treatment-related effects on birds and mammals under field conditions appear to be primarily due to habitat changes after treatment with Roundup.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, AND ANALYTICAL METHODS

2.1 Identity

Glyphosate is the primary name of a weak organic acid that consists of a glycine moiety and a phosphonomethyl moiety. The chemical name is *N*-(phosphonomethyl)glycine according to IUPAC nomenclature. The CAS name is glycine, *N*-(phosphonomethyl)-, and its CAS registry number is 1071-83-6. The empirical formula is $C_3H_8NO_5P$, and the structural formula is as follows:



The relative molecular mass of glyphosate is 169.07. Technical grade glyphosate has a purity of $\geq$ 80%, but the purity generally exceeds 90%. Glyphosate usually is formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine. The CAS registry number of the salt of glyphosate and isopropyl-amine is 38641-94-0.

Surfactants and inerts may be added to formulations of glyphosate. The type of surfactant and its concentration may differ per formulation. A common surfactant in the major formulation Roundup is polyoxyethylene amine. Other known surfactants are ortho X-77 (Mitchell et al., 1987), LI-700, R-11 and Widespread (Monsanto, 1990a). Other additives in formulations may be sulfuric and phosphoric acids.

2.2 Physical and chemical properties

The physical and chemical properties of glyphosate are tabulated in Table 1. Glyphosate is an amphoteric compound of which the ionic species and their pKa values are presented in Fig. 1. Due to its high polarity glyphosate is practically insoluble in, for instance, ethanol, acetone and benzene.

Table 1. Physical and chemical properties of glyphosate[a]

		Remarks
Physical state	crystalline powder	
Colour	white	
Odour	none	
Melting point[b]	184.5 °C	decomposition at 187 °C
Boiling point	n.a.	
Specific gravity (density)[c]	1.704	20 °C
Vapour pressured	< 1 x 10-5 Pa	25 °C
Solubility in water[b,e]	10 100 mg/litre	20 °C
Henry's law constant	< 7 x 10-11	
Octanol-water partition coefficient (log K_{ow})[d]	-2.8	
Surface tension[d]	0.072 N/m	0.5% (w/v) at approx. 25 °C

U.S. Environmental Protection Agency (EPA)

Reregistration Eligibility Decision

September 1993

United States
Environmental Protection
Agency

Office of Prevention, Pesticides
And Toxic Substances
(7508W)

EPA 738-R-93-014
September 1993

EPA

Reregistration Eligibility Decision (RED)

Glyphosate

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 16 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

CERTIFIED MAIL

Dear Registrant:

I am pleased to announce that the Environmental Protection Agency (the "Agency") has completed its reregistration eligibility decision on the pesticide active ingredient glyphosate.

Enclosed is a **Reregistration Eligibility Decision (RED) Document** for the pesticide active ingredients isopropylamine salt of glyphosate and sodium salt of glyphosate, hereafter referred to as glyphosate. The RED is the Agency's evaluation of the glyphosate data base, its conclusions regarding human and environmental risks associated with the current product uses, and its decisions and conditions under which uses and products will be eligible for rereregistration. Also enclosed is the **EPA RED facts** and the **Pesticide Reregistration Handbook** which provides instructions to registrants on how to respond to any labeling and data requirements specified in the RED and how to reregister products.

The RED identifies outstanding product specific data requirements for end-use products and manufacturing-use products. These requirements are listed on the Requirements Status and Registrant's Response Form, which, along with the Data Call-In Response Form listing all of your company's products subject to the RED, is included as an Attachment. Instructions for completing both forms are contained in the RED package. All product specific data must be submitted and found acceptable by the Agency before a product can be reregistered.

Generic data requirements usually will have been fulfilled prior to making a reregistration eligibility decision. However, there may be some instances where additional generic data are required. If generic data requirements need to be fulfilled, all registrants must complete the appropriate Data Call-In Response Form and Requirements Status and Registrant's Response Form. These forms are in the appendices to the RED.

The RED identifies any specific labeling requirements such as restricted use classification, groundwater hazard statements, endangered species precautions, etc., necessary for reregistration based on a review of the generic data for the active ingredient. In addition, in order to be reregistered, all product labeling must be in compliance with format and content labeling as described in 40 CFR §156.10 and all labeling changes imposed by Pesticide Regulation (PR) Notices, and any label changes imposed by this RED.

The Pesticide Reregistration Handbook contains detailed instructions for compliance with the RED and must be followed carefully. There are several key points to remember in preparing your response to the RED:

Within 90 Days of Your Receipt of this Letter

1. For **each** product which is subject to this RED, **you must complete, sign and submit the data call-in (DCI) response forms attached to the RED** [Appendix F, Attachments B and D, has forms for product specific data]. Follow the instructions in Attachments B and D for completing those forms and submit the forms to the appropriate address specified in the Data Call-Ins. **Note that the DCI forms are to be sent to the Special Review and Reregistration Division (use the mailing distribution code RED-SRRD-0178 for your generic response).**

2. **No time extensions will be granted for submitting the 90-day responses.** If the Agency does not receive a response for a product, it may issue a Notice of Intent to Suspend (NOIS) for that product.

3. **Any requests for data waivers or time extensions to the 8-month deadline must be submitted as part of your 90-day response.** Such requests will generally not be considered if submitted later than the 90-day response.

Within 8 Months of the Date of this Letter

1. For **each product**, you must submit a completed **Application for Reregistration** (EPA Form 8570-1), **five copies of the label and labeling** revised as specified by the RED and in accordance with current requirements, **two completed copies of the Confidential Statement of Formula** (CSF) (EPA Form 8570-4), a completed **Certification with Respect to Citation of Data** (EPA Form 8570-31), and **data or references to data** (see item 2 below).

2. You must **submit or cite the required product specific data** as part of your commit-ment for reregistration. For most products, you will probably be citing data which have already been submitted to the Agency. In these cases, you must **submit a list of the studies and the corresponding EPA identifier numbers** (i.e., ACCESSION or MRID numbers). Before citing these studies, you must **make sure that they meet the**

Agency's current acceptance criteria (Appendix F, Attachment E). Be sure to follow data formatting requirements in P.R. Notice 86-5. Failure to adequately comply with the data requirements specified in this RED may result in the Notice of Intent to Suspend your product.

3. The labeling and CSF which you submit for **each** product must **comply with P.R. Notice 91-2** (Appendix D). That Notice requires that the amount of active ingredient declared in the ingredient statement must be stated as the **nominal concentration** rather than the lower certified limit. You have two options for submitting a CSF: (1) accept the standard certified limits (see 40 CFR §158.175) or (2) provide certified limits that are supported by the analysis of five batches. If you choose the second option, you must submit or cite the data for the five batches along with a certification statement as described in 40 CFR §158.175(e).

4. Send your Application for Registration to the **Registration Division Product Manager who is assigned to the product, PM #25 Robert Taylor.** Use the correct address shown on page 6 of the enclosed Product Reregistration Handbook (Appendix E). Note that the mailing distribution code for your response is RED-RD-PM25.

Questions on **product specific data requirements and labeling** (for both End-use and Manufacturing-use products) should be directed to the Special Review and Registration Division Planning and Reregistration Review Manager for glyphosate, Frank Rubis at (703) 308-8184. Questions on the **generic data requirements** should be directed to Eric Feris, the Chemical Review Manager in the Special Review and Reregistration Division at (703) 308-8048 (call via the Virginia Relay: 1-800-828-1140).

The Agency is prepared to meet with any registrants who have questions about responding to the glyphosate RED. **If you wish to meet with the Agency, you must contact Eric Feris within two weeks of your receipt of the RED.** The Agency intends to have one combined meeting with interested registrants. If there are any requests for such a meeting, the Agency will notify all registrants who requested a meeting of the date, location and time. Requests for a meeting will not extend the 90-day or 8-month response deadlines.

Sincerely yours,

Daniel Barolo, Director
Special Review and
Reregistration Division

Enclosures

United States Environmental Protection Agency | Prevention, Pesticides And Toxic Substances (7508W) | EPA-738-F-93-011 September 1993

EPA R.E.D. FACTS

Glyphosate

Pesticide Reregistration

All pesticides sold or distributed in the United States must be registered by EPA, based on scientific studies showing that they can be used without posing unreasonable risks to people or the environment. Because of advances in scientific knowledge, the law requires that pesticides which were first registered years ago be reregistered to ensure that they meet today's more stringent standards.

In evaluating pesticides for reregistration, EPA obtains and reviews a complete set of studies from pesticide producers, describing the human health and environmental effects of each pesticide. The Agency imposes any regulatory controls that are needed to effectively manage each pesticide's risks. EPA then reregisters pesticides that can be used without posing unreasonable risks to human health or the environment.

When a pesticide is eligible for reregistration, EPA announces this and explains why in a Reregistration Eligibility Decision (RED) document. This fact sheet summarizes the information in the RED document for glyphosate.

Use Profile

Glyphosate is a non-selective herbicide registered for use on many food and non-food field crops as well as non-crop areas where total vegetation control is desired. When applied at lower rates, glyphosate also is a plant growth regulator.

Glyphosate is among the most widely used pesticides by volume. It ranked eleventh among conventional pesticides used in the U.S. during 1990-91. In recent years, approximately 13 to 20 million acres were treated with 18.7 million pounds of glyphosate annually. The largest use sites include hay/pasture, soybeans and field corn.

Three salts of glyphosate are used as active ingredients in registered pesticide products. Two of these active ingredients, plus technical grade glyphosate, are contained in the 56 products that are subject to this RED.

The isopropylamine salt, an active ingredient in 53 registered products, is used as a herbicide to control broadleaf weeds and grasses in many food and non-food crops and a variety of other sites including ornamentals, lawns and turf, residential areas, greenhouses, forest plantings and industrial rights-of-way. It is formulated as a liquid, solid or pellet/tablet, and is applied using ground or aerial equipment.

The sodium salt of glyphosate, an active ingredient in two registered pesticide products, is used as a plant growth regulator for peanuts and sugarcane, to modify plant growth and hasten the ripening of fruit. It is applied as a ground spray to peanut fields and as an aerial spray to sugarcane. Preharvest intervals are established for both crops.

The monoammonium salt of glyphosate is an active ingredient in an additional seven herbicide/growth regulator products. This form of glyphosate was initially registered after November 1984, so it is not subject to reregistration or included in this RED. However, in reassessing the existing glyphosate tolerances (maximum residue limits in or on food and feed), EPA included those for the monoammonium salt.

Regulatory History

EPA issued a Registration Standard for glyphosate in June 1986 (NTIS PB87-103214). The Registration Standard required additional phytotoxicity, environmental fate, toxicology, product chemistry and residue chemistry studies. All of the data required have been submitted and reviewed, or were waived.

Human Health Assessment

Toxicity

Glyphosate is of relatively low oral and dermal acute toxicity. It has been placed in Toxicity Category III for these effects (Toxicity Category I indicates the highest degree of acute toxicity, and Category IV the lowest). The acute inhalation toxicity study was waived because glyphosate is non-volatile and because adequate inhalation studies with end-use products exist showing low toxicity.

A subchronic feeding study using rats showed blood and pancreatic effects. A similar study with mice showed reduced body weight gains in both sexes at the highest dose levels. A dermal study with rabbits showed slight reddening and swelling of the skin, decreased food consumption in males and decreased enzyme production, at the highest dose levels.

Several chronic toxicity/carcinogenicity studies using rats, mice and beagle dogs resulted in no effects based on the parameters examined, or resulted in findings that glyphosate was not carcinogenic in the study. In June 1991, EPA classified glyphosate as a Group E oncogen--one that shows evidence of non-carcinogenicity for humans--based on the lack of convincing evidence of carcinogenicity in adequate studies.

In developmental toxicity studies using pregnant rats and rabbits, glyphosate caused treatment-related effects in the high dose groups including diarrhea, decreased body weight gain, nasal discharge and death.

One reproductive toxicity study using rats showed kidney effects in the high dose male pups; another study showed digestive effects and decreased body weight gain. Glyphosate does not cause mutations.

In one metabolism study with rats, most of the glyphosate administered (97.5 percent) was excreted in urine and feces as the parent compound; less than one percent of the absorbed dose remained in tissues and organs, primarily in bone tissue. Aminomethyl phosphonic acid (AMPA) was the only metabolite excreted. A second study using rats showed that very little glyphosate reaches bone marrow, that it is rapidly eliminated from bone marrow, and that it is even more rapidly eliminated from plasma.

Dietary Exposure

The nature of glyphosate residue in plants and animals is adequately understood. Studies with a variety of plants indicate that uptake of glyphosate or AMPA from soil is limited. The material which is taken up is readily translocated throughout the plant and into its fruit. In animals, most glyphosate is eliminated in urine and feces. Enforcement methods are available to detect residues of glyphosate and AMPA in or on plant commodities, in water and in animal commodities.

85 tolerances have been established for residues of glyphosate and its metabolite, AMPA, in or on a wide variety of crops and crop groups, as well as in many processed foods, animal feed and animal tissues (please see 40 CFR 180.364, 40 CFR 185.3500 and 40 CFR 186.3500). EPA has reassessed the existing and proposed tolerances for glyphosate. Though some adjustments will be needed, no major changes in existing tolerances are required. EPA also has compared the U.S. tolerances with international Codex maximum residue limits (MRLs), and is recommending certain adjustments to achieve greater compatibility.

EPA conducted a dietary risk assessment for glyphosate based on a worst-case risk scenario, that is, assuming that 100 percent of all possible commodities/acreage were treated, and assuming that tolerance-level residues remained in/on all treated commodities. The Agency concluded that the chronic dietary risk posed by glyphosate food uses is minimal.

A reference dose (RfD), or estimate of daily exposure that would not cause adverse effects throughout a lifetime, of 2 mg/kg/day has been proposed for glyphosate, based on the developmental toxicity studies described above.

Occupational and Residential Exposure

Occupational and residential exposure to glyphosate can be expected based on its currently registered uses. However, due to glyphosate's low acute toxicity and the absence of other toxicological concerns (especially carcinogenicity), occupational and residential exposure data are not required for reregistration.

Some glyphosate end-use products are in Toxicity Categories I or II for primary eye irritation or skin irritation. In California, glyphosate ranks high among pesticides causing illness or injury to workers, who report numerous incidents of eye and skin irritation from splashes during mixing

and loading. EPA is not adding any personal protective equipment (PPE) requirements at this time, but any existing PPE label requirements must be retained.

The Worker Protection Standard (WPS) for Agricultural Pesticides (please see 40 CFR 156 and 170) established an interim restricted entry interval (REI) of 12 hours for glyphosate. The Agency has decided to retain this REI as a prudent measure to mitigate risks to workers. During the REI, workers may reenter areas treated with glyphosate only in the few, narrow exceptions allowed in the WPS. The REI applies only to glyphosate uses within the scope of the WPS, so homeowner and commercial uses are not included.

Human Risk Assessment

EPA's worst case risk assessment of glyphosate's many registered food uses concludes that human dietary exposure and risk are minimal. Existing and proposed tolerances have been reassessed, and no significant changes are needed to protect the public.

Exposure to workers and other applicators generally is not expected to pose undue risks, due to glyphosate's low acute toxicity. However, splashes during mixing and loading of some products can cause injury, primarily eye and skin irritation. EPA is continuing to recommend PPE, including protective eye wear, for workers using end-use products that are in Toxicity Categories I or II for eye and skin irritation. To mitigate potential risks associated with reentering treated agricultural areas, EPA is retaining the 12 hour REI set by the WPS.

Environmental Assessment

Environmental Fate

Glyphosate adsorbs strongly to soil and is not expected to move vertically below the six inch soil layer; residues are expected to be immobile in soil. Glyphosate is readily degraded by soil microbes to AMPA, which is degraded to carbon dioxide. Glyphosate and AMPA are not likely to move to ground water due to their strong adsorptive characteristics. However, glyphosate does have the potential to contaminate surface waters due to its aquatic use patterns and through erosion, as it adsorbs to soil particles suspended in runoff. If glyphosate reached surface water, it would not be broken down readily by water or sunlight.

Ecological Effects

Glyphosate is no more than slightly toxic to birds and is practically non-toxic to fish, aquatic invertebrates and honeybees. Due to the presence of a toxic inert ingredient, some glyphosate end-use products must be labeled, "Toxic to fish," if they may be applied directly to aquatic environments. Product labeling does not preclude off-target movement of

glyphosate by drift. EPA therefore is requiring three additional terrestrial plant studies to assess potential risks to nontarget plants.

EPA does not expect that most endangered terrestrial or aquatic organisms will be affected by the registered uses of glyphosate. However, many endangered plants as well as the Houston toad (due to its habitat) may be at risk. EPA is deferring any use modifications or labeling amendments until it has published the Endangered Species Protection Plan and has given registrants guidance regarding endangered species precautionary labeling.

Ecological Effects Risk Assessment

Based on current data, EPA has determined that the effects of glyphosate on birds, mammals, fish and invertebrates are minimal. Under certain use conditions, glyphosate may cause adverse effects to nontarget aquatic plants. Additional data are needed to fully evaluate the effects of glyphosate on nontarget terrestrial plants. Risk reduction measures will be developed if needed, once the data from these studies are submitted and evaluated.

Additional Data Required

EPA is requiring three generic studies (Tier II Vegetative Vigor, Droplet Size Spectrum, and Drift Field Evaluation) which are not part of the target data base and do not affect the reregistration eligibility of glyphosate. The Agency also is requiring product-specific data including product chemistry and acute toxicity studies, as well as revised Confidential Statements of Formula and revised labeling.

Product Labeling Changes Required

All end-use glyphosate products must comply with EPA's current pesticide product labeling requirements. In addition:

- **Protection of Aquatic Organisms**

Non-Aquatic Uses - End-use products that are not registered for aquatic uses must bear the following label statement:

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwaters and rinsate.

Aquatic Uses - End-use products registered for aquatic uses must bear the following label statement:

Do not contaminate water when disposing of equipment washwaters and rinsate. Treatment of aquatic weeds can result in oxygen-loss from decomposition for dead plants. This loss can cause fish kills.

- **Worker Protection Standard (WPS) Requirements**

Any product whose labeling permits use in the production of an agricultural plant on any farm, forest, nursery or greenhouse must comply with the labeling requirements of:

- PR Notice 93-7, "Labeling Revisions Required by the Worker Protection Standard (WPS)," and
- PR Notice 93-11, "Supplemental Guidance for PR Notice 93-7."

Unless specifically directed in the RED, all statements required by these two PR Notices must appear on product labeling exactly as instructed in the Notices. Labels must be revised by April 21, 1994, for products distributed or sold by the primary registrant or supplementally registered distributors, and by October 23, 1995, for products distributed or sold by anyone.

- **Personal Protective Equipment (PPE)**

No new PPE requirements must be added to glyphosate labels. However, any existing PPE requirements on labels must be retained.

- **Entry Restrictions**

Products Not Primarily Intended for Home Use:

- Uses Within the Scope of the WPS - A 12-hour restricted entry interval (REI) is required for all products with uses within the scope of the WPS, except products intended primarily for home use. The PPE for early entry should be that required for applicators of glyphosate, except any applicator requirement for an apron or respirator is waived. This REI and PPE should be inserted into the standardized statements required by PR Notice 93-7.
  - Sole Active Ingredient End-Use Products - Labels must be revised to adopt the entry restrictions set forth in this section. Any conflicting entry restrictions on current labeling must be removed.
  - Multiple Active Ingredient Products - Registrants must compare the entry restrictions set forth in this section to those on their current labeling and retain the more protective. A specific time period in hours or days is considered more protective than "until sprays have dried" or "dusts have settled."

- Uses Not Within the Scope of the WPS - No new entry restrictions must be added. However, any entry restrictions on current product labeling with these uses must be retained.

Products Primarily Intended for Home Use:

- No new entry restrictions must be added. However, any entry restrictions on current product labeling must be retained.

Regulatory Conclusion

The use of currently registered pesticide products containing the isopropylamine and sodium salts of glyphosate in accordance with the labeling specified in this RED will not pose unreasonable risks or adverse effects to humans or the environment. Therefore, all uses of these products are eligible for reregistration.

These glyphosate products will be reregistered once the required product-specific data, revised Confidential Statements of Formula and revised labeling are received and accepted by EPA.

Products which contain active ingredients in addition to glyphosate will not be reregistered until all their other active ingredients also are eligible for reregistration.

For More Information

EPA is requesting public comments on the Reregistration Eligibility Decision (RED) document for glyphosate during a 60-day time period, as announced in a Notice of Availability published in the Federal Register. To obtain a copy of the RED document or to submit written comments, please contact the Pesticide Docket, Public Response and Program Resources Branch, Field Operations Division (7506C), Office of Pesticide Programs (OPP), US EPA, Washington, DC 20460, telephone 703-305-5805.

Following the comment period, the glyphosate RED document will be available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, telephone 703-487-4650.

For more information about EPA's pesticide reregistration program, the glyphosate RED, or reregistration of individual products containing glyphosate, please contact the Special Review and Reregistration Division (7508W), OPP, US EPA, Washington, DC 20460, telephone 703-308-8000.

For information about the health effects of pesticides, or for assistance in recognizing and managing pesticide poisoning symptoms, please contact the National Pesticides Telecommunications Network (NPTN). Call toll-free 1-800-858-7378, between 8:00 am and 6:00 pm Central Time, Monday through Friday.

REREGISTRATION ELIGIBILITY DECISION DOCUMENT

GLYPHOSATE

LIST A
CASE 0178

US Environmental Protection Agency
Office of Pesticide Programs
Special Review and Reregsitration Division

GLYPHOSATE REREGISTRATION ELIGIBILITY TEAM

Office of Pesticide Programs:

Special Review and Reregistration Division
Eric Feris . . . . . Reregistration Branch

Health Effects Division
Jane Smith . . . . . Chemical Coordination Branch
Krystyna Locke . . . . . Toxicology Branch I
Jeff Evans . . . . . Occupational and Residential Exposure Branch
Randolph Perfetti . . . . . Chemistry Branch - Reregistration Support

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GLOSSARY OF TERMS AND ABBREVIATIONS

a.i.	Active Ingredient
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CSF	Confidential Statement of Formula
EEC	Estimated Environmental Concentration. The estimated pesticide concentration in an environment, such as a terrestrial ecosystem.
EP	End-Use Product
EPA	U.S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FFDCA	Federal Food, Drug, and Cosmetic Act
FR	Federal Register
HDT	Highest Dose Tested
LC_{50}	Median Lethal Concentration. A statistically derived concentration of a substance that can be expected to cause death in 50% of test animals. It is usually expressed as the weight of substance per weight or volume of water or feed, e.g., mg/l or ppm.
LD_{50}	Median Lethal Dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral or dermal). It is expressed as a weight of substance per unit weight of animal, e.g., mg/kg.
LD_{lo}	Lethal Dose-low. Lowest Dose at which lethality occurs
LEL	Lowest Effect Level

MATC	Maximum Allowable Toxicant Concentration: A range at which the pesticide causes no effect (NOEL) and the lowest dose at which an effect was observed (LOEL).
MP	Manufacturing-Use Product
MPI	Maximum Permissible Intake
MRID	Master Record Identification (number). EPA's system of recording and tracking studies submitted.
N/A	Not Applicable
NPDES	National Pollutant Discharge Elimination System
NOEL	No Observed Effect Level
OPP	Office of Pesticide Programs
PADI	Provisional Acceptable Daily Intake
ppm	Parts Per Million
REI	Restricted Entry Interval
RfD	Reference Dose
RS	Registration Standard
TD	Toxic Dose. The dose at which a substance produces a toxic effect.
TC	Toxic Concentration. The dose at which a substance produces a toxic effect.
TMRC	Theoretical Maximum Residue Contribution.
WPS	Worker Protection Standard

EXECUTIVE SUMMARY

This document addresses the reregistration eligibility of the pesticide glyphosate. There are 63 glyphosate-containing products registered for use in the United States. The isopropylamine salt of glyphosate, the active ingredient in 53 of these registrations, is used as a herbicide to control a number of broadleaf weeds and grasses. The principal food use sites include corn, wheat, sorghum, citrus and stone fruits, potatoes and onions, asparagus, coffee, peanuts, and pineapples. There are also a number of non-food use sites including ornamental, turf, forestry, and industrial rights-of-way. Two registrations contain the sodium salt of glyphosate and are used in sugarcane fields. In addition there are seven herbicide/plant regulation products containing the monoammonium salt of glyphosate which were registered subsequent to the development of List A and are not a subject of this RED. Except where explicitly noted otherwise, the term "glyphosate," when used in this document, refers to either the technical acid or the isoproplyamine and sodium salts of glyphosate. However, the monoammonium salt is included in the tolerance expression. Available data have been sufficient to allow re-assessment of existing tolerances, which includes the monoammonium salt of glyphosate.

In June 1986, the Agency issued the document "Registration Standard for Pesticide Products Containing Glyphosate as the Active Ingredient" (NTIS #PB87-103214). The Registration Standard required scientific studies in the areas of phytotoxicity, environmental fate, toxicology, product chemistry, and residue chemistry. With the exception of a few waived studies, all of the data required have been submitted. After completing its review for reregistration, the Agency now concludes that the data base on glyphosate is substantially complete.

Based on the results of its reregistration review, EPA has concluded that all registered uses of glyphosate are eligible for reregistration. The Agency has classified glyphosate as a Group E carcinogen (signifies evidence of non-carcinogenicity in humans). A Reference Dose of 2 mg/kg/day has been recommended. This proposal is based on a maternal NOEL of 175 mg/kg/day from a rabbit developmental toxicity study and an uncertainty factor of 100. The dietary risk assessment is based on a worst-case scenario, assuming treatment of 100% of acreage and highest legal residue values which likely result in an overestimation of exposure and risk. Even with these values, however, dietary exposure is expected to be minimal. There are 85 tolerances established for various crops and crop groups as well as Federal Food, Drug, and Cosmetic Act §409 tolerances for processed food and animal feed and animal tolerances. A re-assessment of tolerances is included in this document and there are no major changes in the previously-established tolerances. Studies show that glyphosate is no more than slightly toxic to birds and is practically non-toxic to fish and honeybees. However, a toxic inert in glyphosate end use products necessitates the labelling of some

products "toxic to fish" since some glyphosate products are applied directly to aquatic environments.

The Agency does have concerns regarding the potential hazard to endangered plant species and the Houston toad. However, the Agency is not requiring any modification of use or label changes in this document. A Federal Register Notice on the Endangered Species Protection Plan and subsequent guidance to registrants will impose appropriate exposure mitigation measures for areas where endangered plant species and the Houston toad may be encountered. In addition, there have been a number of reported incidents of spray drift damage to non-target crops. Spray drift studies are required as is a Tier II Vegetative Vigor study. These studies are not part of the target data base for reregistration of glyphosate.

Before reregistering each product, the Agency is requiring that product specific data in the areas of product chemistry and acute toxicology, revised Confidential Statements of Formula, and revised labeling be submitted within eight (8) months of the issuance of this document. In an effort to reduce the time, resources, and number of animals needed to fulfill the acute toxicology data requirements for glyphosate-containing end use products, the Agency has "batched" products considered to be similar with respect to acute toxicity testing requirements. After reviewing these data and the revised labels, the Agency will determine whether to re-register a product based on whether or not that product meets the requirements in Section 3(c)(5) of FIFRA. End use products containing glyphosate in combination with other active ingredients will not be re-registered until the Reregistration Eligibility Decisions for all active ingredients contained in that product are issued and all the active ingredients contained in the product are also eligible for reregistration. However, product specific data for these products are being called in at this time.

I. INTRODUCTION

In 1988, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was amended to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984. The amended Act provides a schedule for the reregistration process to be completed in nine years. There are five phases to the reregistration process. The first four phases of the process focus on identification of data requirements to support the reregistration of an active ingredient and the generation and submission of data to fulfill the requirements. The fifth phase is a review by the U.S. Environmental Protection Agency (referred to as "the Agency") of all data submitted to support reregistration.

FIFRA Section 4(g)(2)(A) states that in Phase 5 "the Administrator shall determine whether pesticides containing such active ingredient are eligible for registration" before calling in data on products and either re-registering products or taking "other appropriate regulatory action." Thus, reregistration involves a thorough review of the scientific data base underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential hazards arising from the currently registered uses of the pesticide; to determine the need for additional data on health and environmental effects; and to determine whether the pesticide meets the "no unreasonable adverse effects" criterion of FIFRA.

This document presents the Agency's decision regarding the reregistration eligibility of the registered uses of the isopropylamine salt and the sodium salt formulations of glyphosate. Except where explicitly noted otherwise, the term "glyphosate," when used in this document, refers to either the technical acid or the isoproplyamine and sodium salts of glyphosate but does not cover the monoammonium salt products since the compound was not included in the Federal Register publication of List A. The document consists of six sections. Section I is the introduction. Section II describes glyphosate, its uses, data requirements and regulatory history. Section III discusses the human health and environmental assessment based on the data available to the Agency. Section IV presents the reregistration decision for glyphosate. Section V discusses the reregistration requirements for glyphosate. Finally, Section VI is the Appendices which support this Reregistration Eligibility Document. Additional details concerning the Agency's review of applicable data are available on request.[1]

[1] EPA's reviews of data on the set of registered uses considered for EPA's analysis may be obtained from the OPP P Field Operations Division (H7506C), Office of Pesticide Programs, EPA, Washington, DC 20460.

II. CASE OVERVIEW

A. Chemical Overview

The following active ingredient(s) are covered by this Reregistration Eligibility Document:

Common Name: glyphosate

Chemical Name: N-phosphonomethyl glycine

CAS Registry Number: 38641-94-0

OPP Chemical Codes: 103601 (isopropylamine salt)
103603 (sodium salt)

Empirical Formula: $C_3H_8NO_5P$

Trade Names: Roundup, Rodeo, Shackle

Basic Manufacturer: Monsanto Company
800 N. Lindbergh Blvd.
St. Louis, MO 63167

B. Use Profile

The following is information on the current registered uses with an overview of use sites and application methods. A detailed table of the uses of glyphosate is given in Appendix A.

Chemical: glyphosate, isopropylamine salt (103601)

Type of Chemical: herbicide

Mechanism of Action: not known at this time, but it appears to inhibit the aromatic amino acid biosynthesis pathway and may inhibit or repress chlorismate mutase and/or prephenate hydratase.

Use groups and sites:

AQUATIC FOOD CROP:
agricultural drainage systems, irrigation systems, lakes/ponds/reservoirs (with human or wildlife use), streams/rivers/channeled water.

AQUATIC NON-FOOD INDUSTRIAL:
aquatic areas/water, drainage systems, sewage systems.

AQUATIC NON-FOOD OUTDOOR:
aquatic areas/water

FORESTRY:
conifer release, forest plantings (reforestation programs), forest trees (all or unspecified).

GREENHOUSE FOOD CROP:
greenhouses-in use.

INDOOR NON-FOOD:
greenhouse-empty.

OUTDOOR RESIDENTIAL:
household/domestic dwellings outdoor premises.

TERRESTIAL FEED CROP:
alfalfa, barley, beans, buckwheat, corn, grass forage/fodder/hay, lentils, millet (proso), nongrass forage/fodder/straw/hay, oats, pastures, rye, sorghum, wheat.

TERRESTRIAL FOOD CROP:
acerola (West Indies Cherry), apricot, artichoke (Jerusalem), asparagus, atemoya, avocado, banana, beech nut, beets, blackberry, blueberry, boysenberry, brazil nut, breadfruit (breadnut), broccoli, brussels sprouts, butternut, cabbage, cabbage (Chinese), carambola (jalea), carrot (including tops), cashew, cauliflower, celery, chard (swiss), cherimoya, cherry, chestnut, chicory, cocoa, coffee, collards, cranberry, cress (water), cucumber, currant, date, dewberry, eggfruit tree (canistel), eggplant, elderberry, endive (escarole), fig, filbert (hazelnut), garlic, gooseberry, gourds, groundcherry (strawberry tomato/tomatillo), guava, hickory nut, horseradish, huckleberry, jaboticaba, jackfruit, kale, kitembilla (ceylon gooseberry), kiwi fruit, kohlrabi, leek, lettuce, litchi nut, loganberry, longan, loquat, macadamia nut

(bushnut), mamey (mammee apple), mango, marmaladebox (genipapo), mayhaw (hawthorn), melons, melons (cantaloupe), melons (honeydew), melons (mango), melons (musk), melons (water), melons winter (casaba/crenshaw/honeydew/persian), mustard, nectarine, okra, olive, onion, papaya, parsley, passion fruit, peach, pear, pecan, pepper, persimmon, pistachio, plantain, plum, pomegranate, prune, pumpkin, quince, radish, raspberry (black, red), rhubarb, rutabaga, sapodilla, sapota (white), soursop, spinach, squash (summer), squash (winter), sugar apple (custard apple), sweet potato, tamarind, taro, tea, walnut (English/black), yam.

TERRESTRIAL FOOD + FEED CROP:
agricultural fallow/idleland, almond, apple, barley, beans, beets (unspecified), buckwheat, calamondin, citron (citrus), citrus hybrids other than tangelo, corn (unspecified), corn (field), cotton (unspecified), grapefruit, grapes, kumquat, lemon, lentils, lime, millet proso (broomcorn), mustard, oats, orange, parsnip, peanuts (unspecified), peas (unspecified), pineapple, potato (white/irish), pummelo (shaddock), rape, rice, rice (wild), rye, sorghum, soybeans (unspecified), sugar beet, sugarcane, tangelo, tangerines, tomato, triticale, turnip, wheat.

TERRESTRIAL + GREENHOUSE NON-FOOD CROP:
ornamental and/or shade trees, ornamental woody shrubs and vines.

TERRESTRIAL NON-FOOD CROP:
agricultural fallow/idleland, agricultural rights-of-way/fencerows/hedgerows, agricultural uncultivated areas, airports/landing fields, christmas tree plantations, golf course turf, industrial areas (outdoor), nonagricultural outdoor buildings/structures, nonagricultural rights-of-way/fencerows/hedgerows, nonagricultural uncultivated areas/soils, ornamental and/or shade trees, ornamental lawns and turf, ornamental woody shubs and vines, paths/patios, paved areas (private roads/sidewalks), recreational areas, urban areas.

TERRESTRIAL NON-FOOD+OUTDOOR RESIDENTIAL:
ornamental and/or shade trees, ornamental herbaceous plants, ornamental lawns and turf, ornamental woody shubs and vines.

Pests: many broadleaf and grass weeds

Formulation types registered:

SINGLE ACTIVE INGREDIENT:
Form Not Identified/Liquid
53.50 % glyphosate, isopropylamine salt
41.00 % glyphosate, isopropylamine salt
Form Not Identified/Solid
76.00 % glyphosate, isopropylamine salt
Liquid-Ready to Use
19.70 % glyphosate, isopropylamine salt
18.30 % glyphosate, isopropylamine salt
15.80 % glyphosate, isopropylamine salt
1.00 % glyphosate, isopropylamine salt
0.96 % glyphosate, isopropylamine salt
0.50 % glyphosate, isopropylamine salt
Manufacturing Use
94.00 % glyphosate, isopropylamine salt
Pelleted/Tableted
83.50 % glyphosate, isopropylamine salt
60.00 % glyphosate, isopropylamine salt
Pressurized Liquid
0.96 % glyphosate, isopropylamine salt
0.75 % glyphosate, isopropylamine salt
Soluble Concentrate/Liquid
62.00 % glyphosate, isopropylamine salt
53.80 % glyphosate, isopropylamine salt
41.50 % glyphosate, isopropylamine salt
41.00 % glyphosate, isopropylamine salt
28.60 % glyphosate, isopropylamine salt
25.10 % glyphosate, isopropylamine salt
18.00 % glyphosate, isopropylamine salt
10.00 % glyphosate, isopropylamine salt
8.20 % glyphosate, isopropylamine salt
7.00 % glyphosate, isopropylamine salt
5.00 % glyphosate, isopropylamine salt
Soluble Concentrate/Solid
93.96 % glyphosate, isopropylamine salt

MULTIPLE ACTIVE INGREDIENT:
Liquid-Ready to Use
12.40 % glyphosate, isopropylamine salt + 1 other A.I.
7.70 % glyphosate, isopropylamine salt + 1 other A.I.
0.50 % glyphosate, isopropylamine salt + 1 other A.I.
0.25 % glyphosate, isopropylamine salt + 1 other A.I.
Soluble Concentrate/Liquid
16.50 % glyphosate, isopropylamine salt + 1 other A.I.
14.80 % glyphosate, isopropylamine salt + 1 other A.I.
13.30 % glyphosate, isopropylamine salt + 1 other A.I.
12.90 % glyphosate, isopropylamine salt + 1 other A.I.

Methods and rates of application (Given in maximum active (acid equivalent (ae)) rates, except as otherwise noted):

Broadcast or spray; for example as needed:

Form Not Identified/Liquid - rates were not specified in Appendix A dated 8/12/93;

Form Not Identified/Solid - rates were not specified in Appendix A dated 8/12/93;

Liquid-Ready to Use - applied at rate of 3.08 lb ae/A;

Pelleted/Tableted - applied as a spot treatment, for example from a hand held sprayer;

Pressurized Liquid - applied as a spot treatment, for example from an aerosol can;

Soluble Concentrate/Liquid - applied at rate of 7.5 lb ae/A;

Soluble Concentrate/Solid - applied at rates of 0.09 gal ae/A;

Chemical:	glyphosate, sodium salt (103603)
Type of Chemical:	plant regulator
Mechanism of Action:	modifies plant growth; hastens fruit ripening

Use Groups and Sites:

TERRESTRIAL FOOD + FEED CROP:
peanuts (unspecified); sugarcane

Formulation Types Registered:

SINGLE ACTIVE INGREDIENT:
soluble concentrate/solid
75.0% glyphosate, sodium salt

Methods and Rates of Application:

soluble concentrate/solid - applied as ground spray at peanut bloom stage at 0.0375 lb a.i./A in 10 gal water;

soluble concentrate/solid - applied as aerial spray at sugarcane ratoon stage at 0.525 lb a.i./A in 5 gal water.

Use Limitations:
sugarcane - 21 days preharvest interval; peanuts - 84 days preharvest interval. Do not apply this product through any type of irrigation system.

C. Estimated Usage of Pesticide

This section summarizes the best estimates available for the pesticide uses of glyphosate. These estimates are derived from a variety of published and proprietary sources available to the Agency. The data, reported on an aggregate and site (crop) basis, reflect annual fluctuations in use patterns as well as the variability in using data from various information sources.

The table below summarizes glyphosate useage by site.

Glyphosate Usage

Site	Multiple Acres Treated (x1000)	Pounds AI (x1000)
non-ag areas	unknown	3000-7000
almonds	350-390	500-550

apples	75-275	65-200
barley	550-600	275-325
cherries	15-95	20-125
corn, field	1,300-1,700	1,100-1,200
cotton	300-1,000	225-375
hay/pasture	3,000-3,500	1,500-1,700
dry edible beans/peas	50	20
grapefruit	70-140	183-375
grapes	45-550	25-265
lemons	5-75	10-70
other ag sites	3,000-3,500	1,000-1,500
oranges	300-600	650-1,300
peaches	10-150	10-110
peanuts	10-30	5-10
pears	15-50	15-65
pecans	5-300	5-150
plums/prunes	5-80	5-40
rice	30-55	25-30
sorghum	450-550	100-150
soybeans	2,600-4,800	2,200-2,400
spring wheat	200-225	50-60
sugarcane	10-70	5-35
potatoes	20-40	25-30
sunflowers	60-70	25-40
sweet corn	10-30	5-15
tomatoes	30-40	15-30
green beans/peas	20-40	5-20
walnuts	150-175	100-125
winter wheat	350-1,150	250-450

TOTAL	**12,985-20,280**	**11,398-18,745**

In a typical year between 1989 and 1991, approximately 13-20 million acre treatments were made with 18.7 million pounds active ingredient. Hay/pasture (20%), soybeans (20%), field corn (9%), and other agricultural areas (20%) comprise 71% of the total acreage treated with glyphosate. Non-agricultural areas (33%), soybeans (15%), hay/pasture (11%), and corn (8%) comprise 67% of the total pounds of active ingredient applied.

D. Data Requirements

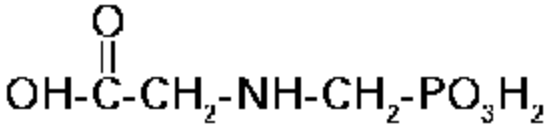
Data required in the June 1986 Registration Standard for glyphosate include studies on product chemistry, ecological effects, environmental fate, toxicology, and residue chemistry. These data were required to support the uses listed in the Registration Standard. Appendix B includes all data requirements identified by the Agency for currently registered uses needed to support reregistration.

E. Regulatory History

Glyphosate is registered in the United States for use as a herbicide. The June 1986 Registration Standard evaluated the studies currently on file at the Agency and required submission of further data. This Reregistration Eligibility Document reflects an assessment of all data which were submitted in response to the Registration Standard.

III. SCIENCE ASSESSMENT

A. Product Chemistry



MOLECULAR STRUCTURE OF GLYPHOSATE

Empirical Formula: $C_3H_8NO_5P$
Molecular Weight: 169.07
CAS Registry No.: 38641-94-0
Shaughnessy No.: 103601 (isopropylamine salt, IPA)
103603 (sodium salt)

The glyphosate (N-phosphonomethyl glycine) salts are nonselective herbicides and plant growth regulators. The technical isopropylamine salt (IPA) is a white crystalline solid with a melting point of 200EC and a bulk density of 1.74 lb/ft^3. It is 1% soluble in water at 25EC and insoluble in ethanol, acetone, or benzene. The technical sodium salt is a white crystalline solid which decomposes at 140EC with a bulk density of 30 lb/ft^3.

B. Human Health Assessment

1. Toxicology Assessment

The toxicological data base on glyphosate is adequate and will support reregistration eligibility.

a. Acute Toxicity

The table below summarizes the toxicity results and categories for technical grade glyphosate. The acute inhalation study was waived by the Agency since glyphosate technical is a nonvolatile solid and adequate inhalation studies were conducted on the end-use product formulations.

Acute Toxicity

Test	Result	Category
Acute Oral (rat) (1)	> 4320 mg/kg	III
Acute Dermal (rabbit)(1)	> 2 g/kg	III
Acute Inhalation (1)	Not Required	N/A

1 - MRID 00067039

The following table is derived from MPs considered toxicologically similar to glyphosate technical.

Acute Toxicity		
Test	**Result**	**Category**
Eye Irritation (1)	mild irritation, clears in 7 days	III
Dermal Irritation (2)	slight irritation	IV
Skin Sensitization (3)	negative	N/A
1 - MRID 41400603 2 - MRID 41400604 3 - MRIDs 00137137, 00137138, 00137139, 00137140		

Other studies submitted to the Agency give similar results. They are acceptable for reregistration (MRIDs 41400601, and 41400602)

b. Subchronic Toxicity

In a 90-day feeding study Sprague-Dawley rats were fed diets containing 0, 1000, 5000 or 20000 ppm of glyphosate for three months. These doses were equivalent to 0, 63, 317 and 1267 mg/kg/day, respectively (males) and 0, 84, 404 and 1623 mg/kg/day, respectively (females). The following findings were regarded as possibly treatment-related: (1) increased serum phosphorus and potassium in all treated groups, males and females; (2) increased serum glucose in the mid-dose and high-dose males; (3) increased blood urea nitrogen (BUN) and serum alkaline phosphatase in the high-dose males; and (4) occurrence of pancreatic lesions in the high-dose males (pancreas was not examined in the low-dose and mid-dose groups). Based on these findings, the systemic NOEL is < 1000 ppm (not determined definitively) for both sexes. (MRIDs 40559401, and 00093879)

In a second 90-day feeding study CD-1 mice were fed diets containing 0, 250, 500 or 2500 mg/kg/day of glyphosate for three months. Body weight gains of the high-dose males and females were about 24% and 18% lower, respectively, than those of the controls. Body weight gains of the low-dose and mid-dose groups were comparable to those of the controls. Based on the reduced

body weight gains in both sexes, the NOEL for systemic toxicity is 500 mg/kg and the LOEL is 2500 mg/kg. (MRID 00036803)

In a 21-day dermal study glyphosate was applied to the skin of New Zealand white rabbits using 10 rabbits/sex/dose (5 with intact and 5 with abraded skin). The levels of glyphosate tested were 10, 1000 or 5000 mg/kg/day. The rabbits were exposed for three consecutive weeks, 6 hours/day, 5 days/week. Treatment-related effects observed only in the high dose groups included: (1) very slight erythema and edema in intact and abraded skin of both sexes; (2) decreased food consumption in males; and (3) decreased serum lactic dehydrogenase in both sexes. Based on these effects, the NOEL for males and females is 1000 mg/kg/day and the LOEL is 5000 mg/kg/day. (MRID 00098460)

The required 90-day feeding study in dogs is satisfied by the one-year dog feeding study. (MRID 00153374)

c. Chronic Toxicity

A chronic feeding/carcinogenicity study was conducted using male and female Sprague-Dawley rats which were fed diets containing 0, 30, 100 or 300 ppm of glyphosate for 26 months. These levels were equivalent to 0, 3, 10 and 31 mg of glyphosate/kg/day, respectively, for the males and 0, 3, 11 and 34 mg of glyphosate/kg/day, respectively, for the females. There were no effects based on any of the parameters examined (toxic signs, mortality, body weights, food consumption, hematology, clinical chemistry, urinalysis, organ weights and organ/tissue pathology). Therefore, the NOEL for systemic toxicity is $ 300 ppm (HDT; males: 31 mg/kg/day and females: 34 mg/kg/day). (MRID 00093879)

A second chronic feeding/carcinogenicity study was conducted using male and female Sprague-Dawley rats which were fed diets containing 0, 2000, 8000 or 20000 ppm of glyphosate for 2 years. These levels were equivalent to 0, 89, 362 or 940 mg/kg/day, respectively, for the males and 0, 113, 457 or 1183 mg/kg/day, respectively, for the females. Treatment-related effects observed only in the high-dose group included: (1) In the females: decreased body weight gains; and (2) In the males: increased incidence of cataracts and lens abnormalities, decreased urinary

pH, increased absolute liver weight and increased liver weight/brain weight ratio (relative liver weight). No significant systemic effects were observed in the low-dose and mid-dose male and female groups. Therefore, the NOEL for systemic toxicity is 8000 ppm (males: 362 mg/kg/day and females: 457 mg/kg/day) and the LOEL is 20000 ppm (HDT; males: 940 mg/kg/day and females: 1183 mg/kg/day). (MRID 41643801)

A chronic study was conducted using male and female beagle dogs which were given glyphosate in gelatin capsules containing 0, 20, 100 or 500 mg/kg/day for one year. There were no effects based on all parameters examined, in all groups. Therefore, the NOEL for systemic toxicity is $ 500 mg/kg/day, for both sexes. (MRID 00153374)

d. Carcinogenicity

A chronic feeding/carcinogenicity study was conducted using Sprague-Dawley rats which were fed diets containing glyphosate (males: 0, 3, 10 or 31 mg/kg/day and females: 0, 3, 11 or 34 mg/kg/day) for 26 months. The following findings were observed in the high-dose groups when compared with the concurrent controls: (1) increased incidence of thyroid C-cell carcinomas in females; and (2) increased incidence of interstitial cell (Leydig cell) testicular tumors. However, the Agency concluded that these neoplasms were not treatment-related and glyphosate was not considered to be carcinogenic in this study because the incidence of thyroid carcinomas was not statistically significant and the incidence of testicular tumors was within the historical incidence. The Agency also concluded that this study was not conducted at high enough dose levels for an adequate negative carcinogenicity. (MRID 00093879)

A chronic feeding/carcinogenicity study was conducted using Sprague-Dawley rats fed diets containing glyphosate (males: 0, 89, 362 or 940 mg/kg/day and females: 0, 113, 457 or 1183 mg/kg/day) for 2 years. The study showed a slightly increased incidence of (1) pancreatic islet cells adenomas in the low-dose and high-dose males; (2) hepatocellular (liver) adenomas in the low-dose and high-dose males; and (3) thyroid C-cells adenomas in the mid-dose and high-dose males and females. The Agency concluded that these

adenomas were not treatment-related and glyphosate was not considered to be carcinogenic in this study. With respect to pancreatic islet cells adenomas, there was no statistically significant positive dose-related trend in their occurrence; there was no progression to carcinomas; and the incidence of pancreatic hyperplasia (non-neoplastic lesion) was not dose-related. With respect to hepatocellular adenomas, the increased incidence of these neoplasms was not statistically significant in comparison with the controls; the incidence was within the historical control range; there was no progression to carcinomas; and the incidence of hyperplasia was not compound-related. With respect to thyroid C-cell adenomas, there was no statistically significant dose-related trend in their occurrence; the increased incidence was not statistically significant; there was no progression to carcinomas; and there was no significant dose-related increase in severity or incidence of hyperplasia in either sex. (MRID 41643801)

A carcinogenicity study in mice was conducted with CD-1 mice fed diets containing 0, 150, 750 or 4500 mg/kg/day of glyphosate for 18 months. No effects were observed in the low-dose and mid-dose groups. The following findings were observed in the high-dose group: (1) decreased body weight gain in males and females; (2) increased incidence of hepatocellular hypertrophy, hepatocellular necrosis and interstitial nephritis in males; (3) increased incidence of proximal tubule epithelial basophilia and hypertrophy in females; and (4) slightly increased incidence of renal tubular adenomas, a rare tumor, in males. Based on these effects, the systemic NOEL and LOEL were 750 mg/kg/day and 4500 mg/kg/day, respectively. The Agency concluded that the occurrence of these adenomas was spontaneous rather than compound-induced because the incidence of renal tubular adenomas in males was not statistically significant when compared with the concurrent controls. An independent group of pathologists and biometricians also conducted extensive evaluations of these adenomas and reached the same conclusion. Therefore, glyphosate was not considered to be carcinogenic in this study. (MRIDs 00130406, and 00150564)

On June 26, 1991, the Agency classified glyphosate in Group E (evidence of non-carcinogenicity for humans), based on a lack of convincing evidence of carcinogenicity in adequate studies with two animal species, rat and mouse.

e. Developmental Toxicity

A developmental toxicity study was conducted with pregnant Charles River COBS CD rats which were administered 0, 300, 1000 or 3500 mg/kg/day of glyphosate by gavage during gestation days 6 through 19. Treatment-related effects observed only in the high-dose dams included: (1) diarrhea; (2) decreased mean body weight gain; (3) breathing rattles; (4) inactivity; (5) red matter around the nose and mouth, and on forelimbs and dorsal head; (6) decreases in total implantations/dam and inviable fetuses/dam; and (7) deaths (6/25 or 24% of the group). Treatment-related developmental effects observed only in the high-dose group included: (1) increased number of litters and fetuses with unossified sternebrae; and (2) decreased mean fetal body weights. Therefore, the NOEL and LOEL for maternal toxicity are 1000 mg/kg/day and 3500 mg/kg/day, respectively. The NOEL and LOEL for developmental toxicity are 1000 mg/kg/day and 3500 mg/kg/day, respectively. (MRID 00046362)

In a second study, pregnant Dutch Belted rabbits were administered 0, 75, 175 or 350 mg/kg/day of glyphosate by gavage during gestation days 6 through 27. Treatment-related findings were observed only in the high-dose group and included: (1) diarrhea; (2) nasal discharge; and (3) death (10/16 or 62.5% of does died by gestation day 21). Developmental toxicity was not observed at any dose tested. Therefore, the NOEL and LOEL for maternal toxicity are 175 mg/kg/day and 350 mg/kg/day, respectively. The NOEL for developmental toxicity is $ 175 mg/kg/day. Due to high maternal mortality at the 350 mg/kg/day dose level, too few litters (only 6) were available to assess adequately developmental toxicity at that level. (MRID 00046363)

f. Reproductive Toxicity

A reproduction study was conducted with male and female Sprague-Dawley rats which were administered 0, 3, 10 or 30 mg/kg/day of glyphosate continuously in the diet for three successive generations. The only effect observed was an increased incidence of focal tubular dilation of the kidney (both unilateral and bilateral combined) in the high-dose male F_{3b} pups. Therefore, the NOEL for systemic and reproductive toxicity is $ 30 mg/kg/day (HDT). The

NOEL and LOEL for developmental toxicity are 10 mg/kg/day and 30 mg/kg/day, respectively. (MRID 00105995)

Another reproduction study was conducted with Sprague-Dawley rats which were administered 0, 100, 500 or 1500 mg/kg/day of glyphosate continuously in the diet for two successive generations. Treatment-related effects observed only in the high-dose group included: (1) soft stools, very frequent, in the F_o and F_1 males and females; (2) decreased food consumption and body weight gain of the F_o and F_1 males and females during the growth (premating) period; and (3) decreased body weight gain of the F_{1a}, F_{2a} and F_{2b} male and female pups during the second and third weeks of lactation. Focal tubular dilation of the kidneys, observed in the previous study (00105995), was not observed at any dose level in this study. Based on the above findings, the systemic NOEL and LOEL are 10000 ppm (500 mg/kg/day) and 30000 ppm (1500 mg/kg/day), respectively. The reproductive NOEL is 30000 ppm (1500 mg/kg/day; HDT); and the developmental NOEL and LOEL are 10000 ppm (500 mg/kg/day) and 30000 ppm (1500 mg/kg/day), respectively. (MRID 41621501)

Since the focal tubular dilation of the kidneys was not observed at the 1500 mg/kg/day level (HDT) in the 2-generation rat reproduction study but was observed at the 30 mg/kg/day level (HDT) in the 3-generation rat reproduction study (00105995), the Agency concluded that the latter was a spurious rather than glyphosate-related effect.

g. Mutagenicity

A Gene mutation assay in an Ames Test was conducted using glyphosate, both with and without metabolic activation. The strains of *Salmonella typhimurium* used were TA98, TA100, TA1535 and TA1537. No increases in reverse mutations were observed at any concentration. (MRID 00078620)

A gene mutation assay in mammalian cells was conducted using glyphosate in the Chinese hamster ovary (CHO) cells/hypoxanthine - guanine -phosphoribosyl transferase (HGPRT) assay, with and without metabolic activation. No mutagenic response was observed either with or without metabolic activation up to the limit of cytotoxicity (10 mg/Ml). (MRID 00132681)

A Structural Chromosomal Aberration Assay was conducted using a single dose of glyphosate administered intraperitoneally (i.p.) to male and female Sprague-Dawley rats. The dose used was 1 g/kg of body weight and the bone marrow cells were examined for clastogenic (chromosome-damaging) effect. No significant clastogenic effects were observed. (MRID 00132683)

In a fourth study, glyphosate was tested in two assays: the rec-assay using *B. subtilis* H17 (rec^+) and M45 (rec^-); and the reverse mutation assays using *E. coli* WP2 hcr and *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation. No increases in mutations were observed in either study. (MRID 00078619)

h. Metabolism

Two metabolism studies with rats are available. In the first study, single or repeated doses of radiolabeled ^{14}C-glyphosate were administered orally to male and female Sprague-Dawley rats. Following a single oral dose of ^{14}C-glyphosate, 30 to 36% of the dose was absorbed and less than 0.27% of the dose was eliminated as CO_2. Ninety-seven point five percent of the administered dose was excreted in the urine and feces as the parent compound, glyphosate. Amino methyl phosphonic acid (AMPA) was the only metabolite found in urine (0.2-0.3% of the administered dose) and feces (0.2-0.4% of the administered dose). Less than 1.0% of the

absorbed dose remained in tissues and organs, primarily in bone tissue. Repeated dosing at 10 mg/kg did not significantly change the metabolism, distribution or excretion of glyphosate. (MRIDs 40767101, and 40767102)

In a second study, male and female Sprague-Dawley rats received single intraperitoneal injections of radiolabeled ^{14}C-glyphosate. The dose level of glyphosate used for male and female rats was 1150 mg/kg. Blood samples were collected 0.25, 0.50, 1, 2, 4, 6 and 10 hours after injection. Femoral bone marrow samples were collected from one third of the male and female rats sacrificed at 0.5, 4, or 10 hours after injection. Thirty minutes after injection of glyphosate, the concentration of radioactivity in the bone marrow of male and female rats was equivalent to 0.0044% and 0.0072%, respectively, of the administered dose. Assuming first order kinetics, the decrease in radioactivity in bone marrow occurred with a half-life of 7.6 and 4.2 hours for males and females, respectively. Similarly, the half-lives of the radioactivity in plasma were approximately 1 hour for both sexes. These findings indicate that very little glyphosate reaches bone marrow, that it is rapidly eliminated from bone marrow and that it is even more rapidly eliminated from plasma. (MRID 00132685)

i. Neurotoxicity

The acute and 90-day neurotoxicity screening battery in the rat (guidelines 81-8-SS, 82-7) is not being required since there was no evidence of neurotoxicity seen in any of the existing studies at very high doses and this chemical lacks a leaving group; therefore, it would not seem likely to inhibit esterases (the presumptive neurotoxic mechanism of concern for all organophosphates).

j. Other Toxicological Endpoints

A dermal penetration study (guideline 85-2) with technical grade glyphosate is not being required because there are no toxicological endpoints to indicate this study is necessary.

Domestic Animal Safety Studies (86-1) are not being required for the use patterns of glyphosate (a plant growth regulator and herbicide).

Technical grade glyphosate contains N-nitrosoglyphosate (NNG) as a contaminant. Carcinogenicity testing of nitroso contaminants is normally required only in those cases in which the level of nitroso compounds exceeds 1.0 ppm. Analyses showed that greater than 92% of the individual technical glyphosate samples contained less than 1.0 ppm NNG. The Agency concluded that the NNG content of glyphosate was not toxicologically significant.

k. Reference Dose

On August 27, 1992, the Agency's Office of Pesticide Programs Reference Dose (RfD) Peer Review Committee recommended that the RfD for glyphosate be established at 2 mg/kg/day. This value was based on the maternal NOEL of 175 mg/kg/day from the rabbit developmental toxicity study (00046363) and an uncertainty factor (UF) of 100. This RfD has not yet been confirmed by the Agency RfD Work Group.

In September of 1986, the Joint Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO) on Pesticides Residues [JMPR] proposed an Allowable Daily Intake (ADI) of 0.3 mg/kg body weight for glyphosate *per se*. The ADI was based on a 26-month feeding study in the rat yielding a NOEL of > 31 mg/kg body weight per day and and uncertainty factor of 100. The Agency places more importance on the developmental rabbit study since no effect was observed in the 26-month study whereas maternal mortality was observed in the developmental rabbit study in the high dose group. JMPR

acknowledged that there is no effect at the highest dose tested in the 26-month rat study.

2. Exposure Assessment

a. Dietary Exposure

The qualitative nature of the residue in plants is adequately understood. Studies with a variety of plants including corn, cotton, soybeans, and wheat indicate that the uptake of glyphosate or its metabolite, aminomethyl phosphonic acid (AMPA), from soil is limited. The material which is taken up is readily translocated. Foliarly applied glyphosate is readily absorbed and translocated throughout the trees or vines to the fruit of apples, coffee, dwarf citrus (calamondin), pears and grapes. Metabolism via N-methylation yields N-methylated glycines and phosphonic acids. For the most part, the ratio of glyphosate to AMPA is 9 to 1 but can approach 1 to 1 in a few cases (e.g., soybeans and carrots). Much of the residue data for crops reflects a detectable residue of parent (0.05 - 0.15 ppm) along with residues below the level of detection (<0.05 ppm) of AMPA. The terminal residue to be regulated in plants is glyphosate *per se*.

The qualitative nature of the residue in animals is adequately understood. Studies with lactating goats and laying hens fed a mixture of glyphosate and AMPA indicate that the primary route of elimination was by excretion (urine and feces). These results are consistent with metabolism studies in rats, rabbits, and cows. The terminal residues in eggs, milk, and animal tissues are glyphosate and its metabolite AMPA; there was no evidence of further metabolism. The terminal residue to be regulated in livestock is glyphosate *per se*.

An adequate enforcement method is available for analysis of residues of glyphosate and its metabolite AMPA in or on plant commodities and in water. This method utilizes GLC (Method I of PAM Vol. II; limit of detection is 0.05 ppm). For enforcement of tolerances in animal commodities, an HPLC method with fluorescence detection is available; the reported limits of detection are 0.01 ppm for glyphosate and 0.012 ppm for AMPA.

The available storage stability data indicate that residues of glyphosate and its metabolite AMPA are stable under frozen storage conditions (-20EC): in or on plant commodities for a period of 1 year, in animal commodities for 2 years, and in water for 1 year. No additional storage stability data are needed.

All data requirements for magnitude of the residue in plants have been evaluated and deemed adequate. Additional potato processing data are being generated. All data requirements for magnitude of the residue in plants as a result of irrigation with glyphosate-treated water have also been submitted and are adequate to support registered use and applicable tolerances. No additional data are required for magnitude of the residue in animals, potable water, and fish. A list of residue chemistry study references is provided on page 24.

b. Occupational and Residential

Occupational and residential exposure can be expected based on the currently registered uses of products containing glyphosate. However, due to the low toxicity (acute category III) of glyphosate and the lack of other toxicological concerns (i.e carcinogenicity) occupational and residential exposure data are not required. Glyphosate is a non-selective herbicide applied to terrestrial food and non-food crops, turf, greenhouse crops, and non-crop areas where total vegetation control is desired. Glyphosate, when applied at lower rates, is also a plant growth regulator.

Although glyphosate meets the Agency's exposure criteria for post-application/reentry and/or mixer/loader/applicator exposure monitoring data, glyphosate does not meet the Agency's toxicity criteria for these data requirements. Acute oral and dermal toxicity data for the technical material are in Toxicity Category III and IV. In addition, glyphosate is poorly absorbed dermally. The acute inhalation toxicity study for the technical material was waived because glyphosate is non-volatile and because there were adequate inhalation studies with end-use products showing low toxicity. Therefore, occupational and residential exposure data are not required to support the reregistration of glyphosate. (For these

same reasons, these data were not required in the 1986 Registration Standard.)

The following information is product-specific related, but is presented here for informational purposes. Some glyphosate end-use products are in Toxicity Category I and II based on primary eye irritation or dermal irritation. In California, where physicians are required to report pesticide poisonings, glyphosate was ranked third out of the 25 leading causes of illnesses or injury due to pesticides used between 1980 and 1984. These mixer/loader/applicator reported incidents consisted of eye and skin irritation. In reports issued by California since then (1987 and 1988), glyphosate continued to be a leading cause of illnesses or injuries (primarily eye and skin irritation). In the 1986 Registration Standard, the Agency recommended personal protective equipment, including protective eyewear for mixer/loader/applicators using end-use products that could cause eye or skin irritation. At that time, it was determined that mixer/loaders were at risk of eye or skin injury from splashes during mixing and loading. The Agency did not require personal protective equipment for users of "homeowner" products (containing up to 10% glyphosate) because of the low concentration of glyphosate and because the products are "ready-to-use", requiring no mixing; therefore, the potential for eye or dermal exposure is minimized.

The Agency, at this time, is not adding any additional personal protective equipment requirements to the labels of end-use products; however, any existing personal protective equipment on those labels must be retained.

The Worker Protection Standard (WPS) for Agricultural Pesticides -- 40 CFR Parts 156 and 170 -- established an interim restricted entry interval (REI) of 12 hours for glyphosate because the acute toxicity categories of glyphosate for acute dermal toxicity, skin irritation potential, and eye irritation potential are Toxicity Category III or IV. The Agency has determined that the 12-hour REI for all WPS sites should be retained as a prudent measure to mitigate risk to workers entering treated areas after application. Furthermore, given the known irritation-effects concerns for glyphosate, the Agency considers the additional protections offered by the requirements in the WPS essential to its decision that a 12-hour REI for this chemical will offer sufficient risk mitigation to workers.

Therefore, during the REI the Agency will allow workers to enter areas treated with glyphosate during the REI only in the few narrow exceptions allowed in the WPS.

The Agency has determined that, at this time, the entry restrictions discussed in this section need not apply to uses of glyphosate ouside the scope of the Worker Protection Standard for Agricultural Chemicals, including out-of-scope commercial uses and homeowner uses. The predicted frequency, duration, and degree of exposure due to post-application as the result of such uses should not warrant the risk mitigation measures being required for persons engaged in the production of agricultural plants for commercial or research purposes.

3. Risk Assessment

a. Dietary

The chronic dietary risk analysis used tolerance level residues and assumed all acreage, of the crops considered, was treated with glyphosate to estimate the Theoretical Maximum Residue Contribution (TMRC) for the overall U.S. population and 22 population subgroups. These exposures (TMRCs) were then compared to the RfD for glyphosate to estimate chronic dietary risk.

The calculated TMRC for the overall U.S. population from food uses of glyphosate is 0.025 mg/kg bwt/day, which represents 1.2% of the RfD. The subgroup most highly exposed, non-nursing infants less than one year old, has a TMRC of 0.058 mg/kg bwt/day, or 2.9% of the RfD. Over one third of the dietary exposure and risk from glyphosate is due to the proposed tolerances on wheat.

This analysis was meant to be a "worst case" scenario of risk. The inclusion of recommended tolerances for reregistration as well as tolerances recommended for revocation; the use of the highest existing, pending, or recommended residue value for each commodity; and the assumptions of tolerance level residues and treatment of 100 percent of the crops for every commodity considered result in an overestimation of exposure and risk values for glyphosate (though there is some underestimation due to the lack of consumption information for some of the commodities to which

glyphosate is expected to be applied). Nonetheless, given the risk values arrived at by this analysis, EPA concludes that the chronic dietary risk posed by this pesticide on these food uses is minimal.

b. Occupational and Residential

As discussed above in the occupational exposure assessment, exposure to humans from proper application of glyphosate to terrestrial food and non-food crops as well as greenhouses, turf, and non-crop areas can result in injury (primarily eye and skin irritation) from splashes during mixing and loading. The Agency continues to recommend protective clothing (including protective eye wear) for mixer/loader/applicators using end-use products that may be in toxicity category I or II for primary eye and dermal irritation.

c. Dietary Exposure References

This table references the residue data used to support the reregistration of glyphosate and includes the commodities eligible for reregistration.

Guideline/Commodity	References[1]
§171-4 (a): Plant Metabolism	00038771, 00039141, 00051983, 00065753, 00108097, 00108129, 00108133, 00108140, 00108151, 00111945
§171-4 (b): Animal Metabolism	00094971, 00108098, 00108099, 00108100, 00108101, 00108116, 00108099, 00108200, 40541301-40541304
§171-4 (c) and (d): Residue Analytical Methods	00028853, 00036222, 00036223, 00036231, 00037688, 00038770, 00038979, 00044423, 00051982, 00053002, 00053005, 00060108, 00061559, 00063714, 00065751, 00065752, 00067425, 00076805, 00078823, 00078824, 00108133, 00108144, 00108149, 00108151, 00108175, 00108176, 00108186, 00108231, 00111945, 00111949, 00122715, 00159419, 00164729, 40502601, 40541304
§171-4 (e): Storage Stability	00039142, 00040083, 00051980, 00053002, 00061553, 00061555, 00108129, 00108132, 40502605, 40532004, 41940701

Guideline/Commodity	References[1]
§171-4 (k) (l): Magnitude of the Residue in Plants	
Root and Tuber Vegetables Group	
- Artichokes, Jerusalem	N/A
- Beets, garden	00108159
- Carrots	00108159
- Chicory	N/A
- Horseradish	N/A
- Parsnips	N/A
- Potatoes	00108151, 41947001
- Radish	00108159
- Rutabagas	N/A
- Salsify	N/A
- Sugar beets	00039381, 00108151
- Sweet potato	00108151
- Turnips	40835201
Leaves of Root and Tuber Vegetables Group	
- Beets, greens	N/A
- Chicory leaves	N/A
- Sugar beet tops	00039381, 00108151
- Turnip tops	40835201
Bulb Vegetables Group	
- Garlic	N/A
- Onions (green and dry bulb)	40783101
Leafy Vegetables (except Brassica) Group	
- Celery	N/A
- Lettuce (head and leaf)	00108159
- Spinach	N/A

Guideline/Commodity	References[1]
Brassica Leafy Vegetables Group	
- Broccoli	40802801, 40802801
- Cabbage	00108159
- Cauliflower	N/A
- Kale	N/A
- Mustard greens	40802801, 40802801
Legume Vegetables (Succulent/Dried) Group	
- Beans (succulent and dried)	00108159
- Lentils	00108159
- Peas (succulent and dried)	00108159
- Soybeans	00015759, 00015760, 00015761, 00015762, 00015763, 00015764, 00015765, 00015766, 00015767, 00024503, 00033954, 00038908, 00040084, 00061555, 00108153, 00108203
(processed commodities)	00061555, 00108153, 00156793
Foliage of Legume Vegetables (Succulent/Dried) Group	
- Bean vines and hay	00108159
- Lentil forage and hay	00108159
- Pea vines and straw	
- Soybean forage and hay	00015759, 00015760, 00015761, 00015762, 00015763, 00015764, 00015765, 00015766, 00015767, 00033954, 00038908, 00040084, 00061555, 00108153, 00108203
Fruiting Vegetables Group	
Cucurbit Vegetables Group	
Citrus Fruits Group	00039142
(processed commodities)	40159401
Pome Fruits Group	00108129
Stone Fruits Group	00111949

Guideline/Commodity	References[1]
- Plums (fresh prunes)	00111949
Small Fruits and Berries Group	
- Blackberries	
- Blueberries	
- Cranberries	00053002
- Grapes	00038770, 00108132
(processed commodities)	40785303
- Raspberries	
Tree Nuts Group	00111945
- Almond hulls	00111945
Cereal Grains Group	
- Barley	00038908, 00040087, 00044422, 00108203
(processed commodities)	N/A
- Corn (field and fresh)	00023336, 00023512, 00037687, 00038908, 00040085, 00048284, 00108203, 40502602
(processed commodities)	40502604, 41478101
- Oats	00038908, 00040087, 00044422, 00108203
(processed commodities)	N/A
- Rice	00038908, 00040087, 00044422
(processed commodities)	N/A
- Rye	N/A
(processed commodities)	N/A
- Sorghum	00038908, 00040087, 00044422, 00108203, 00109271, 40502601
(processed commodities)	40502603
- Wheat	00038908, 00040086, 00044426, 00108203, 00122715, 41484301
(processed commodities)	00150835
Forage, Fodder, and Straw of Cereal Grains Group	
- Barley forage, hay, and straw	00038908, 00040087, 00044422, 00108203

Guideline/Commodity	References[1]
- Corn forage and fodder	00023336, 00023512, 00037687, 00038908, 00040085, 00048284, 00108203, 40502602
- Oat forage, hay, and straw	00038908, 00040087, 00044422, 00108203
- Rice straw	00038908, 00040087, 00044422
- Rye forage and straw	N/A
- Sorghum forage and fodder	00038908, 00040087, 00044422, 00108203, 00109271, 40502601
- Wheat forage and straw	00038908, 00040086, 00044426, 00108203, 00122715
Grass Forage, Fodder, and Hay Group	00076805, 00108147
Non-grass Animal Feeds (forage, fodder, straw, and hay) Group	00076805, 00108147
- Alfalfa seed	40541304
Miscellaneous Commodities	
- Acerola	
- Atemoya	
- Asparagus	00108144, 40642401
- Avocados	00108149
- Bananas	00108175
- Breadfruit	40149401
- Canistel	40149401
- Carambola	
- Cherimoya	
- Cocoa beans	
- Coconut	
- Coffee beans	00051980, 00051981
- Cotton	00060103, 00061553, 00108176, 00108153, 00108203
(processed commodities)	00061553, 00108176, 00108153
- Dates	40149401
- Figs	
- Genip	
- Guavas	00059050
- Jaboticaba	40149401
- Jackfruit	40149401

Guideline/Commodity	References[1]
- Kiwi fruit	
- Litchi Nut (Lychee)	
- Longan	
- Mamey Sapote (Mammee Apple)	
- Mangoes	40580401
- Okra	N/A
- Olives	00108175, 42398401
(processed commodities)	00108175, 42398401
- Palm oil	
- Papayas	00063713
- Passion Fruit	
- Peanuts	00144341, 00028852
(processed commodities)	00144341, 00028852
- Persimmons	40149401
- Pineapple	N/A
- Pistachio	00111945
- Sapodilla	
- Sapote (black and white)	40149401
- Soursop	40149401
- Sugar apple	
- Sugarcane	00108140
(processed commodities)	00108168
- Tamarind	40149401
- Tea	00078823, 00078824
- Watercress	N/A
§171-4 (h): Magnitude of the Residue in Plants Resulting from the Use of Irrigation Water	00039381, 40541305
§171-4 (j): Magnitude of the Residue in Meat, Milk, Poultry, and Eggs	00108115, 40532001-03
§171-4 (g): Magnitude of the Residue in Fish	00036229, 00076491, 00154311, 00155120

Guideline/Commodity	References[1]
§171-4 (f): Nature and Magnitude the Residue in Drinking and Irrigation Water	00039377, 00039381, 00077227, 00077228, 00077229, 00077230, 00077231, 00077232, 00077233, 00077234, 00077235, 00077236, 00077237, 00077238, 00077301, 00108173,
§171-4 (i): Magnitude of the Residue in Food Handling Establishment	
§171-5: Reduction of Residues	

1 N/A means not available by MRID number. Those guidelines/commodities which do not list a MRID reference number, additional reference information can be provided from Table A in the Product and Residue Chemistry Chapters by R.B. Perfetti, Chemistry Branch Reregistration Support (CBRS# 10665) in the Health Effects Division dated 10/27/92 through FOI.

C. Environmental Assessment

1. Environmental Fate

a. Environmental Fate and Transport

(1) Hydrolysis

Glyphosate is stable at pH 3, 6, 9 at 5 and 35EC. (Accession 00108192)

(2) Photodegradation in Water

Glyphosate is stable to photodegradation in pH 5, 7, and 9 buffered solutions under natural sunlight. (MRID 41689101)

(3) Photodegradation on Soil

Glyphosate is stable to photodegradation on soil. (MRID 41335101)

(4) Aerobic Soil Metabolism

Data indicate half-life values of 1.85 and 2.06 days in Kickapoo sandy loam and Dupo silt loam respectively. Aminomethyl phosphonic acid (AMPA) was the major degradate. (MRID 42372501)

(5) Anaerobic Aquatic Metabolism

Glyphosate has a half-life of 8.1 days in anaerobic (flooded plus nitrogen atmosphere) silty clay loam sediment. AMPA was the major degradate. (MRID 42372502)

(6) Aerobic Aquatic Metabolism

Glyphosate has a half-life of 7 days in flooded silty clay loam sediment that was incubated in the dark at 24.6 ± 0.57 C for 30 days. AMPA was the major degradate. (MRID 42372503)

(7) Leaching/Adsorption/Desorption

K_d values of 62, 90, 70, 22, and 175 were reported for Drummer silty clay loam, Ray silt, Spinks sandy loam, Lintonia sandy loam, and Cattail Swamp sediment respectively. After (aged) leaching 7 soils with 20" of water, the recovered radioactivity in the soils was 93-100% of the applied material. (Accessions 00108192, 00076493, 00108140)

(8) Terrestrial Field Dissipation

The Agency has received an interim report on a terrestrial field dissipation study in progress by Monsanto Company. (MRID 42607501)

This report contains data from eight different field sites. Some of the data from the individual field sites are deficient; however, the Agency may use the data from the eight field sites together to satisfy the terrestrial field dissipation 164-1 data requirement.

The interim report results from the first 12 months of bareground field dissipation trials from eight sites show that the median half-life (DT_{50}) for glyphosate applied at maximum annual use rates (7.95 lb a.e./acre, 10.7 lb a.i./acre) was 13.9 days with a range of 2.6 (Texas) to 140.6 (Iowa) days. Acceptable aerobic soil, aerobic aquatic and anaerobic aquatic metabolism studies demonstrate that under those conditions at 25EC in the laboratory glyphosate degrades rapidly with half-lives of approximately 2, 7 and 8 days respectively. The reported half-lives (DT_{50}) from the field studies conducted in the coldest climates, ie. Minnesota, New York and Iowa, were the longest at 28.7, 127.8, and 140.6 days respectively indicating that glyphosate residues in the field are somewhat more persistent in cooler climates as opposed to milder ones (Georgia, California, Arizona, Ohio, and Texas).

Glyphosate (as well as AMPA) was shown to remain predominantly in the 0-6 inch soil layer throughout the duration of the study at all field sites. Iowa was the individual test site to have average glyphosate residues, at all sampling times, greater than 0.01 ppm in the 6-12 inch depth. There were a number of detections from 0.01 to 0.09 ppm in the 6-12 inch layer in Minnesota, New York and Texas, and glyphosate was detected at generally <0.05 ppm at the other 5 field sites (6-12 inch depth).

Glyphosate was detected at three different sites below 12 inches. In California, at 0 DAT, average glyphosate residues were 0.21 ppm and 0.10 ppm in the 12-18 and 18-24 inch soil horizons respectively. Soil core contamination was attributed to these detections since movement of residues to this depth on the first day of sampling is unlikely. In Arizona at 21 DAT the average glyphosate residues were 0.06, in the 18-24 inch soil layer. There were no glyphosate residues in the 6-12 or 12-18 inch soil layer in Arizona on 21 DAT and in subsequent samples below 12 inches which may indicate a problem with sampling technique. In Iowa at 190 DAT the average glyphosate residues were 0.05 ppm in the 12-18 inch soil layer. Since there were no glyphosate residues detected in the 6-12 inch soil layer at 190 DAT, and

the lack of a significant amount of rainfall between sampling intervals in combination with the amount of time between sampling intervals and the high adsorptive characteristics of glyphosate give an indication that there may have been a problem with sampling technique.

AMPA was also shown to remain predominantly in the 0-6 inch soil layer. AMPA was found at every test site on Day 0 samples indicating the rapid degradation of parent glyphosate. The AMPA levels generally reached a maximum between day 14 and day 30. Where the field half-lives were longer (Iowa, Minnesota, New York), the maximum average AMPA levels occurred between 62 and 95 DAT. The maximum average AMPA levels found in the 0-6 inch soil layer were 0.6 ppm and occurred in Ohio and Georgia at 21 DAT and 61 DAT respectively. The AMPA levels at those sites had decreased to 0.12 and 0.44 ppm at 12 months after treatment.

In all samples but three, AMPA residue levels were <0.05 ppm in the 6-12 inch soil layer. In New York at 14 and 30 DAT average residues were detected at 0.06 ppm. In Iowa at the 92 DAT sample average AMPA residues were 0.08 ppm. Iowa and New York also exhibited 50% dissipation times of 140.6 and 127.8 days respectively.

AMPA levels were detected at 0.06 ppm in the 18-24 inch soil layer on 21 DAT in Arizona and 0.04 and 0.03 ppm in the 12-18 inch soil layer at 90 and 180 DAT respectively in New York.

A final report on the terrestrial field dissipation study showed the median half-life (DT_{50}) (of eight sites) of AMPA was 240 days with a range of 119 (Ohio) to 958 (California) days. The half-lives for the dissipation of AMPA for seven of the eight test sites were:

- Arizona 142 days
- California 958 days
- Georgia 896 days
- Minnesota 302 days

- New York 240 days
- Ohio 119 days
- Texas 131 days

Iowa was not calculated because recharging of AMPA residues was greater than degradation. AMPA was shown to remain predominantly in the 0-6 inch soil layer throughout the duration of the study at all eight field sites. AMPA was detected three times (at a concentration greater than 0.05 ppm) at depths greater than 12 inches. The three detections were attributed to contamination during sampling rather than vertical mobility.

(9) Aquatic Field Dissipation

Glyphosate dissipated from water (irrigation source) with a calculated half-life of 7.5 days and 120 days from the sediment of the farm pond in Missouri. (MRID 40881601)

In Michigan, Georgia and Oregon pond and stream water, the maximum glyphosate concentrations were measured immediately posttreatment and dissipated rapidly. Glyphosate accumulated in the pond sediment, and to a lesser extent in the stream sediments; glyphosate was present in pond sediment at $1 ppm in Michigan and Oregon at approximately 1 year posttreatment. (MRID 41552801)

(10) Forestry Dissipation

When aerially applied at 3.75 lb/A to forested sites in Michigan, Oregon, and Georgia, glyphosate averaged 652-1273 ppm in tree foliage immediately posttreatment. It then declined rapidly with half-lives of <1 day at the Michigan and Georgia sites and <14 days at the Oregon site.

The forestry dissipation study results demonstrate that when used under normal silviculture practices according to label directions, the maximum combined glyphosate and AMPA residue level in soil is less than 5 ppm. Glyphosate and AMPA residues in soil dissipate with time. The average half-life for the dissipation of glyphosate was 100 days, and

ranged from 35 to 158 days. The average half-life for the dissipation of AMPA was 118 days, and ranged from 71 days to 165 days. (MRID 41552801)

(11) Accumulation in Confined Rotational Crops

Glyphosate residues (expressed as fresh weight) accumulated in lettuce, carrots, and barley planted 30, 119, and 364 days after sandy loam soil was treated with glyphosate at 3.71 lb ai/A. Accumulation decreased as the length of the rotation increased. In crops planted at 30 days, posttreatment, [^{14}C]residues at harvest were 0.097 ppm in lettuce, 0.051 and 0.037 ppm in carrot tops and roots, respectively, and 0.188 and 0.175 ppm in barley grain and straw, respectively. In immature lettuce harvested at 40 and 60 days postplanting, [^{14}C]residues were 0.108 and 0.048 ppm, respectively. In crops planted at 119 days posttreatment, [^{14}C]residues at harvest were 0.037 ppm in lettuce, 0.028 and 0.017 ppm in carrot tops and roots, respectively, and 0.078 and 0.056 ppm in barley grain and straw, respectively. In immature lettuce harvested at 28 and 48 days postplanting, [^{14}C]residues were 0.059 and 0.055 ppm, respectively. In crops planted at 364 days posttreatment, [^{14}C]residues at harvest were 0.028 ppm in lettuce, 0.018 and 0.0096 ppm in carrot tops and roots, respectively, and 0.047 and 0.061 ppm in barley grain and straw, respectively. In immature lettuce harvested at 35 and 61 days postplanting, [^{14}C]residues were 0.057 and 0.043 ppm, respectively; in barley forage harvested at 48 days postplanting, [^{14}C]residues were 0.056 ppm. (MRID 41543201 and 41543202)

(12) Accumulation in Irrigated Crops

Alfalfa, corn (grain and forage), grass (fescue or sudan) and lettuce were irrigated five to eight times during the 1987 growing season with glyphosate treated water containing a maximum of 21.3 ppm (on treatment day then fell to 0.46 ppm by 1 day after treatment) of glyphosate. Residues in the sediment beneath the treated water reached a maximum of 3.5 ppm at 14 days after treatment. Residues

of glyphosate in the sprinkler water at the pond site were the highest 7 days after treatment at 0.12 ppm. One lettuce sample from the Missouri location (the pond site) at 29 days after treatment (of water source) and 5 irrigation events was found to contain 0.06 ppm glyphosate. (MRID 40541305)

(13) Bioaccumulation in Fish

Maximum bioconcentration factors were 0.38X for edible tissues, 0.63X for nonedible tissues, and 0.52X for whole fish. (MRID 41228301)

(14) Laboratory and Field Volatility

The requirement of these studies was waived based on the low vapor pressure of glyphosate.

b. Environmental Fate and Groundwater Assessment

In general, the available field and laboratory data indicate glyphosate adsorbs strongly to soil and would not be expected to move vertically below the 6 inch soil layer. Based on unaged batch equilibrium studies glyphosate and glyphosate residues are expected to be immobile with $Kd_{(ads)}$ values ranging from 62 to 175. The mechanism of adsorption is unclear; however, it is speculated that it may be associated with vacant phosphate sorption sites or high levels of metallic soil cations. The data indicate that chemical and photochemical decomposition is not a significant pathway of degradation of glyphosate in soil and water. However, glyphosate is readily degraded by soil microbes to aminomethyl phosphonic acid (AMPA), which is degraded to CO_2, although at a slower rate than parent glyphosate. Even though glyphosate is highly water soluble it appears that parent glyphosate and AMPA have a low potential to move to ground-water due to their strong adsorptive characteristics demonstrated in the laboratory and field studies. However, glyphosate does have the potential to contaminate surface waters due to its aquatic use patterns and erosion via transport of residues adsorbed to soil particles suspended in runoff water. If glyphosate were to reach surface water it would be resistant to hydrolysis and aqueous photolysis.

Based on the low vapor pressure of glyphosate, volatilization from soils will not be an important dissipation mechanism. The low octanol/water coefficient suggests that glyphosate will have a low tendency to accumulate in fish.

2. Ecological Effects

a. Ecological Hazard

(1) Effects to Nontarget Birds

To establish the toxicity of glyphosate to birds, tests were required using the technical grade material.

(a) Avian Single-Dose Oral LD_{50} - Technical

Acute Oral Toxicity Findings			
Species	**% AI**	**LD_{50} (95% CL)**	**Conclusions**
Bobwhite quail	83%	> 2000 mg/kg	practically non-toxic to upland game birds

One avian single-dose oral study on either a waterfowl species (preferably mallard duck) or an upland species (preferably bobwhite quail) was required. These data indicate that technical glyphosate is practically non-toxic to an upland bird species on an acute oral basis. The guideline requirement for an avian acute oral study is fulfilled. (Study ID 234395)

(b) Avian Dietary - Technical

Avian Subacute Dietary Toxicity Findings			
Species	**% AI**	**Reproductive Impairment**	**Conclusions**
Mallard duck	98.5% Tech	> 4640 ppm	no more than slightly toxic to upland game birds and waterfowl
Bobwhite quail	98.% Tech	> 4640 ppm	

Two subacute dietary studies, one study on a species of waterfowl (preferably mallard duck) and one on an upland game bird species (preferably a bobwhite quail), were required. These data indicate that the technical glyphosate is no more than slightly toxic to birds on a dietary basis. The guideline requirement is fulfilled for both studies. (Study IDs 94171 and 00086492)

(c) Avian Reproduction

Avian Reproduction Findings			
Species	**% AI**	**Reproductive Impairment**	**Conclusions**
Mallard duck	83% Tech	No effects up to 1000 ppm	not expected to cause reproductive impairment
Mallard duck	90.4% Tech	No effects up to 30 ppm	
Bobwhite quail	83% Tech	No effects up to 1000 ppm	

An avian reproduction test was required to support registration of the end-use products of glyphosate since the following guideline criteria have been exceeded. The labeling for several use patterns contains directions for use under which birds may be subject to repeated exposure to glyphosate. The labeling allows repeat application for certain uses, such as alfalfa, barley, oats, apples, cherries, and oranges. These data indicate that technical glyphosate is not expected to cause reproductive impairment. The guideline requirements for an avian reproduction study on both upland game bird and waterfowl are fulfilled. (Study IDs 235924, 00036328, and 235924)

(d) Summary of Findings

Glyphosate is practically non-toxic to bobwhite quail on the basis of acute oral toxicity. An LD_{50} greater than 2000 mg/kg was determined for bobwhite quail given a single oral dose of technical glyphosate. Studies indicate that the 8-day dietary LC_{50} of the chemical is greater than 4000 ppm for both mallard ducks and bobwhite quail. These data indicate that the chemical is slightly toxic to birds. Avian reproduction studies indicate reproductive impairment would not be expected at a dietary level of up to 1000 ppm. The available acute toxicity data do not indicate a requirement of precautionary labeling for birds on products containing glyphosate.

(2) Effects on Non-Target Fish

(a) Acute Toxicity to Freshwater Fish

Acute Toxicity to Freshwater Fish Findings			
Species	**% AI**	**48-hr LC_{50} (95%CL)**	**Conclusions**
Bluegill sunfish	96.5%	> 24 mg/l	ranges in toxicity from slightly non-toxic to practically non-toxic to both cold water and warm water fish
Fathead Minnow	87.3%	84.9 mg/l (72.9-99.3)	
Bluegill sunfish	83%	120 mg/l (111-130)	
Rainbow Trout	83%	86 mg/l (70-106)	
Rainbow Trout	96.7%	140 mg/l (120-170)	
Fathead minnow	96.7%	97 mg/l (79-120)	
Channel catfish	96.7%	130 mg/l (110-160)	
Bluegill sunfish	96.7%	140 mg/l (110-160)	

The minimum data required for establishing the acute toxicity of glyphosate to freshwater fish are the results of two 96-hour studies with the technical grade product. One study was to be performed on a cold water fish species (preferably rainbow trout) and one study was to be performed using a warm water species (preferably bluegill sunfish). The results of these eight studies indicate that technical glyphosate is slightly to practically nontoxic to both cold water and warm water fish. The guidelines requirement for acute toxicity testing of the technical on freshwater fish is fulfilled. (Study IDs 00108112, 00108171, 234395, 097661, and 249160)

(b) Chronic Toxicity to Freshwater Fish

Chronic Toxicity to Freshwater Fish Findings			
Species	**% AI**	**Results**	**Conclusions**
Fathead Minnow	87.3% tech	MATC > 25.7 mg/l	no effects at or below this level

Due to the aquatic use of the chemical, its presence in water is likely to be continuous or recurrent regardless of toxicity; therefore, chronic testing was required. This fish full life cycle study satisfies the generic guideline requirement for chronic freshwater fish testing. (Study ID 00108171)

Acute Toxicity to Freshwater Fish Findings from Studies using Formulated Products			
Species	**% AI (IPA salt)**	**96-hr LC_{50} (95% CL)**	**Conclusions**
Bluegill sunfish	41.8%	5.8 mg/l (4.4-8.3)	ranges in toxicity from moderately toxic to practically non-toxic to both warmwater and coldwater fish
Rainbow Trout	41.8%	8.2 mg/l (6.4-9.0)	
Channel catfish	41.36%	16 mg/l (9.4-26)	
Rainbow Trout	41.36	11 mg/l (8.7-14)	
Bluegill sunfish	41.36%	14 mg/l (8.7-24)	
Fathead Minnow	41.36%	9.4 mg/l (5.6-16)	
Rainbow Trout	62.4%	>1000 mg/l	
Bluegill sunfish	62.4%	>1000 mg/l	
Rainbow Trout	*41.2% + 15.3 "AA" surfactant	120 mg/l (56-180)	

Acute Toxicity to Freshwater Fish Findings from Studies using Formulated Products			
Rainbow Trout	*40.7% + 15% "W" surfactant	150 mg/l (100-320)	
Bluegill sunfish	*40.7% + 15% "W" surfactant	>100 mg/l	
Bluegill sunfish	*41.2% + 15.3% "AA" surfactant	>180 mg/l	
Rainbow Trout	7.03% + 0.5% "X-77"	240 mg/l (180-320 mg/l)	
Bluegill sunfish	7.03%+ 0.5% "X-77"	830 mg/l (620-1600)	
Rainbow Trout	51%	8.3 mg/l (7.0-9.9)	
Fathead minnows	41%	2.3 mg/l (1.9-2.8)	
Rainbow Trout	41%	9.0 mg/l (7.5-11)	
Bluegill sunfish	41%	4.3 mg/l (3.4-5.5)	
Channel catfish	41%	13 mg/l (11-16)	
Bluegill sunfish	41%	5 mg/l (3.8-6.6)	
Rainbow Trout	41%	1.3 mg/l (1.1-16)	

Testing of an end-use product is required if the pesticide will be introduced directly into an aquatic environment when used as directed by the label. Drainage systems would be included in such a category. Therefore, formulated product testing was required. According to the surfactant selected, the formulated product toxicity ranges from moderately toxic to practically non-toxic. (Study ID 249159, 00070894,

00070895, 00070897, 00070896, 00078661, 00078662, 00078658, 00078655, 00078656, 00078659, 00078664, 00078665, 249160)

Surfactant Test Findings			
Species	**% AI**	**96-hour LC_{50} (95% CL)**	**Conclusions**
Fathead minnow	MONO818 Tech 100%	1.0 mg/l (1.2-1.7)	ranges in toxicity from highly toxic to slightly toxic to warmwater and coldwater fish
Rainbow trout	MONO818 Tech 100%	2.0 mg/l (1.5-2.7)	
Rainbow Trout	MONO818	0.65 mg/l (.54-.78)	
Channel Catfish	MONO8l8 Tech 100%	13 mg/l (10-17)	
Bluegill sunfish	MONO8l8 Tech 100%	3.0 (2.5-3.7)	
Bluegill sunfish	MONO818 Tech 100%	1 mg/l (.72-1.4)	

Testing of the surfactant may be required under unusual circumstances. When inerts are likely to be toxic, testing can be required. These data indicate that MONO818 ranges from moderately toxic to very highly toxic to both cold and warm water fish after 96 hour exposure. (Study ID 249160)

(c) Summary of Findings

Three tests on warm water species, one bluegill and two with fathead minnow, produced the 96-hour LC_{50}s of 120 ppm, 84.9 ppm, and 97 ppm, respectively (McAllister and Forbis 1978, ID #234395; EG & G Bionomics 1975, ID #00108171 and Folmar, Sanders, and Julin 1979, ID #249160). Two rainbow trout 96-hour LC_{50}s provided values of 86 ppm and 140 ppm. Based on these tests, technical glyphosate ranges from slightly to practically non-toxic to freshwater fish species.

Surfactant testing was performed with both cold water and warm water fish. In this case, the initial formulation demonstrated an application rate much

lower than technical glyphosate. The LC_{50} for rainbow trout was 1.3 mg/l or moderately toxic. The surfactant (MON0818) when tested alone produced an LC_{50} value of 0.65 mg/l for rainbow trout indicating a highly toxic category (Folmar et al. 1979, ID #249160). In contrast, the formulation of 41.2 percent isopropylamine salt and 15.3 percent "AA" surfactant provided a rainbow trout LC_{50} of 120 mg/l, indicating a practically non-toxic compound (Thompson and Griffen 1980, ID #00078658). Bluegill are in the same category of toxicity with an even higher LC_{50} of greater than 180 mg/l (Thompson and Griffen 1980, ID #00078659). The bluegill and rainbow trout were similar in sensitivity to the formulation containing the "W" surfactant with LC_{50} values of 150 and >100 mg/l, respectively. Also, neither rainbow trout (LC_{50} 240 mg/l) nor bluegill (LC_{50} 830 mg/l) were very sensitive to the x-77(.5) surfactant and glyphosate(7.03%).

The surfactant MON0818 has been tested separately, producing an LC_{50} of 13 mg/l on *Chironomous* indicating it is a slightly toxic material. For fish, the catfish appears to be the most tolerant with an LC_{50} value of 13 mg/l, and rainbow trout the most sensitive with an LC_{50} value of 0.65 mg/l. Based upon available data products containing MONO8l8 must include the statement, "This pesticide is toxic to fish."

(3) Effects on Aquatic Invertebrates

(a) Acute Toxicity to Freshwater Invertebrates

Acute Toxicity to Freshwater Invertebrates Findings			
Species	**% AI**	**48-hr LC_{50} (ppm)**	**Conclusions**
Daphnia magna	83% tech	780	ranges in toxicity from slightly toxic to practically non-toxic to freshwater invertebrates
Chironomus plumosus	96.7% tech	55 (31-97)	

The minimum data requirement to establish the acute toxicity of glyphosate to freshwater invertebrates is a 48-hour acute study using the technical material. Test organisms should be first instar *Daphnia magna* or early instar amphipods, stone flies or mayflies. The results of these studies indicate that technical glyphosate is slightly toxic to *Chironomus plumosus* and is practically non toxic to *Daphnia magna*. The guideline requirement for acute testing on a freshwater invertebrate has been fulfilled. (Study ID 00108172, and 249160)

(b) Chronic Toxicity to Freshwater Invertebrates

Chronic Toxicity to Freshwater Invertebrates Findings			
Species	**% AI**	**Results**	**Conclusions**
Daphnia magna	99.7% tech	MATC > 50 -< 96 mg/L	caused reduced reproductive capacity

Due to the aquatic use of the chemical its presence in water is likely to be continuous or recurrent regardless of toxicity; therefore, chronic testing was required. This study satisfies the guideline requirement for chronic freshwater invertebrate testing. (Study ID 249160)

Acute Toxicity to Freshwater Invertebrates Findings from Studies using Formulated Products			
Species	**% AI (IPA salt)**	**48-hr LC_{50} (ppm)**	**Conclusions**
Daphnia magna	62.4%	869 (703-1019)	ranges in toxicity from moderately toxic to practically non-toxic to freshwater invertebrates

Acute Toxicity to Freshwater Invertebrates Findings from Studies using Formulated Products			
Daphnia magna	7.03% + X-77 surfactant @0.5%	>1000	
Daphnia magna	41.2% + "AA" surfactant @ 15.3%	310 (250-400)	
Daphnia magna	40.7% MON2139 + 15% "W" surfactant	72 (62-83)	
Daphnia magna	41%	3 (2.6-3.4)	
Gammarus pseudolimnaeus	41%	62 (40-98)	
Chironomus plumosus	41%	18 (9.4-32)	
Daphnia pulex	51% MON 2139	242(224-261.5)	
Daphnia magna	41.36%	5.3 (4.4-6.3)	
Gammarus pseudolimnaeus	41.83%	41.9 (30.7-62)	
		Other results	
Ephemerella walkeri	41%	Mayfly nymphs avoided glyphosate at concentrations of 10 mg/L but not at 1.0 mg/l.	

Acute Toxicity to Freshwater Invertebrates Findings from Studies using Formulated Products			
Chironomus plumosus	41%	Significant increases in stream drift of midge larvae was observed after the 2.0 mg/l, but not at the 0.02 or 0.2 mg/l level.	

Testing of an end-use product is required if the pesticide will be introduced directly into an aquatic environment when used as directed by the label. Drainage systems (wet and dry) would be included in such a category. Therefore, formulated product testing was required. According to the surfactant selected, the formulated product toxicity ranges from moderately toxic to practically non-toxic. (Study ID 00078663, 00078666, 00078660, 00078657, 249160, 00108109, 00070893, and 249159)

Surfactant Test Findings			
Species	**% AI**	**48-hr LC_{50} (95%CL)**	**Conclusions**
Daphnia magna	100% MONO818 surfactant	13 mg/L (7.1-24)	slightly toxic to freshwater invertebrates

Testing of the surfactant may be required under unusual circumstances. One test on the surfactant was received and determined as acceptable for use in a risk assessment. (Study ID 249160)

(d) Summary of Findings

A 48-hour LC_{50} of 780 ppm (mg/l) was found for *Daphnia magna* exposed to technical glyphosate (McAllister and Forbis 1978, ID #00108172). The results of this study indicate that the chemical is practically non-toxic to aquatic invertebrates.

In addition to these acute studies, a fish life-cycle study indicates technical glyphosate has a

MATC greater than 25.7 ppm. No effect was observed at the highest level tested. A *Daphnia magna* life cycle study with an MATC of >50 - <96 ppm reported reduced reproductive capacity, the most sensitive parameter.

The available acute toxicity data indicate that precautionary labeling for freshwater intervertebrates is not required for products containing glyphosate.

In order to determine the effect of the three surfactants ("W", "AA", and "X-77") on invertebrates, additional *Daphnia* studies were conducted. The 7.03 percent isopropylamine salt of glyphosate with a surfactant at 0.5 percent identified as X-77 resulted in an LC_{50} of greater than 1000 mg/l or practically non-toxic category for *Daphnia*. The second combination was 41.2 percent isopropylamine and 15.3 percent of a surfactant identified as "AA." This LC_{50} was 310 ppm which would indicate it is practically non-toxic to *Daphnia*. The third combination consisted of 40.7 percent isopropylamine and 15 percent of a surfactant identified as "W." The resultant LC_{50} of 72 ppm reveals that this material is slightly toxic to *Daphnia*.

A glyphosate formulation was tested several times with different invertebrates. The LC_{50} values ranged from 3 mg/l for *Daphnia* to 62 mg/l for *Gammarus* indicating a moderately toxic material for *Daphnia* and no more than slightly toxic for *Gammarus*.

(4) Effects on Marine/Estuarine Organisms

(a) Acute Toxicity

Acute toxicity testing for estuarine and marine organisms on technical glyphosate is required. The guidelines require estuarine and marine studies when exposure of such waters is likely. Crops, such as cotton, corn, sugarcane, turf, citrus, berries, forestry, sorghum, watermelon, etc. would allow this type of exposure to occur.

Acute toxicity testing for estuarine and marine organisms on formulated glyphosate may be required when exposure to estuarine and marine water is expected. The use in drainage systems (wet or dry) would allow this type of exposure. Minimum requirements are results from testing the technical on one estuarine fish (96 hrs LC_{50}) and either a 48 hrs oyster larvae study or a 96 hrs shell deposition study. Again, since there is such an extensive data set for this chemical, the Agency can determine that glyphosate demonstrates low toxicity to fish and oyster species, and therefore is waiving the marine fish and oyster acute toxicity studies on the formulated product.

Acute Toxicity to Estuarine and Marine Organisms Findings

Species	% AI	Results	Conclusions
Grass shrimp	96.7% tech	LC_{50} 281 ppm (207-381)	ranges in toxicity from slightly to practically non-toxic to marine organisms
Fiddler crab	96.7% tech	LC_{50} 934 ppm (555-1570)	
Atlantic oyster	96.7% tech	TL_{50} > 10 mg/L for 48 hours	

These data on marine/estuarine species are acceptable for use in a risk assessment. These data indicate that technical glyphosate is practically non-toxic to grass shrimp, fiddler crab, and slightly toxic to the Atlantic oyster. Acute toxicity testing on an estuarine fish species is normally required. However, since there is such an extensive data set for this chemical, the Agency can determine that glyphosate

demonstrates low toxicity to fish species, and therefore is waiving the marine fish acute toxicity study. (Study ID 00108110, and 00108111)

(b) Summary of Findings

A series of studies were performed on marine/ estuarine species. A 96-hour LC_{50} of 281 ppm was determined for grass shrimp (*Palaemonetas vulgaris*). In a study on fiddler crabs (*Uca pugilator*), it was determined that the 96-hour LC_{50} is 934 ppm glyphosate. Both of these studies indicate technical glyphosate is practically non-toxic to grass shrimp and fiddler crabs. An embryo-larvae 48-hour TL_{50} for Atlantic oyster greater than 10 ppm indicating glyphosate is slightly toxic.

(5) Effects on Non-Target Insects

(a) Acute Toxicity Testing

Acute Toxicity to Honeybees Data

Species	AI %	Results	Conclusions
Honeybee acute oral	tech*CP67573	oral LD_{50} > 100µg/bee	practically non-toxic to honeybees on an acute oral and acute contact basis
Honeybee acute oral	36 % MON2139	oral LD_{50} > 100µg/bee	
Honeybee acute contact	tech*CP67573	contact LD_{50} > 100µg/bee	
Honeybee acute contact	36 % MON2139	contact LD_{50} > 100µg/bee	

* - The percentage of active ingredient used was not reported.

The guidelines require acute toxicity testing to honeybees on the technical when a herbicide is registered as a general use herbicide. Given the multitude of use patterns for which this chemical is registered, acute honeybee toxicity studies are required. Based on these data, glyphosate (CP67573) is considered practically nontoxic on the basis of acute contact toxicity, as well as on acute oral toxicity. These data satisfy guideline requirements for nontarget insect studies when glyphosate is used as a general use herbicide. (Fiche No. 00026489)

(b) Summary of Findings

Four studies were conducted, two on technical glyphosate and two on the formulation MON2139, consisting of 36 % active ingredient. Results from the honeybee acute oral toxicity study indicates both technical and formulated glyphosate are practically nontoxic to the honey bee with LD_{50} values greater than 100 µg/bee. Results from the honeybee acute contact toxicity study indicates both technical and formulated glyphosate are practically nontoxic to the honey bee with LD_{50} values greater than 100 µg/bee.

(6) Effects to Non-Target Plants

When a herbicide is applied as a terrestrial nonfood use, aquatic nonfood use, or as a forestry use, Tier I nontarget phytotoxicity studies are required in order to evaluate the effects of the herbicide on nontarget plants.

(a) Phytotoxicity Testing

Effects on Non-Target Plant Findings

Species	%AI	Results
Selenastrum capricornutum	96.6	4 day EC_{50} = 12.5 mg/l
Navicula pelliculosa	96.6	4 Day EC_{50} = 39.9 mg/l
Skeletonema costatum	96.6	4 day EC_{50} = 0.85 mg/l
Anabaena flos-aquae	96.6	4 day EC_{50} = 11.7 mg/l
Lemna gibba	96.6	7 day EC_{50} = 21.5 mg/l

Based on the results of the preceding studies, the data indicates that the 4 day EC_{50} ranged from 0.85 mg/l to 39.9 mg/l for four aquatic plant species, and a 7 day EC_{50} of 21.5 mg/l for one aquatic species. Based

on the data submitted, the requirements for Tier I and Tier II Aquatic Plant Growth Studies (122-2 and 123-2) have been fulfilled.

A seed germination/seedling emergence study was conducted (MRID 40159301) on isopropylamine salt of glyphosate CP-70139 (Tech) 50% acid basis. The results indicate that CP-70139 applied at a rate up to 10.0 lb ai/A resulted in <25 % effect on the spectrum of monocots and dicots tested. Based on the results of this study, Tier I data requirements for seed germination/seedling emergence guideline reference 122-1 have been satisfied. (MRIDs 40236901, 40236902, 40236903, 40236934, and 40236905)

(b) Summary of Findings

Based on the results of the aquatic plant growth studies which were conducted on 5 species, the data indicates that the 4 day EC_{50} ranged from 0.85 mg/l to 39.9 mg/l for four aquatic plant species, and a 7 day EC_{50} of 21.5 mg/l for one aquatic species.

A seed germination/seedling emergence study was conducted on isopropylamine salt of glyphosate CP-70139 (Tech) 50% acid basis. The results indicate that CP-70139 applied at a rate up to 10.0 lb ai/A resulted in <25 % effect on the spectrum of monocots and dicots tested.

Based on the use patterns, the method of application, and the chemical properties of glyphosate, additional studies are required to evaluate the effects on nontarget plants. The recommended labels do not preclude off-target movement of glyphosate by drift. Nor do they address the potential off-target movement via terrestrial plants as well as aquatic plants. Therefore, the Agency is requiring terrestrial plant test data to assess potential risk to nontarget plants. The data required are the Tier II Vegetative Vigor Guideline Reference No. 123-1. In addition, droplet size spectrum (201-1) and drift field evaluation (202-1) data are required.

These three guideline studies, Vegetative Vigor, Droplet Size Spectrum, and Drift Field Evaluation are not considered part of the target data base for reregistration. These data do not affect the

reregistration eligibility of glyphosate. If, upon review of the data from these studies, modification in use practices and/or precautionary measures are necessary, the Agency will require all registrants to make label changes as appropriate.

b. Ecological Effects Risk Assessment

Based on the current data, it has been determined that effects to birds, mammals, fish and invertebrates are minimal. Under certain use conditions, glyphosate is expected to cause adverse effects to nontarget aquatic plants. Additional data are needed in order to fully evaluate the effects of glyphosate on nontarget terrestrial plants. This includes results from vegetative vigor testing (123-1), droplet size spectrum (201-1). In addition, the drift field evaluation (202-1) study must be submitted and reviewed. Risk reduction measures cannot be recommended until data are submitted and evaluated.

(1) Non-Endangered Species

(a) Terrestrial Species

The acute oral LD_{50} found for bobwhite quail dosed with technical glyphosate is greater than 3851 mg/kg. This indicates that the chemical is practically non-toxic to an upland game species. On a dietary basis, the available data indicate that, at most, technical glyphosate is slightly toxic to both mallards and bobwhite (LC_{50} > 4640). The articles of Hoerger and Kenaga (1972) and Kenaga (1973) were consulted in order to estimate the maximum concentration of glyphosate which may occur at the highest application rate for such sites as, cotton and corn. The following chart addresses the major vegetation categories upon which fauna are expected to feed.

Feed Category Concentrations (ppm) @ 5.0625 lbs ai/A	
Short grass	1215

Long grass	557
Leafy crops	632
Forage; small insects	294
Pods; large insects	61
Fruit	35

Comparing these residues to the dietary data for both bobwhite and mallards (LC_{50} > 4640; 1/5th the LC_{50} > 928), higher use rates may produce potentially toxic residues on short grass only (assuming the LC_{50} is just over > 4640). Wildlife ingesting significant amounts of insects, pods and/or fruits should not be affected by single applications.

Directions for some of the use patterns do indicate that applications can be repeated. Multiple treatments could potentially increase residues on dietary items within an extended time period. Also, the available information suggest that glyphosate is relatively persistent. The half-life in soil is as high as 90.2 days. However, avian reproduction studies demonstrated no adverse effects at the highest level tested, 1000 parts per million. Similarly, 90-day dietary studies with dogs and rats indicate no significant abnormalities when the maximum level tested is 2000 parts per million. Based on this, minimal risk is expected.

(b) Aquatic Species

Aquatic organisms do not appear to be sensitive to technical glyphosate. The most sensitive aquatic invertebrate tested is *Chironomus plumosus* with a 48-hr LC_{50} of 55 ppm which is very near to the lower limit of the *Daphnia* chronic MATC of 50 mg/l. The most sensitive fish species are fathead minnow and rainbow trout which have 96-hour LC_{50}s of 84.9 and 86 mg/l. Chronic testing for the technical with fathead minnow provided an MATC of > 25.7 mg/l. Based on the toxicity and the various EEC's the Agency has determined technical glyphosate should not cause acute or chronic adverse effects to aquatic environments. Therefore, minimal risk is expected to aquatic organisms from the technical glyphosate.

(c) Terrestrial Plants and Aquatic Macrophytes

A seed germination/seedling emergence study was conducted on isopropylamine salt of glyphosate CP-70139 (Tech) 50% acid basis. The results indicate that CP-70139 applied at a rate up to 10.0 lb ai/A resulted in <25 % effect on the spectrum of monocots and dicots tested. Considering the use patterns that are terrestrial food crop and non-food crop the above EEC's were considered for evaluating the effects to nontarget plants. The highest exposure of 0.404 lb a.i. (from aerial application, mist blower and sprinkler irrigation) is well below the 10.0 lb a.i./A rate which resulted in < 25 % effect on the monocots and dicots tested. Therefore, it has been determined that the use of glyphosate is not expected to cause adverse effects on seed germination/seedling emergence with the various registered use patterns. (MRID 40159301)

No vegetative vigor (123-1) plant studies have been conducted. Based on the use patterns, the method of application and the chemical properties of

glyphosate, additional studies are required to evaluate these effects on nontarget terrestrial plants. The recommended labeling precautions do not preclude off-target movement of glyphosate by drift. To assess potential risk to terrestrial plants the Agency is requiring additional terrestrial plant test data, including results from vegetative vigor testing, droplet size spectrum testing and drift field evaluation. These data are not part of the target data base for reregistration. Risk reduction measures cannot be recommended until data are submitted and evaluated. If, upon review of the data from these studies, modification in use practices and/or precautionary measures are necessary, the Agency will require all registrants to make label changes as appropriate.

The aquatic EEC from direct application of 3.72 ppm was used to estimate exposure. Based on the results of the aquatic macrophyte toxicity data, the 4 day EC_{50} was reported to be as low as 0.85 ppm indicating that there may be adverse effects to nontarget aquatic plant species.

(2) Endangered Species

Based on the toxicity data and the estimated exposure, it is not expected that endangered terrestrial or aquatic organisms will be affected from the use of glyphosate on the registered uses since the EEC's are well below the endangered species criteria (birds= 1/10 LC_{50}, aquatic organisms= 1/20 LC_{50}). However, many endangered plants may be at risk from the use of glyphosate on the registered use patterns. In addition, as discussed in the 1986 Glyphosate Registration Standard, it was determined that based on habitat, the Houston Toad may be at risk from the use of glyphosate on alfalfa.

IV. RISK MANAGEMENT AND REREGISTRATION DECISION

A. Determination of Eligibility

Section 4(g)(2)(A) of FIFRA calls for the Agency to determine, after submission of relevant data concerning an active ingredient, whether products containing the active ingredients are eligible for reregistration. The Agency has previously identified and required the submission of the generic (i.e. active ingredient specific) data required to support reregistration of products containing glyphosate active ingredients. The Agency has completed its review of these generic data, and has determined that the data are sufficient to support reregistration of all products containing the isopropylamine and sodium salts of glyphosate. Appendix B identifies the generic data requirements that the Agency reviewed as part of its determination of reregistration eligibility of glyphosate, and lists the submitted studies that the Agency found acceptable.

The data identified in Appendix B were sufficient to allow the Agency to assess the registered uses of glyphosate and to determine that glyphosate can be used without resulting in unreasonable adverse effects to man and the environment. The Agency therefore finds that all products containing glyphosate as the active ingredients are eligible for reregistration. The reregistration of particular products is addressed in Section V of this document.

The Agency made its reregistration eligibility determination based upon the target data base required for reregistration, the current guidelines for conducting acceptable studies to generate such data and the data identified in Appendix B. Although the Agency has found that all uses of glyphosate (isopropylamine and sodium salt formulations) are eligible for reregistration, it should be understood that the Agency may take appropriate regulatory action, and/or require the submission of additional data to support the registration of products containing glyphosate, if new information comes to the Agency's attention or if the data requirements for registration (or the guidelines for generating such data) change.

1. Eligibility Decision

Based on the reviews of the generic data for the active ingredient glyphosate, the Agency has sufficient information on the health effects of glyphosate and on its potential for causing adverse effects in fish and wildlife and the environment. The Agency concludes that products containing glyphosate for all uses are eligible for reregistration.

The Agency has determined that glyphosate products, labeled and used as specified in this Reregistration Eligibility Document, will not pose unreasonable risks or adverse effects to humans or the environment.

2. Eligible and Ineligible Uses

The Agency has determined that all uses of glyphosate are eligible for reregistration.

B. Regulatory Position

The following is a summary of the regulatory positions and rationales for glyphosate. Where labeling revisions are imposed, specific language is set forth in Section V of this document.

1. Tolerance Re-assessment

The Agency has determined that aminomethyl phosphonic acid (AMPA), the metabolite of glyphosate, no longer needs to be regulated and therefore this compound will be dropped from the tolerance expression. Also, although the monoammonium salt of glyphosate is not subject to reregistration, the available data are to allow re-assessment of existing tolerances for residues resulting from the application of the monoammonium salt of glyphosate.

Tolerances Listed Under 40 CFR §180.364(a):

The tolerances listed in 40 CFR §180.364(a) are for the combined residues of glyphosate and its metabolite AMPA resulting from application of the isopropylamine salt of glyphosate and/or the monoammonium salt of glyphosate.

Sufficient data are available to ascertain the adequacy of the established tolerances listed in 40 CFR §180.364(a) for: acerola; alfalfa, forage, seed, and hay; almonds, hulls; artichokes, Jerusalem; asparagus; atemoya; avocados; Bahiagrass; bananas; beets, garden, roots; Bermudagrass; bluegrass; Brassica leafy vegetables group; bromegrass; bulb vegetables group; carambola; carrots; cereal grains group; citrus fruits group; coffee beans, green; clover; cotton forage; cotton hay; cottonseed; cranberries; cucurbit vegetables group; fescue; figs; foliage of legume vegetables group; fruiting vegetables group; grapes; grass forage, fodder, and hay group; guavas; horseradish; kiwifruit; leafy vegetables group; leaves of the root and tuber vegetables group; legume vegetables group; longan fruit; lychee; mangoes; non-grass animal feeds group, forage and hay; orchardgrass; papayas; parsnips; passion fruit; peanuts; peanuts,

vines; pineapple; pistachio; pome fruits group; radishes; rutabagas; ryegrass; sapodilla; sapote; small fruits and berries group; soybeans; soybean, forage; stone fruits group; sugar apple; sugar beets; sweet potatoes; timothy; tree nuts group; turnip roots; wheatgrass; and yams. Certain commodity definitions of the above tolerances are not in accordance with the definitions listed in Table II of Subdivision O; see the tolerance re-assessment table on page 63 for modifications in commodity definitions.

The established crop group tolerances for the now-obsolete "seed and pod vegetables" (0.2 ppm) and "seed and pod vegetables, forage and hay" (0.2 ppm) are inappropriate and are to be replaced with "legume vegetables group (except soybeans)" and "legume vegetables group, foliage of (except soybean forage and hay)," respectively. Soybeans must be excluded from the crop group tolerances because the use pattern for soybeans is different from other legume vegetables, and the established tolerance for soybeans and soybean forage and hay differ by a factor >5x from other legume vegetables. To achieve compatibility with Codex MRLs for selected commodities, the following actions must be taken (see the table on page 68): (i) increase U.S. tolerance for legume vegetables group (except soybeans) from 0.2 ppm to 5 ppm; and (ii) increase U.S. tolerance for soybean hay from 15 ppm to 20 ppm.

The individual tolerances for cranberries (0.2 ppm) and grapes (0.2 ppm) should be revoked since these fruits are covered by the crop group tolerance (0.2 ppm) for small fruits and berries. The tolerance for cotton hay is to be revoked since this is not a raw agricultural commodity of cotton.

Tolerances for wheat, grain and wheat, straw at 4 and 85 ppm, respectively, have been proposed (PP0F3865/FAP2H5635). When these tolerances have been established, the tolerances for the cereal grains group and the cereal grains group, forage, fodder, and straw should be modified to "cereal grains group (except wheat)" and "cereal grains group, forage, fodder, and straw (except wheat straw)", respectively. To achieve compatibility with the Codex MRL for wheat grain, the U.S. tolerance should be established at 5 ppm (see the table on page 68).

The existing and conflicting tolerances for alfalfa (200 ppm), alfalfa fresh and hay (0.2 ppm), clover (200 ppm), and forage legumes (except soybeans and peanuts; 0.4 ppm) should be deleted. Concomitant with the deletion of these tolerances, a tolerance of 100 ppm for residues in or on the non-grass animal feeds group, forage and hay, is to be established. The available data from alfalfa, lespedeza, and trefoil will support this crop group tolerance.

The established tolerances for "forage grasses" (0.2 ppm), "grasses, forage" (0.2 ppm), Bahiagrass (200 ppm), Bermudagrass (200 ppm), bluegrass (200 ppm), bromegrass (200 ppm), fescue (200 ppm), orchardgrass (200 ppm), ryegrass (200 ppm), timothy (200 ppm), and wheatgrass (200 ppm) is to be deleted. Concomitant with the deletion of these tolerances, a tolerance for residues in on or on the grass forage, fodder, and hay group is to be established at 100 ppm. The available data indicate that following registered use, residues in or on the grass forage, fodder, and hay group will not exceed 100 ppm.

Individual tolerances exist for residues in or on salsify and the following tropical/subtropical crops: breadfruit; canistel; cherimoya; cocoa beans; coconut; dates; genip; jaboticaba; jackfruit; persimmons; sapote (black and white); soursop; and tamarind. There are currently no registered uses of glyphosate on these crop sites. These tolerances will be revoked.

A tolerance of 200 ppm has recently been established for residues in or on soybean straw (FR 42701, 9/16/92). However, this tolerance is to be revoked since this is not a raw agricultural commodity of soybeans. The tolerance for soybeans, hay should be raised to cover this desiccant use.

The expression negligible residues (N) should be deleted. For a complete listing of appropriate commodity definition changes and recommendations, see the table on page 63.

Tolerances Listed Under 40 CFR §180.364(b):

The tolerances listed in 40 CFR §180.364(b) are for the combined residues of glyphosate and its metabolite AMPA resulting from application of the glyphosate isopropylamine salt and/or glyphosate monoammonium salt for herbicidal and plant growth regulator purposes and/or the sodium sesqui salt for plant regulator purposes.

Sufficient data are available to ascertain the adequacy of the established tolerances listed in 40 CFR §180.364(b) for: liver and kidney of cattle, goats, hogs, horses, poultry, and sheep; peanuts; peanuts, hay; peanuts, hulls; sugarcane; fish; and shellfish. See the table on page 63 for modifications in commodity definitions.

Tolerances Listed Under 40 CFR §180.364(c):

The tolerances listed in 40 CFR §180.364(c) are for the combined residues of glyphosate and its metabolite AMPA resulting from the use of irrigation water containing residues of 0.5 ppm following applications on or around aquatic sites, and are established at 0.1 ppm. The Agency's Office of Water has established a maximum contaminant level (MCL) of 0.7 ppm for glyphosate *per se* in drinking water (FR Notice: Vol. 57, No. 138, page 31776, dated July 17, 1992).

Sufficient data are available to ascertain the established tolerances listed in 40 CFR §180.364(c) for the crop groupings Brassica leafy vegetables group; bulb vegetables group; cereal grains group; citrus fruits group; cucurbit vegetables group; foliage of legume vegetables group; forage, fodder, and straw of the cereal grains group; fruiting vegetables group; grass forage, fodder and hay group; leafy vegetables group; leaves of the root and tuber vegetables group; legume vegetables group; non-grass animal feeds group, forage and hay; pome fruits group; root and tuber vegetables group; stone fruits group; tree nuts group; and the individual commodities avocados, cottonseed, and hops. See the table on page 63 for modifications in commodity definitions.

Tolerances Listed Under 40 CFR §185.3500:

The tolerances listed in 40 CFR §185.3500(1) are for the combined residues of glyphosate and its metabolite AMPA resulting from the

application of the glyphosate for herbicidal purposes and/or the sodium sesqui salt for plant regulator purposes.

Sufficient data are available to ascertain the adequacy of the established food additive tolerances listed in 40 CFR §185.3500(1) for sugarcane, molasses. See the table on page 63 for modifications in commodity definitions.

The tolerances listed in 40 CFR §185.3500(2) are for the combined residues of glyphosate and its metabolite AMPA resulting from the application of the isopropylamine salt of glyphosate for herbicidal purposes.

Sufficient data are available to ascertain the adequacy of the established food additive tolerances listed in 40 CFR §185.3500(2) for olives (imported), palm oil, dried tea and instant tea. See the table on page 63 for modifications in commodity definitions.

A 12-ppm food additive tolerance for wheat milling fractions (except flour) has been proposed (FAP2H5635). To achieve compatibility with the Codex MRL for wheat bran, unprocessed, the U.S. tolerance should be established at 40 ppm (see the table on page 68).

Tolerances Listed Under 40 CFR §186.3500:

The tolerances listed in 40 CFR §186.3500(a) are for the combined residues of glyphosate and its metabolite AMPA.

Sufficient data are available to ascertain the adequacy of the established feed additive tolerances listed in 40 CFR §186.3500(a) for dried citrus pulp and soybean hulls. See the table on page 63 for modifications in commodity definitions.

A tolerance has recently been established at 1.0 ppm for the combined residues of glyphosate and AMPA in citrus, molasses (FR 42701, 9/16/92).

Existing tolerances of glyphosate are currently established in the Title 40 of the Code of Federal Regulations, §180.364. The reassessment of the established tolerances is set forth in the Tolerance Reassessment Table as follows.

Commodity	Current Tolerance [1] (ppm)	Tolerance [2] Reassessment (ppm)	Comment/*Correct Commodity Definition*
Tolerances listed under 180.364(a):			
Acerola	0.2		
Alfalfa Alfalfa, fresh and hay Clover Forage legumes (except soybeans and peanuts)	200.0 0.2 200.0 0.4	Revoke and establish at 100	*Non-grass animal feeds group, forage and hay*
Almond hulls	1		*Almonds, hulls*
Artichokes, Jerusalem	0.2		
Asparagus	0.5		
Atemoya	0.2		
Avocados	0.2		
Bahiagrass Bermudagrass Bluegrass Bromegrass Fescue Forage grasses Grasses, forage Orchardgrass Ryegrass Timothy Wheatgrass	200.0 200.0 200.0 200.0 200.0 0.2 0.2 200.0 200.0 200.0 200.0	Revoke and establish at 100	*Grass forage, fodder, and hay group*
Bananas	0.2		
Beets	0.2		*Beets, garden, roots*
Beets, sugar	0.2		*Sugar beets*
Breadfruit	0.2	Revoke	No registered uses
Canistel	0.2	Revoke	No registered uses
Carambola	0.2		
Carrots	0.2		
Cherimoya	0.2	Revoke	No registered uses
Chicory	0.2		*Chicory, roots*
Citrus fruits	0.2		*Citrus fruits group*
Cocoa beans	0.2	Revoke	No registered uses
Coconut	0.1	Revoke	No registered uses
Coffee beans	1		*Coffee beans, green*
Cotton, forage	15		

Commodity	Current Tolerance [1] (ppm)	Tolerance [2] Reassessment (ppm)	Comment/*Correct Commodity Definition*
Cotton, hay	15	Revoke	Not in Table II, Subdivision O, PAG
Cottonseed	15		
Cranberries	0.2	Revoke	Covered under small fruits and berries group
Dates	0.2	Revoke	No registered uses
Figs	0.2		
Forage grasses Grasses, forage	0.2 0.2	0.2	*Forage, fodder, and straw of cereal grains group (except wheat straw)*
Fruits, small and berries	0.2		*Small fruits and berries group*
Genip	0.2	Revoke	No registered uses
Grain crops	0.1		*Cereal grains group (except wheat)*
Grapes	0.2	Revoke	Covered under small fruits and berries group
Guavas	0.2		
Horseradish	0.2		
Jaboticaba	0.2	Revoke	No registered uses
Jackfruit	0.2	Revoke	No registered uses
Kiwifruit	0.2	0.1	see Codex Harmonization Table
Leafy vegetables	0.2		*Leafy vegetables (except Brassica) group* and *Leaves of root and tuber vegetables group*
Longan	0.2		*Longan fruit*
Lychee	0.2		
Mamy sapote	0.2		*Sapote*
Mangoes	0.2		
Nuts	0.2		*Tree nuts group*
Olives	0.2		
Papayas	0.2		
Parsnips	0.2		*Parsnips, roots*
Passion fruit	0.2		
Peanut, forage	0.5		*Peanuts, vines*
Persimmons	0.2	Revoke	No registered uses

Commodity	Current Tolerance [1] (ppm)	Tolerance [2] Reassessment (ppm)	Comment/*Correct Commodity Definition*
Pineapple	0.1		*Pineapples*
Pistachio nuts	0.2		*Pistachios*
Pome fruits	0.2		*Pome fruits group*
Potatoes	0.2		
Radishes	0.2		*Radishes, root*
Rutabagas	0.2		*Rutabagas, root*
Salsify	0.2	Revoke	No registered uses
Sapodilla	0.2		
Sapote, black	0.2	Revoke	No registered uses
Sapote, white	0.2	Revoke	No registered uses
Seed and pod vegetables	0.2	5	see Codex harmonization Table; *Legume vegetables group (except soybeans)*
Seed and pod vegetables, forage Seed and pod vegetables, hay	0.2 0.2	0.2	*Foliage of legume vegetables group (except soybean forage and hay)*
Soursop	0.2	Revoke	No registered uses
Soybeans	20		
Soybeans, forage	15		
Soybeans, hay	15	200	Raised to cover desiccant use.
Soybeans, straw	200	Revoke	Not in Table II, Subdivision O, PAG
Stone fruit	0.2		*Stone fruits group*
Sugar apple	0.2		
Sweet potatoes	0.2		
Tamarind	0.2	Revoke	No registered uses
Turnips	0.2		*Turnips, roots*
Vegetables, bulb	0.2		*Bulb vegetables group*
Vegetables, cucurbit	0.5		*Cucurbit vegetables group*
Vegetables, fruiting (except cucurbits) group	0.1		*Fruiting vegetables group*
Vegetables, leafy, Brassica (cole)	0.2		*Brassica leafy vegetables group*
Yams	0.2		
Wheat, grain	N/A	5.0	see Codex harmonization Table
Wheat, straw	N/A	85 (proposed)	

Commodity	Current Tolerance [1] (ppm)	Tolerance [2] Reassessment (ppm)	Comment/*Correct Commodity Definition*
Tolerances listed under 40 CFR §180.364(b):			
Cattle, kidney	0.5	2.0	see Codex harmonization Table
Cattle, liver	0.5	2.0	see Codex harmonization Table
Fish	0.25		
Goats, kidney	0.5		
Goats, liver	0.5		
Hogs, kidney	0.5	1.0	see Codex harmonization Table
Hogs, liver	0.5	1.0	see Codex harmonization Table
Horses, kidney	0.5		
Horses, liver	0.5		
Peanuts	0.1		
Peanut, hay	0.5		*Peanuts, hay*
Peanut, hulls	0.5		*Peanuts, hulls*
Poultry, kidney	0.5		
Poultry, liver	0.5		
Sheep, kidney	0.5		
Sheep, liver	0.5		
Shellfish	3.0		
Sugarcane	2.0		
Tolerances listed under 40 CFR 180.364(c):			
Avocados	0.1		
Citrus	0.1		*Citrus fruits group*
Cottonseed	0.1		
Cucurbits	0.1		*Cucurbit vegetables group*
Forage grasses	0.1		*Grass forage, fodder, and hay group*
Forage legumes	0.1		*Non-grass animal feeds group, forage and hay*
Fruiting vegetables	0.1		*Fruiting vegetables group*
Grain crops	0.1		*Cereal grains group* and *Forage, fodder, and straw of cereal grains group*
Hops	0.1		

Commodity	Current Tolerance [1] (ppm)	Tolerance [2] Reassessment (ppm)	Comment/*Correct Commodity Definition*
Leafy vegetables	0.1		*Leafy vegetables (except Brassica) group* and *Brassica (cole) leafy vegetables group*
Nuts	0.1		*Tree nuts group*
Pome fruits	0.1		*Pome fruits group*
Root crop vegetables	0.1		*Root and tuber vegetables group* and *Leaves of root and tuber vegetables group* and *Bulb vegetables group*
Seed and pod vegetables	0.1		*Legume vegetables group* and *Foliage of legume vegetables group*
Stone fruit	0.1		*Stone fruits group*
Tolerances listed under 40 CFR §185.3500(a)(1):			
Molasses, sugarcane	30.0		*Sugarcane, molasses*
Tolerances listed under 40 CFR §185.3500(a)(2):			
Oil, palm	0.1		*Palm oil, refined*
Olives, imported	0.1		
Tea, dried	1.0		
Tea, instant	7.0	Revoke	Not in Table II, Subdivision O, PAG
Wheat milling fractions (except flour)	N/A	40	see Codex harmonization Table
Tolerances listed under 40 CFR §186.3500(a):			
Citrus, pulp, dried	1.0		
Citrus molasses	1.0		*Citrus, molasses*
Soybean hulls	100		*Soybeans, hulls*

1 Tolerances are for the combined residues of glyphosate and its metabolite AMPA.
2 Tolerances are now for glyphosate *per se*.

CODEX HARMONIZATION TABLE

Several maximum residue limits (MRLs) for glyphosate have been established by Codex in various commodities. The Codex MRLs (currently expressed in terms of glyphosate *per se*) and applicable U.S. tolerances (expressed in terms of the combined residues of glyphosate and its metabolite AMPA) are listed in the table below. The Agency has determined that AMPA no longer needs to be regulated and therefore will be deleted from the tolerance expression. Based on this determination, the expression of the U.S. tolerances and the Codex MRLs will be harmonized, and both will now be expressed in terms of glyphosate *per se*.

Codex MRLs and applicable U.S. tolerances. Recommendations for compatibility are based on conclusions following reassessments of U.S. tolerances (see Tolerance Reassessment Table, above).

Commodity	MRL (Step) (mg/kg)	U.S. Tolerance (ppm)	Recommendation
Barley	20 (CXL)	0.1 (Cereal grains group, except wheat)	
Beans (dry)	2 (CXL)	0.2 (Legume vegetables group, except soybeans)	
Cattle meat	0.1 (CXL)		
Cattle milk	0.1 (CXL)		
Cattle, edible offal	2 (CXL)	0.5 (Cattle, liver & kidney)	increase U.S. tolerances
Cottonseed	0.5 (CXL)	15	
Eggs	0.1 (CXL)		
Hay or fodder (dry) of grasses	50 (CXL)	100 (Grass forage, fodder, and hay group)	
Kiwifruit	0.1 (CXL)	0.2	decrease U.S. tolerance
Maize	0.1 (CXL)	0.1	
Oats	20 (CXL)	0.1 (Cereal grains group, except wheat)	
Peas (dry)	5 (CXL)	0.2 (Legume vegetables group, except soybeans)	increase U.S. tolerance
Pig meat	0.1 (CXL)		
Pig, edible offal	1 (CXL)	0.5 (Hogs, liver & kidney)	increase U.S. tolerances
Poultry meat	0.1 (CXL)		
Rape seed	10 (CXL)		
Rice	0.1 (CXL)	0.1 (Cereal grains group, except wheat)	

Commodity	MRL (Step) (mg/kg)	U.S. Tolerance (ppm)	Recommendation
Sorghum	0.1 (CXL)	0.1 (Cereal grains group, except wheat)	
Soya bean fodder	20 (Step 8)	15 (Soybeans, hay)	
Soya bean forage (green)	5 (Step 8)	15 (Soybeans, forage)	
Soya bean (dry)	5 (Step 8)	20 (Soybeans)	
Soya bean (immature seeds)	0.2 (CXL)		
Straw and fodder (dry) of cereal grains	100 (CXL)	0.2 (Forage, fodder, and straw of cereal grains group, except wheat straw)	
Sweet corn (corn-on-the-cob)	0.1 (CXL)	0.1 (Cereal grains group, except wheat)	
Wheat	5 (CXL)	4 (proposed)	increase U.S. tolerance proposal
Wheat bran, unprocessed	40 (Step 6)	12 (proposed)	increase U.S. tolerance proposal
Wheat flour	0.5 (Step 8)		
Wheat whole meal	5 (Step 8)	12 (proposed)	

The following conclusions can be made regarding efforts to harmonize the U.S. tolerances with the Codex MRLs:

- Compatibility between the U.S. tolerances and permanent Codex MRLs exists in or on: corn (field and sweet); rice; and sorghum.

- The levels of U.S. tolerances should be increased, toxicological and DRES considerations permitting, to achieve compatibility with the Codex MRLs in or on the following commodities: (i) liver and kidney of cattle (from 0.5 to 2.0 ppm); (ii) liver and kidney of hogs (from 0.5 to 1.0 ppm); and (iii) legume vegetables group (except soybeans) (from 0.2 to 5 ppm);

- The level of the U.S. tolerance should be decreased to achieve compatibility with the Codex MRLs in or on kiwifruit (from 0.2 to 0.1 ppm).

- The U.S. tolerances in or on the following commodities were based on registered use patterns in the U.S. and cannot be lowered to achieve compatibility with the Codex MRLs: (i) grass forage, fodder, and hay group; (ii) soybeans; and (iii) soybeans, forage.

- Wheat grain and wheat bran tolerances of 4 and 12 ppm, respectively, have been proposed. To achieve compatibility with Codex, these tolerance levels should be increased, toxicological and DRES considerations permitting, to 5 and 40 ppm, respectively.

- Ë Wide differences (>5x) exist between the U.S. tolerances and permanent Codex MRLs in or on the following commodities: barley; beans (dry); soybeans, hay; cottonseed; oats; forage, fodder, and straw of cereal grains. The decision to harmonize residue levels in or on these commodities cannot be made at this time.

- Ë No questions of compatibility exist with respect to commodities where: (i) no Codex MRLs have been established, but U.S. tolerances exist; and (ii) Codex MRLs have been established, but U.S. tolerances do not exist.

2. Labeling Rationale

While studies show that glyphosate is no more than slightly toxic to birds and is practically non-toxic to fish and honeybees, a toxic inert in glyphosate end use products necessitates the labelling of some products "toxic to fish" since some glyphosate products are applied directly to aquatic environments.

3. Endangered Species Statement

The Agency does have concerns regarding exposure of endangered plant species to glyphosate. In the June 1986 Registration Standard, the Agency discussed consultations with the US Fish and Wildlife Service (FWS) on hazards to crops, rangeland, silvicultural sites, and the Houston toad which may result from the use of glyphosate. Because a jeopardy opinion resulted from these consultations, the agency imposed endangered species labeling requirements in the Registration Standard to mitigate the risk to endangered species. Since that time, additional plant species have been added to the list of endangered species. At the present time, EPA is working with the FWS and other federal and state agencies to develop a program to avoid jeopardizing the continued existence of all listed species by the use of pesticides. When the Endangered Species Protection Program is implemented and subsequent guidance is given, endangered species labeling amendments may be required on affected end-use products. Labeling statements for end use products will likely refer users to county specific bulletins specifying detailed limitations on use to protect endangered species.

V. ACTIONS REQUIRED BY REGISTRANTS

This section specifies the data requirements and responses necessary for the reregistration of both manufacturing-use and end-use products.

A. Manufacturing-Use Products

1. Additional Generic Data Requirements

The generic data base supporting the reregistration of glyphosate for the above eligible uses has been reviewed and determined to be substantially complete. The Agency will be calling in data on processed potatoes in a separate DCI. However, the following additional generic data are required at this time. These additional generic data are not part of the target data base for glyphosate and do not affect the reregistration eligibility of glyphosate. (See Appendices for the Generic Data Call-In Notice.)

Name of Study	Guideline Number
Tier II Vegetative Vigor	123-1
Droplet Size Spectrum	201-1
Drift Field Evaluation	202-1

2. Labeling Requirements for Manufacturing-Use Products

Effluent Discharge Labeling Statement

All manufacturing-use or end-use products that may be contained in an effluent discharged to the waters of the United States or municipal sewer systems must bear the following revised effluent discharge labeling statement.

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA."

All affected products distributed or sold by registrants and distributors (supplemental registrants) must bear the above labeling by October 1, 1995. All products distributed or sold by persons other than registrants or supplemental registrants after October 1, 1997 must bear the correct labeling. Refer to PR Notice 93-10 or 40 CFR 152.46(a)(1) for additional information.

B. End-Use Products

1. Additional Product-Specific Data Requirements

Section 4(g)(2)B) of FIFRA calls for the Agency to obtain any needed product-specific data regarding the pesticide after a determination of eligibility has been

made. The product specific data requirements are listed in Appendix G, the Product Specific Data Call-In Notice.

Registrants must review previous data submissions to ensure that they meet current EPA acceptance criteria (Appendix F; Attachment E) and if not, commit to conduct new studies. If a registrant believes that previously submitted data meet current testing standards, then study MRID numbers should be cited according to the instructions in the Requirement Status and Registrants Response Form provided for each product.

2. Labeling Requirements for End-Use Products

The labels and labeling of all products must comply with EPA's current regulations and requirements as specified in 40 CFR §156.10 and other applicable documents. Please follow the instructions in the Pesticide Reregistration Handbook with respect to labels and labeling. Furthermore, the following additional labeling must be present on glyphosate end-use product labels.

a. Nonaquatic

"Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwaters and rinsate."

b. Aquatic

"Do not contaminate water when disposing of equipment washwaters and rinsate. Treatment of aquatic weeds can result in oxygen loss from decomposition for dead plants. This loss can cause fish kills."

c. Worker Protection Standard

Compliance

Any product whose labeling reasonably permits use in the commercial or research production of an agricultural plant on any farm, forest, nursery, or greenhouse must comply with the labeling requirements of PR Notice 93-7, "Labeling Revisions Required by the Worker Protection Standard (WPS), and PR Notice 93-11, "Supplemental Guidance for PR Notice 93-7," which reflect the requirements of EPA's labeling regulations for worker protection statements (40 CFR part 156, subpart K). These labeling revisions are necessary to implement the Worker Protection Standard for Agricultural Pesticides (40 CFR Part 170) and must be completed in accordance with, and within the deadlines

specified in, PR Notices 93-7 and 93-11. Unless otherwise specifically directed in this RED, all statements required by PR Notices 93-7 and 93-11 are to be on the product labeling exactly as instructed in those notices.

After April 21, 1994, except as otherwise provided in PR Notices 93-7 and 93-11, all products within the scope of those notices must bear WPS PR-Notice-complying labeling when they are distributed or sold by the primary registrant or any supplementally registered distributor.

After October 23, 1995, except as otherwise provided in PR Notices 93-7 and 93-11, all products within the scope of those notices must bear WPS PR-Notice-complying labeling when they are distributed or sold by any person.

Personal Protective Equipment

Do not add any additional personal protective equipment requirements to the labels of glyphosate end-use products, however, any existing personal protective equipment on those labels must be retained.

Entry Restrictions

Products not Primarily Intended for Home Use

Uses Within the Scope of the WPS: A 12-hour restricted entry interval (REI) is required for all uses within the scope of the WPS (see PR Notice 93-7) on all end-use products, except those intended primarily for home use (see tests in PR Notice 93-7 and 93-11). This REI should be inserted into the standardized REI statement required by PR Notice 93-7. The personal protective equipment for early entry should be the PPE required for applicators of glyphosate, except any applicator requirement for an apron or respirator is waived. This PPE should be inserted into the standardized early entry PPE statement required by PR Notice 93-7."

Sole-active-ingredient end-use products that contain glyphosate must be revised to adopt the entry restrictions set forth in this section. Any conflicting entry restrictions on their current labeling must be removed.

Multiple-active-ingredient end-use products that contain glyphosate must compare the entry restrictions set forth in this section to the entry restrictions on their current labeling and retain the more protective. A specific time-period in hours or days is considered more protective than "sprays have dried" or "dusts have settled."

Uses Not Within the Scope of the WPS: Do not add any additional entry restrictions for uses not within the scope of the WPS, however, any entry restrictions on the current product labeling for those uses must be retained.

Products Primarily Intended for Home Use: For products primarily intended for home use (see tests in PR Notice 93-7 and 93-11), do not add any additional entry restrictions for such products, however, any entry restrictions on the current product labeling must be retained.

C. Existing Stocks

Registrants may generally distribute and sell products bearing old labels/labeling for 26 months from the date of the issuance of this RED. Persons other than the registrant may generally distribute or sell such products for 50 months from the date of the issuance of this RED. However, existing stocks time frames will be established case-by-case, depending on the number of products involved, the number of label changes, and other factors. Refer to "Existing Stocks of Pesticide Products; State of Policy"; Federal Register, Volume 56, No. 123, June 26, 1991.

The Agency has determined that registrants may distribute and sell glyphosate products bearing old labels/labeling for 26 months from the date of issuance of this RED. Persons other than registrants may distribute or sell such products for 50 months from the date of issuance of this RED.

VI. APPENDICES

1. **Bolded** references were reviewed on 4/26/90. Unbolded references were reviewed in the Residue Chemistry Science Chapter of the Reregistration Standard dated 7/15/85. Otherwise, references were reviewed as noted.

Appendix A

Use Patterns Subject to Reregistration

Appendix A is approximately 200 pages long and is not being included in the mailing of the RED. Instead, a summary of eligible sites and use groups is provided. Interested parties may order a copy of the full Appendix A per the instructions in Appendix D.

103601 - Glyphosate, isopropylamine salt

LUIS
General Chemical Report
Site and Use Groups

Site	Use Group	Use Group Category Desc..
ACEROLA (WEST INDIES CHERRY)	TERRESTRIAL FOOD CROP	Food/Feed Uses
AGRICULTURAL DRAINAGE SYSTEMS	AQUATIC FOOD CROP	Food/Feed Uses
AGRICULTURAL FALLOW/IDLELAND	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
ALFALFA	TERRESTRIAL FEED CROP	Food/Feed Uses
ALMOND	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
APPLE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
APRICOT	TERRESTRIAL FOOD CROP	Food/Feed Uses
ARTICHOKE, JERUSALEM	TERRESTRIAL FOOD CROP	Food/Feed Uses
ASPARAGUS	TERRESTRIAL FOOD CROP	Food/Feed Uses
ATEMOYA	TERRESTRIAL FOOD CROP	Food/Feed Uses
AVOCADO	TERRESTRIAL FOOD CROP	Food/Feed Uses
BANANA	TERRESTRIAL FOOD CROP	Food/Feed Uses
BARLEY	TERRESTRIAL FEED CROP	Food/Feed Uses
BARLEY	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
BEANS	TERRESTRIAL FEED CROP	Food/Feed Uses
BEANS	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
BEECH NUT	TERRESTRIAL FOOD CROP	Food/Feed Uses
BEETS	TERRESTRIAL FOOD CROP	Food/Feed Uses
BEETS (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
BLACKBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
BLUEBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
BOYSENBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
BRAZIL NUT	TERRESTRIAL FOOD CROP	Food/Feed Uses
BREADFRUIT (BREADNUT)	TERRESTRIAL FOOD CROP	Food/Feed Uses
BROCCOLI	TERRESTRIAL FOOD CROP	Food/Feed Uses
BRUSSELS SPROUTS	TERRESTRIAL FOOD CROP	Food/Feed Uses
BUCKWHEAT	TERRESTRIAL FEED CROP	Food/Feed Uses
BUCKWHEAT	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
BUTTERNUT	TERRESTRIAL FOOD CROP	Food/Feed Uses
CABBAGE	TERRESTRIAL FOOD CROP	Food/Feed Uses
CABBAGE, CHINESE	TERRESTRIAL FOOD CROP	Food/Feed Uses
CALAMONDIN	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
CARAMBOLA (JALEA)	TERRESTRIAL FOOD CROP	Food/Feed Uses
CARROT (INCLUDING TOPS)	TERRESTRIAL FOOD CROP	Food/Feed Uses
CASHEW	TERRESTRIAL FOOD CROP	Food/Feed Uses
CAULIFLOWER	TERRESTRIAL FOOD CROP	Food/Feed Uses
CELERY	TERRESTRIAL FOOD CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
CHARD, SWISS	TERRESTRIAL FOOD CROP	Food/Feed Uses
CHERIMOYA	TERRESTRIAL FOOD CROP	Food/Feed Uses
CHERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
CHESTNUT	TERRESTRIAL FOOD CROP	Food/Feed Uses
CHICORY	TERRESTRIAL FOOD CROP	Food/Feed Uses
CITRON (CITRUS)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
CITRUS HYBRIDS OTHER THAN TANGELO	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
COCOA	TERRESTRIAL FOOD CROP	Food/Feed Uses
COFFEE	TERRESTRIAL FOOD CROP	Food/Feed Uses
COLLARDS	TERRESTRIAL FOOD CROP	Food/Feed Uses
CORN	TERRESTRIAL FEED CROP	Food/Feed Uses
CORN (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
CORN, FIELD	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
COTTON (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
CRANBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
CRESS, WATER	TERRESTRIAL FOOD CROP	Food/Feed Uses
CUCUMBER	TERRESTRIAL FOOD CROP	Food/Feed Uses
CURRANT	TERRESTRIAL FOOD CROP	Food/Feed Uses
DATE	TERRESTRIAL FOOD CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
DEWBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
EGGFRUIT TREE (CANISTEL)	TERRESTRIAL FOOD CROP	Food/Feed Uses
EGGPLANT	TERRESTRIAL FOOD CROP	Food/Feed Uses
ELDERBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
ENDIVE (ESCAROLE)	TERRESTRIAL FOOD CROP	Food/Feed Uses
FIG	TERRESTRIAL FOOD CROP	Food/Feed Uses
FILBERT (HAZELNUT)	TERRESTRIAL FOOD CROP	Food/Feed Uses
GARLIC	TERRESTRIAL FOOD CROP	Food/Feed Uses
GOOSEBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
GOURDS	TERRESTRIAL FOOD CROP	Food/Feed Uses
GRAPEFRUIT	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
GRAPES	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
GRASS FORAGE/FODDER/HAY	TERRESTRIAL FEED CROP	Food/Feed Uses
GREENHOUSES-IN USE	GREENHOUSE FOOD CROP	Food/Feed Uses
GROUNDCHERRY (STRAWBERRY TOMATO/TOMATILLO)	TERRESTRIAL FOOD CROP	Food/Feed Uses
GUAVA	TERRESTRIAL FOOD CROP	Food/Feed Uses
HICKORY NUT	TERRESTRIAL FOOD CROP	Food/Feed Uses
HORSERADISH	TERRESTRIAL FOOD CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
HUCKLEBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
IRRIGATION SYSTEMS	AQUATIC FOOD CROP	Food/Feed Uses
JABOTICABA	TERRESTRIAL FOOD CROP	Food/Feed Uses
JACKFRUIT	TERRESTRIAL FOOD CROP	Food/Feed Uses
KALE	TERRESTRIAL FOOD CROP	Food/Feed Uses
KITEMBILLA (CEYLON GOOSEBERRY)	TERRESTRIAL FOOD CROP	Food/Feed Uses
KIWI FRUIT	TERRESTRIAL FOOD CROP	Food/Feed Uses
KOHLRABI	TERRESTRIAL FOOD CROP	Food/Feed Uses
KUMQUAT	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
LAKES/PONDS/RESERVOIRS (WITH HUMAN OR WILDLIFE USE)	AQUATIC FOOD CROP	Food/Feed Uses
[illegible]EER	TERRESTRIAL FOOD CROP	Food/Feed Uses
LEMON	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
LENTILS	TERRESTRIAL FEED CROP	Food/Feed Uses
LENTILS	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
LETTUCE	TERRESTRIAL FOOD CROP	Food/Feed Uses
LIME	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
LITCHI NUT	TERRESTRIAL FOOD CROP	Food/Feed Uses
LOGANBERRY	TERRESTRIAL FOOD CROP	Food/Feed Uses
LONGAN	TERRESTRIAL FOOD CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
LOQUAT	TERRESTRIAL FOOD CROP	Food/Feed Uses
MACADAMIA NUT (BUSHNUT)	TERRESTRIAL FOOD CROP	Food/Feed Uses
MAMEY (MAMMEE APPLE)	TERRESTRIAL FOOD CROP	Food/Feed Uses
MANGO	TERRESTRIAL FOOD CROP	Food/Feed Uses
MARMALADEBOX (GENIPAPO)	TERRESTRIAL FOOD CROP	Food/Feed Uses
MAYHAW (HAWTHORN)	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS, CANTALOUPE	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS, HONEYDEW	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS, MANGO	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS, MUSK	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS, WATER	TERRESTRIAL FOOD CROP	Food/Feed Uses
MELONS, WINTER (CASABA/CRENSHAW/HONEYDEW/PERSIAN)	TERRESTRIAL FOOD CROP	Food/Feed Uses
MILLET (PROSO)	TERRESTRIAL FEED CROP	Food/Feed Uses
MILLET, PROSO (BROOMCORN)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
MUSTARD	TERRESTRIAL FOOD CROP	Food/Feed Uses
MUSTARD	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
NECTARINE	TERRESTRIAL FOOD CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
RONGRASS FORAGE/FODDER/STRAW/HAY	TERRESTRIAL FEED CROP	Food/Feed Uses
OATS	TERRESTRIAL FEED CROP	Food/Feed Uses
OATS	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
OKRA	TERRESTRIAL FOOD CROP	Food/Feed Uses
OLIVE	TERRESTRIAL FOOD CROP	Food/Feed Uses
ONION	TERRESTRIAL FOOD CROP	Food/Feed Uses
ORANGE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
PAPAYA	TERRESTRIAL FOOD CROP	Food/Feed Uses
PARSLEY	TERRESTRIAL FOOD CROP	Food/Feed Uses
PARSNIP	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
ASSION FRUIT	TERRESTRIAL FOOD CROP	Food/Feed Uses
PASTURES	TERRESTRIAL FEED CROP	Food/Feed Uses
PEACH	TERRESTRIAL FOOD CROP	Food/Feed Uses
PEANUTS (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
PEAR	TERRESTRIAL FOOD CROP	Food/Feed Uses
PEAS (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
PECAN	TERRESTRIAL FOOD CROP	Food/Feed Uses
PEPPER	TERRESTRIAL FOOD CROP	Food/Feed Uses
PERSIMMON	TERRESTRIAL FOOD CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc.
PINEAPPLE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
PISTACHIO	TERRESTRIAL FOOD CROP	Food/Feed Uses
PLANTAIN	TERRESTRIAL FOOD CROP	Food/Feed Uses
PLUM	TERRESTRIAL FOOD CROP	Food/Feed Uses
POMEGRANATE	TERRESTRIAL FOOD CROP	Food/Feed Uses
POTATO, WHITE/IRISH	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
PRUNE	TERRESTRIAL FOOD CROP	Food/Feed Uses
PUMMELO (SHADDOCK)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
PUMPKIN	TERRESTRIAL FOOD CROP	Food/Feed Uses
QUINCE	TERRESTRIAL FOOD CROP	Food/Feed Uses
RADISH	TERRESTRIAL FOOD CROP	Food/Feed Uses
RAPE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
RASPBERRY (BLACK, RED)	TERRESTRIAL FOOD CROP	Food/Feed Uses
RHUBARB	TERRESTRIAL FOOD CROP	Food/Feed Uses
RICE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
RICE, WILD	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
RUTABAGA	TERRESTRIAL FOOD CROP	Food/Feed Uses
RYE	TERRESTRIAL FEED CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
RYE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
SAPODILLA	TERRESTRIAL FOOD CROP	Food/Feed Uses
SAPOTA, WHITE	TERRESTRIAL FOOD CROP	Food/Feed Uses
SITE NOT SPECIFIED	USE GROUP FOR SITE 00000	Food/Feed Uses
SORGHUM	TERRESTRIAL FEED CROP	Food/Feed Uses
SORGHUM	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
SOURSOP	TERRESTRIAL FOOD CROP	Food/Feed Uses
SOYBEANS (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
SPINACH	TERRESTRIAL FOOD CROP	Food/Feed Uses
SQUASH (SUMMER)	TERRESTRIAL FOOD CROP	Food/Feed Uses
SQUASH (WINTER)	TERRESTRIAL FOOD CROP	Food/Feed Uses
STREAMS/RIVERS/CHANNELED WATER	AQUATIC FOOD CROP	Food/Feed Uses
SUGAR APPLE (CUSTARD APPLE)	TERRESTRIAL FOOD CROP	Food/Feed Uses
SUGAR BEET	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
SUGARCANE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
SWEET POTATO	TERRESTRIAL FOOD CROP	Food/Feed Uses
TAMARIND	TERRESTRIAL FOOD CROP	Food/Feed Uses
TANGELO	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
TANGERINES	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses

Site	Use Group	Use Group Category Desc..
TARO	TERRESTRIAL FOOD CROP	Food/Feed Uses
TEA	TERRESTRIAL FOOD CROP	Food/Feed Uses
TOMATO	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
TRITICALE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
TURNIP	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
WALNUT (ENGLISH/BLACK)	TERRESTRIAL FOOD CROP	Food/Feed Uses
WHEAT	TERRESTRIAL FEED CROP	Food/Feed Uses
WHEAT	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
YAM	TERRESTRIAL FOOD CROP	Food/Feed Uses
AGRICULTURAL FALLOW/IDLELAND	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
AGRICULTURAL RIGHTS-OF-WAY/FENCEROWS/HEDGEROWS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
AGRICULTURAL UNCULTIVATED AREAS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
AIRPORTS/LANDING FIELDS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
AQUATIC AREAS/WATER	AQUATIC NON-FOOD INDUSTRIAL	Non-Food/Non-Feed Uses
AQUATIC AREAS/WATER	AQUATIC NON-FOOD OUTDOOR	Non-Food/Non-Feed Uses
CHRISTMAS TREE PLANTATIONS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
CONIFER RELEASE	FORESTRY	Non-Food/Non-Feed Uses
DRAINAGE SYSTEMS	AQUATIC NON-FOOD INDUSTRIAL	Non-Food/Non-Feed Uses

Site	Use Group	Use Group Category Desc..
FOREST PLANTINGS (REFORESTATION PROGRAMS)	FORESTRY	Non-Food/Non-Feed Uses
FOREST TREES (ALL OR UNSPECIFIED)	FORESTRY	Non-Food/Non-Feed Uses
GOLF COURSE TURF	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
GREENHOUSE-EMPTY	INDOOR NON-FOOD	Non-Food/Non-Feed Uses
HOUSEHOLD/DOMESTIC DWELLINGS OUTDOOR PREMISES	OUTDOOR RESIDENTIAL	Non-Food/Non-Feed Uses
INDUSTRIAL AREAS (OUTDOOR)	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
NONAGRICULTURAL OUTDOOR BUILDINGS/STRUCTURES	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
NONAGRICULTURAL RIGHTS-OF-WAY/FENCEROWS/HEDGEROWS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
NONAGRICULTURAL UNCULTIVATED AREAS/SOILS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
ORNAMENTAL AND/OR SHADE TREES	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
ORNAMENTAL AND/OR SHADE TREES	TERRESTRIAL NON-FOOD+OUTDOOR RESIDE	Non-Food/Non-Feed Uses
ORNAMENTAL AND/OR SHADE TREES	TERRESTRIAL+GREENHOUSE NON-FOOD CRO	Non-Food/Non-Feed Uses
ORNAMENTAL HERBACEOUS PLANTS	TERRESTRIAL NON-FOOD+OUTDOOR RESIDE	Non-Food/Non-Feed Uses
ORNAMENTAL LAWNS AND TURF	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
ORNAMENTAL LAWNS AND TURF	TERRESTRIAL NON-FOOD+OUTDOOR RESIDE	Non-Food/Non-Feed Uses
ORNAMENTAL WOODY SHRUBS AND VINES	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
ORNAMENTAL WOODY SHRUBS AND VINES	TERRESTRIAL NON-FOOD+OUTDOOR RESIDE	Non-Food/Non-Feed Uses
ORNAMENTAL WOODY SHRUBS AND VINES	TERRESTRIAL+GREENHOUSE NON-FOOD CRO	Non-Food/Non-Feed Uses
PATHS/PATIOS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses

Site	Use Group	Use Group Category Desc..
PAVED AREAS (PRIVATE ROADS/SIDEWALKS)	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
RECREATIONAL AREAS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses
SEWAGE SYSTEMS	AQUATIC NON-FOOD INDUSTRIAL	Non-Food/Non-Feed Uses
SITE NOT SPECIFIED	USE GROUP FOR SITE 00000	Non-Food/Non-Feed Uses
URBAN AREAS	TERRESTRIAL NON-FOOD CROP	Non-Food/Non-Feed Uses

Site	Use Group	Use Group Category Desc..
PEANUTS (UNSPECIFIED)	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses
SUGARCANE	TERRESTRIAL FOOD+FEED CROP	Food/Feed Uses

Appendix B

Table of Generic Data Requirements and Studies Used to Make the Reregistration Decision

GUIDE TO APPENDIX B

Appendix B contains listings of data requirements which support the reregistration for the pesticide glyphosate covered by this Reregistration Eligibility Document. It contains generic data requirements that apply to glyphosate in all products, including data requirements for which a "typical formulation" is the test substance.

The data table is organized in the following format:

1. Data Requirement (Column 1). The data requirements are listed in the order in which they appear in 40 CFR, Part 158. The reference numbers accompanying each test refer to the test protocols set in the Pesticide Assessment Guidelines, which are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703) 487 - 4650.

2. Use Pattern (Column 2). This column indicates the use patterns for which the data requirements apply. The following letter designations are used for the given use patterns:

    A Terrestrial food
    B Terrestrial feed
    C Terrestrial non-food
    D Aquatic food
    E Aquatic non-food outdoor
    F Aquatic non-food industrial
    G Aquatic non-food residential
    H Greenhouse food
    I Greenhouse non-food
    J Forestry
    K Residential
    L Indoor food
    M Indoor non-food
    N Indoor medical
    O Indoor residential

3. Bibliographic citation (Column 3). If the Agency has acceptable data in its files, this column lists the identifying number of each study. This normally is the Master Record Identification (MRID) number, but may be a "GS" number if no MRID number has been assigned. Refer to the Bibliography appendix for a complete citation of the

study.

Data Supporting Guideline Requirements for the Reregistration of Glyphosate

REQUIREMENT		USE PATTERN	CITATION(S)
PRODUCT CHEMISTRY			
61-2A	**Start. Mat. & Mnfg. Process**	all	00161333
61-2B	**Formation of Impurities**	all	00161333
62-1	**Preliminary Analysis**	all	40405401, 00161333
63-2	**Color**	all	00161333
63-3	**Physical State**	all	00161333
63-4	**Odor**	all	00161333
63-5	**Melting Point**	all	00161333
63-6	**Boiling Point**	all	00161333
63-7	**Density**	all	00161333
63-8	**Solubility**	all	00161333
63-9	**Vapor Pressure**	all	41096101, 00161333
63-10	**Dissociation Constant**	all	00161333
63-11	**Octanol/Water Partition**	all	00161333
63-12	**pH**	all	00161333
63-13	**Stability**	all	00161333, 40559301
63-17	**Storage stability**	A C	41573601, 00039142, 00061553, 00040083, 00061555, 00051980, 00108129, 00053002, 00108102

Data Supporting Guideline Requirements for the Reregistration of Glyphosate

REQUIREMENT		USE PATTERN	CITATION(S)
ECOLOGICAL EFFECTS			
71-1A	**Acute Avian Oral - Quail/Duck**	A B C D F G H	00108204
71-2A	**Avian Dietary - Quail**	A B C D F G H	00108107
71-2B	**Avian Dietary - Duck**	A B C D F G H	00076492
71-3	**Wild Mammal Toxicity**	A B C D F G H	00076492
71-4A	**Avian Reproduction - Quail**	A B C D G	00108207
71-4B	**Avian Reproduction - Duck**	A B C D G	00036328, 00111953
72-1A	**Fish Toxicity Bluegill**	A B C D F G H	00136339, GS-0178025
72-1B	**Fish Toxicity Bluegill - TEP**	A B C D G	15296, 152599, 152601, 152767
72-1C	**Fish Toxicity Rainbow Trout**	A B C D F G H	00108112, 00108205
72-1D	**Fish Toxicity Rainbow Trout - TEP**	A B C D G	00070895, 00078661, 00070897, 00078662, 00078655, 00078664, 00078656, 00078665, 00078658, 00108205, 00078659, 00124760, GS0178025, 5298, 152766, 152903, 155477
72-2A	**Invertebrate Toxicity**	A B C D F G H	00108172
72-2B	**Invertebrate Toxicity - TEP**	A B C D G	00070893, 00078666, 00078657, 00124762, 00078660, GS0178025, 0078663, 152597, 152600, 152602, 152768
72-3B	**Estuarine/Marine Toxicity - Mollusk**	A B C D	00108110

Data Supporting Guideline Requirements for the Reregistration of Glyphosate

REQUIREMENT		USE PATTERN	CITATION(S)
72-3C	**Estuarine/Marine Toxicity - Shrimp**	A B C D	00108111
72-4B	**Life Cycle Invertebrate**	A B C D G H	00124763
72-5	**Life Cycle Fish**	A B C D G H	00108171
122-1A	**Seed Germination/Seedling Emergence**	B D G	40159301
122-2	**Aquatic Plant Growth**	B D G	40236901, 40236902, 40236903, 40236904, 40236905
123-2	**Aquatic Plant Growth**	B D G	40236901, 40236902, 40236903, 40236904, 40236905
141-1	**Honey Bee Acute Contact**	A B G H	00026489
TOXICOLOGY			
81-1	**Acute Oral Toxicity - Rat**	A B C D F G H	00067039, 41400601
81-2	**Acute Dermal Toxicity - Rabbit/Rat**	A B C D F G H	00067039, 41400602
81-4	**Primary Eye Irritation - Rabbit**		41400603, 41400604
81-6	**Dermal Sensitization - Guinea Pig**		00137137, 00137138, 00137139, 00137140
82-1A	**90-Day Feeding - Rodent**		00036803, 40559401
82-2	**21-Day Dermal - Rabbit/Rat**	A B C D F G H	00098460
83-1A	**Chronic Feeding Toxicity - Rodent**	A C D F H	00098460, 00093879

Data Supporting Guideline Requirements for the Reregistration of Glyphosate

REQUIREMENT		USE PATTERN	CITATION(S)
83-1B	**Chronic Feeding Toxicity - Non-Rodent**	A C D F H	00162912, 41728701, 00153374
83-2A	**Oncogenicity - Rat**	A C D F H	41728701, 41643801, 00093879
83-2B	**Oncogenicity - Mouse**	A C D F H	00130406, 00150564
83-3A	**Developmental Toxicity - Rat**	A B C D F G H	00046362
83-3B	**Developmental Toxicity - Rabbit**	A B C D F G H	00046363
83-4	**2-Generation Reproduction - Rat**	A C D H	00081674, 00105995, 41621501
84-2A	**Gene Mutation (Ames Test)**	A B C D F G H	00078620, 00132683
84-2B	**Structural Chromosomal Aberration**	A B C D F G H	00046364, 00132681, 00132685
84-4	**Other Genotoxic Effects**	A B C D F G H	00078619, 00132686, 00132685
85-1	**General Metabolism**	A C D F G H	40767101, 40767102
ENVIRONMENTAL FATE			
161-1	**Hydrolysis**	A B C D F G H	00108192
161-2	**Photodegradation - Water**	A B C D G	41689101
161-3	**Photodegradation - Soil**	A G	41335101
162-1	**Aerobic Soil Metabolism**	A B F G H	42372501
162-3	**Anaerobic Aquatic Metabolism**	C D	42372502
162-4	**Aerobic Aquatic Metabolism**	C D	42372503
163-1	**Leaching/Adsorption/ Desorption**	A B C D	00108192

Data Supporting Guideline Requirements for the Reregistration of Glyphosate

REQUIREMENT		USE PATTERN	CITATION(S)
164-1	**Terrestrial Field Dissipation**	A B H	42765001
164-2	**Aquatic Field Dissipation**	C D	42383201
164-3	**Forest Field Dissipation**	G	41552801
165-1	**Confined Rotational Crop**	A C	42372504, 41543201, 41543202
165-3	**Accumulation - Irrigated Crops**	C D	42372505, 40541305
165-4	**Bioaccumulation in Fish**	A B C D G	41228301

RESIDUE CHEMISTRY REFERENCES ARE CONTAINED IN THE BODY OF THE RED UNDER SECTION III, B

Appendix C

Citations Considered to be Part of the Data Base
Supporting the Reregistration of Glyphosate

GUIDE TO APPENDIX C

1. **CONTENTS OF BIBLIOGRAPHY.** This bibliography contains citations of all studies considered relevant by EPA in arriving at the positions and conclusions stated elsewhere in the Reregistration Eligibility Document. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions. Selections from other sources including published literature, in those instances where they have been considered, are included.

2. **UNITS OF ENTRY.** The unit of entry in this bibliography is called a "study". In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review and can be described with a conventional bibliographic citation. The Agency has also attempted to unite basic documents and commentaries upon them, treating them as a single study.

3. **IDENTIFICATION OF ENTRIES.** The entries in this bibliography are sorted numerically by Master Record Identifier, or "MRID Number". This number is unique to the citation, and should be uses whenever a specific reference is required. It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted studies (see paragraph 4(d)(4) below for further explanation). In a few cases, entries added to the bibliography late in the review may be preceded by a nine character temporary identifying number is also to be used whenever specific reference is needed.

4. **FORM OF ENTRY.** In addition to the Master Record Identifier (MRID), each entry consists of a citation containing standard elements followed, in the case of material submitted to EPA, by a description of the earliest known submission. Bibliographic conventions used reflect the standard of the American National Standards Institute (ANSI), expanded to provide for certain special needs.

a. **Author.** Whenever the author could confidently be identified, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown a identifiable laboratory or testing facility as the author. When no author or laboratory could be identified, the Agency has shown the first submitter as the author.

b. **Document Date.** The date of the study is taken directly from the document. When the date is followed by a question mark, the bibliographer has deduced the date from the evidence contained in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c. **Title.** In some cases, it has been necessary for the Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. **Trailing Parentheses.** For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission Date. The date of the earliest known submission appears immediately following the word "received".

(2) Administrative Number. The next element immediately following the word "under" is the registration number, experimental use permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is the submitter. When authorship is de-faulted to the submitter, this element is omitted.

(4) Volume Identification (Accession Numbers). The final element in the trailing parentheses identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL", which stands for "Company Data Library". This accession number is in turn followed by an alphabetic

suffix which shows the relative position of the study within the volume.

00015759 Kahrs, R.A.; Cheung, M.W. (1979) Tank Mixes of Metolachlor (plus Linuron or Metribuzin plus Glyphosate--Soybeans; Tank M of Metolachlor (8E) plus Linuron or Metribuzin plus Paraquat Soybeans: No and Minimum Tillage Applications: Report No. AB 79029. Summary of studies 237821-B through 237821-Q. (Unpub- lished study received Mar 16, 1979 under 100-583; submitted Ciba-Geigy Corp., Greensboro, N.C.; CDL:237821-A)

00015760 Kincaid, L. (1979) Metolachlor + Glyphosate + Linuron; Dual Roundup 4E + Lorox 50W: AG-A No. 4763 I,II. (Unpublished stu including letter dated May 23, 1978 from J.D. Riggleman to R ert A. Kahrs, received Mar 16, 1979 under 100-583; prepared cooperation with E.I. du Pont de Nemours & Co., Inc. and ADC Laboratories, submitted by Ciba-Geigy Corp., Greensboro, N.C CDL:237821-B)

00015761 Schnappinger, M.G. (1979) Metolachlor + Glyphosate + Linuron Dual 8E + Roundup 4E + Lorox 50W: AG-A No. 4886 I,II. (Unpublished study including letter dated May 23, 1978 from Riggleman to Robert A. Kahrs, received Mar 16, 1979 under 100-583; prepared in cooperation with E.I. du Pont de Nemour Co., Inc. and ADC Laboratories, submitted by Ciba-Geigy Corp Greensboro, N.C.: CDL:237821-C)

00015762 Searcy, V.; Herman, D. (1979) Metolachlor + Glyphosate + Linuron; Dual 8E + Roundup 4E + Lorox 50W: AG-A No. 4893 I,I (Unpublished study including letter dated May 23, 1978 from Riggleman to Robert A. Kahrs, received Mar 16, 1979 under 100-583; prepared in cooperation with E.I. du Pont de Nemour Co., Inc. and ADC Laboratories, submitted by Ciba-Geigy Corp Greensboro, N.C.; CDL:237821-D)

00015763 Rose, W.; Worsham, D. (1979) Metolachlor + Glyphosate + Linu Dual 8E + Roundup 4E + Lorox 50W: AG-A No. 4956 I,II A. (Unp lished study including letter dated May 23, 1978 from J.D. R gleman to Robert A. Kahrs, received Mar 16, 1979 under 100-5 prepared in cooperation with Rocky Mount Experiment Station, Laboratories and E.I. du Pont de Nemours & Co., Inc., submit by Ciba-Geigy Corp., Greensboro, N.C.; CDL:237821-E)

00015764 Kincaid, L. (1979) Metolachlor (Dual(R) 8E); Glyphosate (Rou 4E); Metribuzin (Sencor 50W): AG-A No. 4765 I,II. (Unpublish study including letter dated May 23, 1978 from J.D. Rigglema Robert A. Kahrs, received Mar 16, 1979 under 100-583; prepar

in cooperation with ADC Laboratories and E.I. du Pont de Nem & Co., Inc., submitted by Ciba-Geigy Corp., Greensboro, N.C. CDL:237821-F)

00015765 Schnappinger, M.G. (1978) Metolachlor (Dual 8E); Glyphosate (Roundup 4E); Metribuzin (Sencor 50W): AG-A No. 4887 I,II. (Unpublished study including letter dated May 23, 1978 from Riggleman to Robert Kahrs, received Mar 16, 1979 under 100-5 prepared in cooperation with ADC Laboratories and E.I. du Po de Nemours Co., Inc., submitted by Ciba-Geigy Corp., Greensb N.C.; CDL:237821-G)

00015766 Searcy, S.; Herman, D. (1979) Metolachlor (Dual 8E); Glyphos (Roundup 4E); Metribuzin (Sencor 50W): AG-A No. 4895 I,II. (Unpublished study including letter dated May 23, 1978 from Riggleman to Robert A. Kahrs, received Mar 16, 1979 under 10 583; prepared in cooperation with ADC Laboratories and E.I. Pont de Nemours Co., Inc., submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:237821-H)

00015767 Rose, W.; Worsham, D. (1979) Metolachlor (Dual 8E); Glyphosa (Roundup 4E); Metribuzin (Sencor 50W): AG-A No. 4958 I,II A. (Unpublished study including letter dated May 23, 1978 from Riggleman to Robert A. Kahrs, received Mar 16, 1979 under 10 583; prepared in cooperation with ADC Laboratories and E.I. Pont de Nemours & Co., Inc., submitted by Ciba-Geigy Corp., Greensboro, N.C.; CDL:237821-I)

00023336 Monsanto Company (1974) Residues of Glyphosate, Atrazine and Simazine in or on Field Corn Grain, Sweet Corn and Corn Fora and Fodder following a Tank Mix, Pre-emergent, Minimum Till Application of Roundup, Atrazine and Simazine. (Unpublished study received Dec 19, 1977 under 524-308; CDL:232518-B)

00023512 Houseworth, L.D.; Schnappinger, H.G.; Slagowski, J.L.; et al (1979) Tank Mixes of Metolachlor (6E, 8E) plus Simazine and/ Atrazine plus Paraquat or Glyphosate--Corn: Summary of Resid Data: Report No. ABR-79105. (Unpublished study received Dec 1979 under 100-583; prepared in cooperation with Chevron Che ical Co. and others, submitted by Ciba-Geigy Corp., Greensbo N.C.; CDL:241647-A)

00024503 Monsanto Company (1974) Summary of Residue Data. (Unpublishe study received Jan 16, 1978 under 524-285; CDL:232680-B)

00027235 Monsanto Company (1979) Analytical Residue Method for N-Phosphonomethylglycine (Glyphosate) and Aminomethylphospho acid in Sugarcane, Bagasse, Raw Sugar and Molasses. (Unpubli study received Dec 28, 1979 under 524-332; CDL:099157-B)

00028852 Monsanto Company (1976) Glyphosate Residues in Peanuts follo Preemergent Treatment with Roundup Herbicide. (Unpublished s received Feb 22, 1980 under 524-308; CDL:099306-A)

00028853 Monsanto Company (19??) Analytical Residue Method for N-(Phosphonomethyl) glycine, Aminomethylphosphonic acid and N-Nitroso-N(phosphonomethyl) glycine in Peanuts. (Unpublishe study received Feb 22, 1980 under 524-308; CDL:099306-B)

00033954 Monsanto Company (1973) Summary and Conclusion: Residue Data (Unpublished study received Dec 30, 1975 under 524-308; CDL: 224062-A)

00036222 Monsanto Company (1974) Analytical Residue Method for N-Phosphonomethyl glycine and Aminomethylphosphonic acid in and Water. Method B dated Nov 21, 1974. (Unpublished study received Sep 25, 1975 under 6G1679; CDL:095356-A)

00036223 Monsanto Company (1974) Analytical Residue Method for N-Phosphonomethyl glycine and Aminomethylphosphonic acid in Forage and Grain. Method B dated Mar 1, 1974. (Unpublished s received Sep 25, 1975 under 6G1679; CDL:095356-B)

00036229 Kramer, R.M.; Beasley, R.K.; Steinmetz, J.R.; et al. (1975) Interim Report on CP 67573, Residue and Metabolism. Part 28: Determination of Residues of Glyphosate and Its Metabolite i Fish: Agricultural Research Report No. 378. (pp. 1-13 only; unpublished study received Sep 25, 1975 under 6G1679; submit by Monsanto Co., Washington, D.C.; CDL:095356-I)

00036231 Monsanto Company (1975) Analytical Residue Method for N-Phosphonomethylglycine and Aminomethylphosphonic acid in F Tissue. Method dated Sep 2, 1975. (Unpublished study receive Sep 25, 1975 under 6G1679; CDL:095356-K)

00036328 Fink, R. (1975) Final Report: One Generation Reproduction Study--Mallard Duck: Project No. 139-101. (Unpublished study received Sep 26, 1975 under 6G1679; prepared by Truslow Farm Inc., submitted by Monsanto Co., Washington, D.C.; CDL:09648

00037687 Monsanto Company (1976) Residues of Glyphosate, Alachlor and Cyanazine in or on Field Corn Forage, Fodder, and Grain following a Tank Mix, Pre-emergent, Minimum Till Application Roundup, Lasso and Blades. (Unpublished study received Apr 1 1979 under 524-285; CDL:238167-B)

00037688 Monsanto Company (1979) Analytical Residue Method for N-Phosphonomethyl Glycine, Aminomethylphosphonic acid and N-Nitrosoglyphosate in Field Corn Forage, Fodder and Grain. Method dated Jan 22, 1979. (Unpublished study received Apr 1 1979 under 524-285; CDL:238167-C)

00038770 Cowell, J.E.; Taylor, A.L.; Stranz, J.L.; et al. (1974) Fina Report on CP 67563, Residue and Metabolism: Part 21: Determination of CP 67573 and CP 50435 Residues in Grapes: Agricultural Research Report No. 337. Includes undated metho entitled: Roundup and metabolite residue analytical method. (Unpublished study received Oct 4, 1974 under 5fl560; submit by Monsanto Co., Washington, D.C.; CDL:094261-A)

00038771 Rueppel, M.L.; Suba, L.A.; Moran, S.J.; et al. (1974) Final Report on CP 67573, Residue and Metabolism: Part 20: The Metabolism of CP 67573 in Grape Plants: Agricultural Researc Report No. 335. (Unpublished study received Oct 4, 1974 unde 5F1560; submitted by Monsanto Co., Washington, D.C.; CDL:094261-B)

00038908 Beasley, R.K.; Daniels, R.J.; Lauer, R.; et al. (1974) Final Report on CP 67573, Residue and Metabolism--Part 17: Determination of Crop Residues in Corn, Wheat, Soybeans, Sma Grains, Soil and Water: Agricultural Research Report No. 325 (Unpublished study received Jan 31, 1977 under 524-308; submitted by Monsanto Co., Washington, D.C.; CDL:095787-B)

00038979 Cowell, J.E.; Taylor, A.L.; Stranz, J.L.; et al. (1974) Roun and Metabolite Residue Analytical Method. (Unpublished study ceived 1974 under 5G1561; submitted by Monsanto Co., Washing D.C.; CDL:094264-B)

00039141 Sutherland, M.L.; Marvel, J.T.; Banduhn, M.C.; et al. (1975) Summary of Metabolism Studies of Glyphosate in Citrus Plants (Unpublished study received Jan 26, 1976 under 524-308; submitted by Monsanto Co., Washington, D.C.; CDL:094958-B)

00039142 Beasley, R.K.; Kramer, R.M.; Carstarphen, B.A.; et al. (1975

Summary of Glyphosate (Roundup) Residue Studies in Citrus Fr and Processed Fractions. (Unpublished study received Jan 26, 1976 under 6G1734; submitted by Monsanto Co., Washington, D. CDL:095065-A)

00039377 Conkin, R.A.; Hannah, L.H.; Stewart, E.R. (1975) Residue Dat for Roundup on Rice and in Fish. (Unpublished study received 26, 1975 under 6H5106; submitted by Monsanto Co., Washington D.C.; CDL:094900-C)

00039381 Kramer, R.M.; Arras, D.D.; Beasley, R.K.; et al. (1975) Fina Report on CP 67573 Residue and Metabolism: Agricultural Rese Report No. 372. (Unpublished study received Sep 25, 1975 und 6G1679; prepared in cooperation with Washington State Univ. others, submitted by Monsanto Co., Washington, D.C.; CDL: 095355-A)

00040083 Monsanto Company (1975) Storage Stability of Field Residue Samples and Glyphosate-14C Treated Crops. (Unpublished study received Aug 13, 1975 under 5F1536; CDL:094866-A)

00040084 Monsanto Company (1975) Glyphosate Residues in Soybeans. (Un lished study received Aug 13, 1975 under 5F1536; CDL:094866-

00040085 Monsanto Company (1975) Glyphosate Residues in Corn. (Unpub- lished study received Aug 13, 1975 under 5F1536; CDL:094866-

00040086 Monsanto Company (1975) Glyphosate Residues in Wheat Grain. (Unpublished study received Aug 13, 1975 under 5F1536; CDL: 094866-D)

00040087 Monsanto Company (1975) Glyphosate Residues in Small Grains. (Unpublished study received Aug 13, 1975 under 5F1536; CDL: 094866-E)

00044422 Monsanto Company (19??) Summary and Conclusions: Roundup on Barley, Buckwheat, Oats, Rice, Rye and Sorghums. (Unpublishe study received on unknown date under 5G1523; CDL:094036-B)

00044423 Monsanto Company (1974) Analytical Residue Method for N-Phosphonomethyl glycine and Aminomethylphosphonic acid in Forage and Grain. Method dated Mar 1, 1974. (Unpublished stu received on unknown date under SG1523; CDL:094036-C)

00044426 Monsanto Company (1973) Roundup Metabolite in Various Grains

(Unpublished study received on unknown date under 5G1523, CD 094155-F)

00046362 Rodwell, D.E.; Tasker, E.J.; Blair, A.M.; et al. (1980) Teratology Study in Rats: IRDC No. 401-054. (Unpublished st including IRDC no. 999-021; received May 23, 1980 under 524- prepared by International Research and Development Corp., submitted by Monsanto Co., Washington, D.C.; CDL:242516-A)

00046363 Rodwell, D.E.; Tasker, E.J.; Blair, M.; et al. (1980) Terato Study in Rabbits: IRDC No. 401-056. (Unpublished study recei May 23, 1980 under 524-308; prepared by International Resear and Development Corp., submitted by Monsanto Co., Washington D.C.; CDL:242516-B)

00048284 Monsanto Company (1973) Residue Data. (Compilation; unpublis study received on unknown date under 524-EX-21; CDL:223373-E

00051980 Monsanto Company (1975) Residue Results. (Unpublished study ceived Jun 3, 1976 under 524-308; CDL:096177-D)

00051982 Monsanto Company (1976) Analytical Residue Method for N-Phosphonomethylglycine and Aminomethylphosphonic acid in G Coffee Beans. Method dated May 1, 1976. (Unpublished study received Jun 3, 1976 under 524-308; CDL:096177-F)

00051983 Malik, J.M.; Curtis, T.S.; Marvel, J.T. (1975) Final Report CP67573, Residue and Metabolism; Part 24: The Metabolism of 67573 in Coffee Plants: Agricultural Research Report No. 344 (Unpublished study received Jun 3, 1976 under 524-308; submi by Monsanto Co.xx Washington, D.C.; CDL:096177-I)

00053005 Beasley, R.K.; Steinmetz, J.R.; Taylor, A.L.; et al. (1977) lytical Residue Method for N-Phosphonomethyl glycine and Ami methylphosphonic acid in Forage Legumes and Grasses: Report MSL-0061. Method dated Jun 28, 1977. (Unpublished study rece Sep 16, 1980 under 524-308; submitted by Monsanto Co., Washington, D.C.; CDL:099625-B)

00059050 Interregional Research Project Number 4 (1978) Summary of Glyphosate Residues in Guava. (Unpublished study received No 19, 1980 under lE2443; CDL:099739-A)

00060103 Baszis, S.R.; Cowell, J.; Lottman, M.; et al. (1980) Glyphos Residues in Cotton following Topical Treatment with Roundup

Herbicide: Report No. MSL-1283. Final rept. Includes method dated Aug 12, 1980 entitled: Analytical residue method for N (Phosphonomethyl)glycine, Aminomethylphosphonic acid and N-N troso-N-(Phosphonomethyl)glycine in forages and grains. (Unp lished study received Nov 12, 1980 under 524-EX-54; submitte Monsanto Co., Washington, D.C.; CDL:099720-A)

00061555 Monsanto Company (1974) Residue Results. (Unpublished study ceived on unknown date under 524-EX-24; CDL:095345-J)

00061559 Monsanto Company (19??) Analytical Residue Method for N-Phosphonomethyl glycine (Glyphosate) and Aminomethylphosph acid in Sugarcane, Sugarcane Leaves, Bagasse, Sugar and Molasses, Irrigation Water and Soil. (Unpublished study rece Mar 11, 1976 under 524-308; CDL:095141-E)

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40502602 Kunstman, J. (1987) Glyphosate Residues in Corn Grain and Fodder Following Preharvest Applications with Roundup Herbicide: MSL-6638. Unpublished study prepared by Monsanto Co. 176 p.

40502604 Kunstman, J. (1987) Glyphosate Residues in Corn Grain Fractions Following Preharvest Applications to Corn with Roundup Herbicide: MSL-6917. Unpublished study prepared by Monsanto Co. 195 p.

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40541305 Kunstman, J. (1988) Volume 5: Irrigated Crops Study--Determinatic of Glyphosate Residues in Crops, Irrigation Water, Sediment, ar Soil following Treatment of Irrigation Source with Rodeo: Labo- ratory Project No. MSL-7633. Unpublished study prepared by Mor santo Agricultural Co. 203 p.

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Appendix D

List of Available Related Documents

The following is a list of available documents related to glyphosate. Its purpose is to provide a path to more detailed information if it is required. These accompanying documents are part of the Administrative Record for glyphosate and are included in the EPA's Office of Pesticide Programs Public Docket.

1. Health and Environmental Effects Science Chapters

2. Detailed Label Usage Information System (LUIS) Report

3. Glyphosate RED Fact Sheet (included in this RED)

4. PR Notice 91-2 (Included in this RED) Pertains to the Label Ingredient Statement

5. Complete Appendix A which details the use patterns subject to reregistration

Federal publications on glyphosate are available and may be purchased from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161.

1. Pesticide Fact Sheet (No. EPA-738-F-93-011) for Glyphosate

2. Registration Standard for Pesticide Products Containing Glyphosate as the Active Ingredient (The 1986 Registration Standard): NTIS Stock No. PB87-103214

Appendix E

Pesticide Reregistration Handbook

PESTICIDE REREGISTRATION HANDBOOK

HOW TO RESPOND TO THE
REREGISTRATION ELIGIBILITY DOCUMENT (RED)

OFFICE OF PESTICIDE PROGRAMS

ENVIRONMENTAL PROTECTION AGENCY

OCTOBER 1991

PRODUCT REREGISTRATION HANDBOOK

TABLE OF CONTENTS

PESTICIDE REREGISTRATION HANDBOOK

I. INTRODUCTION

A. Purpose and Content of this Handbook

This Handbook provides instructions to registrants on how to respond to the Reregistration Eligibility Document (hereafter referred to as the "RED") and how to reregister products.

Section I is this introduction.

Section II contains step-by-step instructions which must be followed by registrants responding to the RED.

Section III provides additional instructions on the format, content and other aspects of generic data, product specific data and labels/labeling which may be required to be submitted.

Detailed instructions are in the Appendix.

B. The Reregistration Eligibility Document (RED)

Under Section 4 of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended in 1988, EPA is required to reregister pesticides that were first registered before November 1, 1984. The RED describes in detail the subject chemical, its uses and its regulatory history; describes EPA's decision concerning the eligibility of the uses of the chemical for reregistration; and explains the scientific and regulatory bases for this decision. EPA's reviews[1] of the data by scientific discipline are available upon request. Appendices to the RED contain: (1) a Data Dall-In Notice which requires submission of generic and product specific data and which gives directions for responding, (2) a listing of existing studies that satisfy generic data requirements and (3) a bibliography of the generic studies EPA has reviewed.

C. The Reregistration Process

Reregistration involves a thorough review of the scientific data base underlying a pesticide's registration. The purpose of EPA's review is to reassess the potential hazards arising from the currently registered uses of the pesticide, to determine whether the data base is substantially complete or there is need for additional generic data, and to determine whether the pesticide is eligible for reregistration. This decision is issued as the RED.

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[1] EPA's science reviews and information on the registered uses considered for EPA's analyses may be obtained from: EPA, Freedom of Information, 401 M St., S.W., Washington, D.C. 20460.

If the RED declares that some or all uses of the chemical are eligible for reregistration, affected registrants must first respond within 90 days of receipt to the data call-in portion of the RED. Within 8 months of receiving the RED, registrants must submit or cite any data and labels/labeling required for each product. EPA has until 14 months after the RED is issued (i.e., 6 months after the registrants' 8 month deadline) to review the submission for each product and decide whether to reregister it based on the following criteria:

--whether all of the product specific data and labels/labeling are acceptable,

--whether all of the uses on the label/labeling are eligible,

--whether all of the active ingredients in the product are eligible, and

--if no List 1 toxic inert ingredient is contained in the product (a List 1 inert is permitted only if all data for it have been submitted and EPA determines that the inert does not pose any unreasonable adverse effects in that product).

Products which meet all of these criteria will be reregistered. Products which do not meet all of these criteria, but which have acceptable product specific data and labeling, will be processed as amendments in order to implement label changes required by the RED.

II. INSTRUCTIONS FOR RESPONDING

A. How and When to Respond

This section provides directions for submitting timely and adequate responses necessary to reregister products containing the active ingredient covered by the RED. Registrants must follow these steps exactly to avoid suspension of their products. All products containing the active ingredient in the RED [i.e., manufacturing use products, end use products and special local need (SLN or Section 24c) registrations] are subject to the requirements of the RED. Figure 1 summarizes how and when to respond to the RED. A step-by-step explanation follows.

Step 1. Are Expedited Label Changes Required? In some instances, EPA may conclude that certain changes to product labels/labeling must be implemented rapidly. If the RED requires expedited label/labeling changes, registrants must submit the items below by the deadline specified in the RED. If expedited label changes are not required, go to Step 2.

a. Application for Registration (EPA Form 8570-1). Complete

and sign the form. In Section II, insert the phrase "Expedited Amendment in Response to the Reregistration Eligibility Document for (insert case name for chemical)." Applications for expedited label changes will be processed as applications for amended registration. Use only an original application form with a red identifier number in the upper right-hand corner.

b. Five (5) copies of revised draft label and labeling. Refer to the RED for label/labeling changes and follow the instructions in Section III.C. and the Appendix of this Handbook for revising the label and labeling for each product.

Step 2. Are data required? If the RED requires generic or product specific data, you must follow the directions in the data call-in notice in the RED. All registrants must respond for all products within 90 days of receipt; products for which an adequate response is not received on time will be subject to suspension. No time extensions will be given for responding within 90 days.

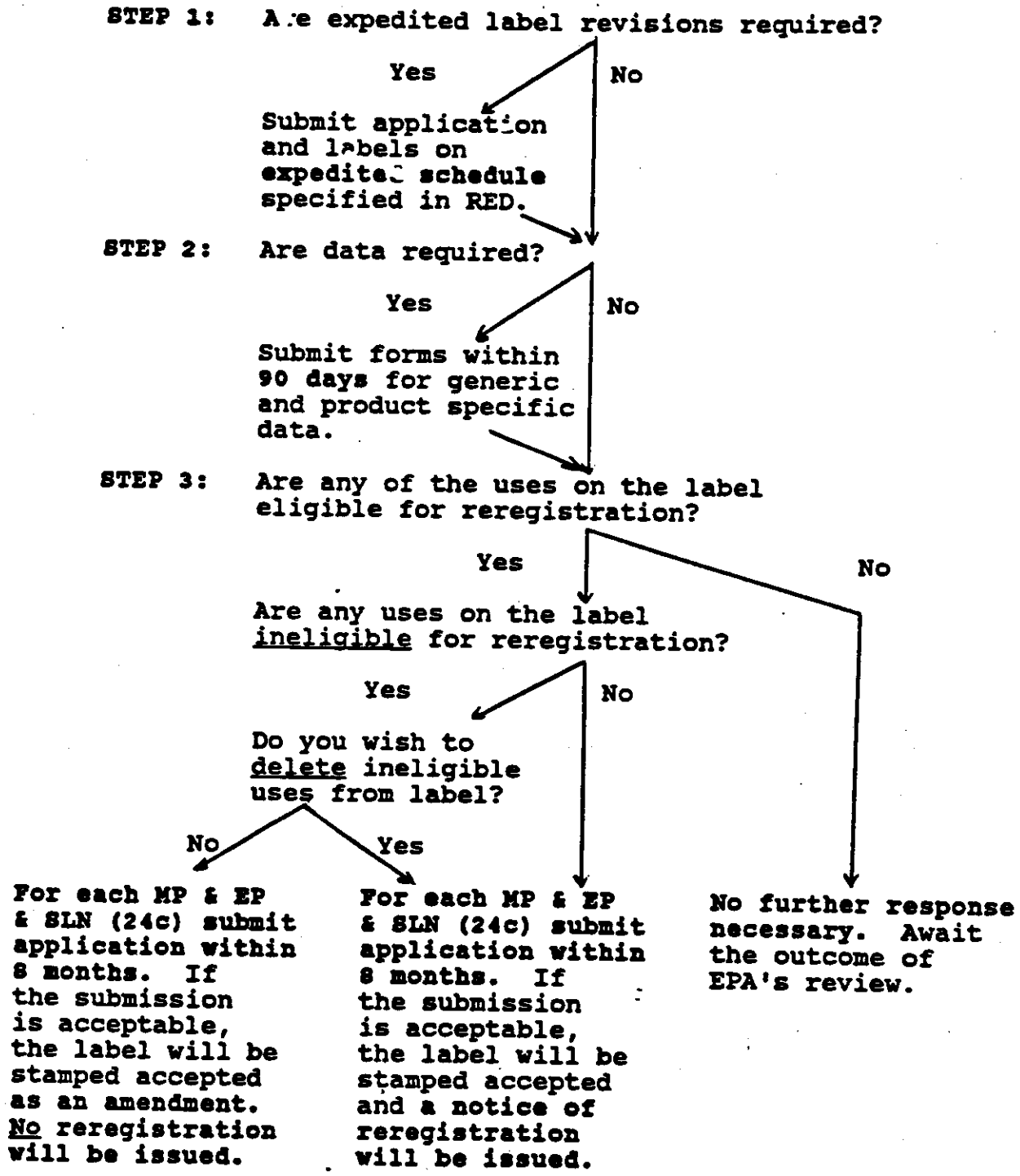
Step 3. Are Uses of a Pesticide Eligible for Reregistration? If any uses of the active ingredient(s) covered by the RED are eligible for reregistration, follow these instructions. If no uses are eligible, no further response may be needed (see page 5).

EPA's decision on the eligibility of each of the uses of the active ingredient(s) is presented in the RED. If any uses of a chemical are eligible for reregistration, registrants for manufacturing-use products (MPs), end-use products (EPs) and special local needs registrations (SLNs), must submit the items below for each product within 8 months of the date of issuance of the RED:

a. Application for Reregistration (use EPA Form 8570-1). Complete and sign the form. In Section II of that form, check the box "Other" and insert the phrase "Application for Reregistration." Use only an original application form with a red identifier number in the upper right-hand corner.

b. Five (5) copies of revised draft label and labeling. Refer to the RED for labeling changes specific to the active ingredient, follow the instructions in Section III.C. of this Handbook and refer to the Appendix of this Handbook for guidance on current requirements for labels and labeling. If there are ineligible uses on the label or labeling, you may delete such uses and avoid all requirements and consequences which may be associated with ineligible uses (e.g, generic data requirements, cancellation, suspension, etc.). If you delete certain uses now and those uses become eligible for reregistration later, you must submit an amendment application to add those uses back to the label.

FIGURE 1. HOW AND WHEN TO RESPOND TO THE REREGISTRATION ELIGIBILITY DOCUMENT (RED) FOR MANUFACTURING USE PRODUCTS (MPs), END-USE PRODUCTS (EPs) and SPECIAL LOCAL NEEDS REGISTRATIONS (SLNs).



c. Product Specific Data. You must follow the instructions in the Data Call-In Notice in the RED and in Section III of this Handbook. Responses to the data call in are due within 90 days of receipt of the RED and submission or citation of data is due within 8 months of the issuance of the RED.

d. Two (2) copies of the current Confidential Statement of Formula (EPA Form 8570-4, revised February 85). Two completed and signed CSF forms must be submitted for the basic formulation and for each alternate formulation. If CSFs are not provided for the alternate formulas, they will not be reregistered and will no longer be acceptable. The Appendix of this Handbook has specific instructions for completing the CSF form.

e. Certification With Respect to Citation of Data (EPA Form 8570-31). This form must be completed, signed and submitted for each product to assure that the data compensation provisions of FIFRA are met.

B. When No Response is Needed

If no uses of a pesticide are eligible for reregistration, it is unlikely that you will be required to submit product specific data or labeling. Uses of an active ingredient may be declared ineligible for reregistration for two possible reasons:

--Available data indicate that one or more of the criteria for an in-depth special review have been met;

--Additional generic data are required.

In the first instance, if the active ingredient is placed into special review, reregistration activities associated with those uses of the chemical are stopped until EPA makes a final determination. At that time, EPA will indicate which uses may be eligible for reregistration and which uses are to be cancelled. If some or all of the previously ineligible uses become eligible for reregistration, EPA will start the reregistration process for products containing only eligible uses.

In the second instance, based upon the review of studies for an active ingredient during reregistration, additional generic data (e.g., second- or third-tier studies) may be needed (see the RED). In such cases, the chemical's uses will not be eligible for reregistration until the additional generic data have been submitted to and reviewed and found acceptable by EPA. If the data are reviewed and found to be acceptable, EPA will indicate which uses will be eligible for reregistration and will initiate reregistration of products containing previously ineligible uses. If the data are not submitted, products containing the active ingredient may be suspended.

C. Where to Respond

By U.S. Mail:

Document Processing Desk (insert distribution code)
Office of Pesticide Programs (H7504C)
Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460-0001

By express mail or by hand delivery:

Document Processing Desk (insert distribution code)
Office of Pesticide Programs (H7504C)
Room 266A, Crystal Mall 2
1921 Jefferson Davis Highway
Arlington, VA 22202

These mailing addresses and the following distribution codes must be used to assure the timely receipt and processing of your submissions. Not using them may significantly delay the handling of your submissions:

RED-SRRD-xxx (where xxx is the case code given on the front of the RED)--use this distribution code for all responses pertaining to or containing generic data. Such responses include the 90-day response forms for generic data or hard copies of generic data.

RED-RD-PMxx (where xx is the Product Manager team number)--use this distribution code for all responses pertaining to or containing product specific data or labeling. Such responses would include expedited labeling amendments, 90-day responses to product specific data requirements, hard copies of product specific data and applications for reregistration.

III. SUBMISSION OF DATA AND LABELS/LABELING

This section provides additional instructions concerning responses required for generic data, product specific data and labels/labeling.

A. Generic Data

During EPA's evaluation of an active ingredient for reregistration, additional generic data requirements may be identified that registrants must fulfill. In some instances these data requirements would have to be satisfied before an active ingredient or some of its uses could be declared eligible for reregistration. In other cases, these new data requirements would not affect the eligibility of the active ingredient, but would be necessary to confirm EPA's assessment of that chemical.

Any new data requirements and how they affect reregistration eligibility of a chemical are discussed in the RED. If new generic data requirements are imposed in a Data Dall-In Notice in the RED, registrants must respond as described in that Notice. The RED also contains instructions for completing these forms, a citation of EPA's legal authority for requiring the new data, a listing of options available to registrants for satisfying the data requirements and the name of the contact person for inquiries.

B. Product Specific Data

Product specific data may be required for the reregistration of each pesticide product in three areas--product chemistry, acute toxicity and efficacy.

1. Product Chemistry

Following are instructions for submitting product-specific data and a discussion of EPA's policy on inert ingredients.

a. Data

All data requirements for MPs, EPs and SLNs (24c's) are specified in the Data Call-In Notice in the RED. In addition:

--If you cite data from another identical, registered product, you must identify the EPA registration number of that product.

--If the product-specific data submitted or cited do not pertain to an identical formulation to the product submitted for reregistration, then new product-specific data are required to be submitted by the deadline specified in the Data Call-In Notice. The only exception is for products which EPA "groups" together a being similar enough to depend on the same data. Such groupings are discussed in the appendix to the RED (for acute toxicity purposes, for example), if it was feasible to do so.

b. Inert Ingredients

EPA has implemented a strategy for regulating inert ingredients which affects the reregistration of pesticide products. This strategy, issued on April 22, 1987 (52 FR 13305-13309) and updated on November 22, 1989 (54 FR 48314-48316), adopted certain policies designed to reduce the potential for adverse effects from pesticide products containing intentionally added inert ingredients. EPA divided the known inert ingredients into four categories:

--Inerts of toxicological concern (List 1) for which available data demonstrate toxic effects of concern (includes about 50 chemicals).

--Potentially toxic inerts (List 2) for which only limited data are available, but such data or the chemical structure suggest the potential for toxicity (includes about 60 chemicals).

--Inerts of unknown toxicity (List 3) for which no data or bases for suspecting toxic effects are available (includes up to 2,000 chemicals).

--Inerts of minimal concern (List 4) which are generally regarded as innocuous (includes about 290 chemicals).

When a RED is issued and any uses of an active ingredient are declared eligible for reregistration, all products containing that active ingredient will be subject to reregistration. EPA will, as part of the reregistration review, examine the inert ingredients of each product prior to reregistration to ensure that they do not present unreasonable risks. In reviewing the product chemistry data, EPA will identify List 1 inerts. EPA will continue to encourage registrants to eliminate any List 1 inerts present. Reregistration of products containing only List 2, 3 or 4 inerts will be unaffected by the inerts strategy.

Consistent with the strategy on inerts, a product containing a List 1 inert ingredient will <u>not</u> be reregistered until a full risk assessment of the product has been conducted, based on the data called in for that inert ingredient. However, the existing registration of a product containing a List 1 inert will remain valid as long as the product bears the required label warning and is in compliance with any outstanding DCI, or other activity under the inerts strategy.

Any product containing a List 2, 3 or 4 inert <u>may</u> be reregistered <u>if</u> it meets all other requirements for reregistration. As the inerts strategy is implemented and data for the List 2 and 3 inerts are reviewed, EPA may move these inerts to the other Lists. If an inert were moved to List 1, products containing that inert would become ineligible for reregistration. Inert ingredients must also meet normal registration and tolerance requirements, as applicable.

2. <u>Acute Toxicity</u>

The data call-in notice in the RED specifies the acute toxicity data required for reregistration of each MP or EP. It indicates whether any of the standard tests have been waived and, if so, why.

If feasible, EPA will "batch" products that are similar with respect to their acute toxicity so that one set of tests can support reregistration of each baatch of products. This approach will impose the least amount of testing necessary to adequately support the registration and labeling for pesticide products. The

main benefits of this approach are to minimize the need for animal testing, reduce the expense to registrants to generate the tests and decrease the resources EPA must spend on reviewing data. Registrants may contact other registrants with products in the same "batch" to decide whether to provide or depend on one set of data; alternatively, registrants may choose to conduct their own studies.

3. Product Performance

Consult the Data Call-In section of the RED to determine whether Product Performance data are required for your product.

Product performance (efficacy) data are generated in studies designed to document how candidate pesticide formulations perform as pest control agents. These data include tests run to determine whether a formulation is lethal to certain pest species, to document the effectiveness of the formulation in controlling pest species in actual use situations, and to determine whether certain claims beyond mere control of a pest (e.g., "six-month residual effect," "kills Warfarin resistant house mice," etc.) are justified.

EPA has standard protocols for certain efficacy tests. In general, standard methods have been developed for tests needed to substantiate claims that have been made frequently for pesticide products. As the scope of potential pesticidal claims is extremely broad, the Agency does not have standard methods for tests needed to substantiate many pesticide claims, especially those that are uncommon. The Product Performance Guidelines, Subdivision G, offer general guidance for developing protocols for efficacy testing. Proposed protocols should be submitted to EPA for review before tests are initiated.

a. Efficacy Data Submission Waiver Policy

FIFRA gives the Administrator of EPA authority "to waive data requirements pertaining to efficacy" but does not require that efficacy data requirements be waived for any class of pesticide product registered under Section 3 of the Act. As a matter of policy, EPA does not require submission of efficacy data to support many types of pesticidal claims but does require submission of such data for certain types of claims. As noted in 40 CFR 158.640, this waiver applies to the submission of efficacy data rather than to the generation of efficacy data. EPA expects each registrant to "ensure through testing that his products are efficacious when used in accordance with commonly accepted pest control practices."

This general policy notwithstanding, EPA may, at any time, require a registrant to submit efficacy data to support any claim made for a product. EPA also may require that certain claims of effectiveness be established before a Section 3 registration is granted.

b. Claims and Products for Which Efficacy Data Generally Are Required

Submission of efficacy data at reregistration typically is required for the following types of products:

1. products claimed to control microorganisms that pose potential threats to public health;

2. products claimed to control vertebrate pests that may directly or indirectly transmit diseases to humans;

3. potentially very hazardous products for which EPA determines that it is necessary to conduct a "risk-benefits" analysis;

4. products of types for which EPA has reasons (e.g., consumer complaints, unlikely claims, unusual use patterns, etc.) to question claims; and

C. Labels and Labeling

To remain in compliance with FIFRA, the label and labeling of each product must be revised to meet the requirements for reregistration as described below. "Labeling" includes the container label and any written, printed or graphic matter that accompanies the pesticide in U.S. commerce at any time (such as technical bulletins, collateral labeling, etc.). Applications for new uses or labeling changes that do not pertain to reregistration must be filed separately from the application for reregistration described in Step 3 earlier. Changes to labeling which must be made for reregistration include, but are not limited to:

1. Labeling changes specified in the RED. Such changes may include statements on RESTRICTED USE, groundwater hazards, protective clothing/equipment, endangered species, environmental hazards, etc.

2. The format and content of labeling as described in 40 CFR 156.10. When further acute testing is needed, the currently accepted precautionary statements will usually be retained until testing is completed and the data are reviewed.

3. Labeling changes required by Pesticide Regulatory (PR) Notices, regulations, regulatory decisions and policies issued by EPA which are relevant to the pesticide. Your product's labeling must reflect any applicable requirements which are in effect at the time the RED is issued. Some existing notices are referred to in Section B. of the Appendix.

APPENDIX

A. Confidential Statement of Formula and Instructions

B. Instructions for Label Contents

C. Sample Label Formats--General Use & Restricted Use

D. Label Regulations (40 CFR 156.10)

Confidential Business Information: Does Not Contain National Security Information (E.O. 12065)

Form Approved. OMB No. 2070-0060. Approval expires 9-30-88

EPA

United States Environmental Protection Agency
Office of Pesticide Programs (TS-767)
Washington, DC 20460

Confidential Statement of Formula

A. ☐ Basic Formulation ☐ Alternate Formulation

B. Page of

See Instructions on Back

1. Name and Address of Applicant/Registrant *(Include ZIP Code)*

2. Name and Address of Producer *(Include ZIP Code)*

3. Product Name

4. Registration No./File Symbol

5. EPA Product Mgr./Team No.

6. Country Where Formulated

7. Pounds/Gal or Bulk Density

8. pH

9. Flash Point/Flame Extension

EPA USE ONLY	10. Components in Formulation *(List as actually introduced into the formulation. Give commonly accepted chemical name, trade name, and CAS number.)*	11. Supplier Name & Address	12. EPA Reg. No.	13. Each Component in Formulation a. Amount	b. % by Weight	14. Certified Limits % by Weight a. Upper Limit	b. Lower Limit	15. Purpose in Formulation

16. Typed Name of Approving Official

17. Total Weight 100%

18. Signature of Approving Official

19. Title

20. Phone No. *(Include Area Code)*

21. Date

EPA Form 8570-4 (Rev. 2-85) Previous editions are obsolete

Instructions for Completing the Confidential Statement of Formula

The Confidential Statement of Formula (CSF) Form 8570-4 must be used. Two legible, signed copies of the form are required. Following are basic instructions:

a. All the blocks on the form must be filled in and answered completely.

b. If any block is not applicable, mark it N/A.

c. The CSF must be signed, dated and the telephone number of the responsible party must be provided.

d. All applicable information which is on the product-specific data submission must also be reported on the CSF.

e. All weights reported under item 7 must be in pounds per gallon for liquids and pounds per cubic feet for solids.

f. Flashpoint must be in degrees Fahrenheit and flame extension in inches.

g. For all active ingredients, the EPA Registration Numbers for the currently registered source products must be reported under column 12.

h. The Chemical Abstracts Service (CAS) Numbers for all actives and inerts and all common names for the trade names must be reported.

i. For the active ingredients, the percent purity of the source products must be reported under column 10 and must be exactly the same as on the source product's label.

j. All the weights in columns 13.a. and 13.b. must be in pounds, kilograms, or grams. In no case will volumes be accepted. Do not mix English and metric system units (i.e., pounds and kilograms).

k. All the items under column 13.b. must total 100 percent.

l. All items under columns 14.a. and 14.b. for the active ingredients must represent pure active form.

m. The upper and lower certified limits for all active and inert ingredients must follow the 40 CFR 158.175 instructions. An explanation must be provided if the proposed limits are different than standard certified limits.

n. When new CSFs are submitted and approved, all previously submitted CSFs become obsolete for that specific formulation.

B. INSTRUCTIONS FOR LABEL CONTENTS

40 CFR 156.10 and Pesticide Regulatory (P.R.) Notices require that specific labeling statements appear at certain locations on the label. The sample label formats in Appendix C show where these statements are to be placed.

Item 1. PRODUCT NAME - The name, brand or trademark is required to be located on the front panel, preferably centered in the upper part of the panel. The name of a product will not be accepted if it is false or misleading. [40 CFR 156.10(b)]

Item 2. COMPANY NAME AND ADDRESS - The name and address of the producer, registrant or person for whom the product is produced are required on the label and should be located at the bottom of the front panel or at the end of the label text. [40 CFR 156.10(c)]

Item 3. NET CONTENTS - A net contents statement is required on all labels or on the container of the pesticide. The preferred location is the bottom of the front panel immediately above the company name and address, or at the end of the label text. The net contents must be expressed in the largest suitable unit, e.g., "1 pound 10 ounces" rather than "26 ounces." In addition to English units, net contents may be expressed in metric units. [40 CFR 156.10(d)]

Item 4. EPA REGISTRATION NUMBER - The registration number assigned to the pesticide product must appear on the label, preceded by the phrase "EPA Registration No.," or "EPA Reg. No." The registration number must be set in type of a size and style similar to other print on that part of the label on which it appears and must run parallel to it. The registration number and the required identifying phrase must not appear in such a manner as to suggest or imply recommendation or endorsement of the product by the Agency. [40 CFR 156.10(e)]

Item 5. EPA ESTABLISHMENT NUMBER - The EPA establishment number, preceded by the phrase "EPA Est." is the final establishment at which the product was produced, and may appear in any suitable location on the label or immediate container. It must also appear on the wrapper or outside container of the package if the EPA establishment number on the immediate container cannot be clearly read through such wrapper or container. [40 CFR 156.10(f)]

Item 6A. INGREDIENTS STATEMENT - An ingredients statement is normally required on the front panel. The ingredients statement must contain the name and percentage by weight of each active ingredient and the total percentage by weight of all inert ingredients. The preferred location is immediately below the product name. The ingredients statement must run parallel with, and be clearly distinguished from, other text on the panel. It must not be placed in the body of other text. [40 CFR 156.10(g)]

Item 6B. POUNDS PER GALLON STATEMENT - For liquid agricultural

formulations, the pounds per gallon of active ingredient must be indicated on the label. [40 CFR 156.10(h)(iv)]

Item 6C. NAMES TO BE USED IN INGREDIENT STATEMENT - The acceptable common name, if there is one, shall be used, followed by the chemical name. If no common name has been established, the chemical name alone shall be used. Chemicals related to the active ingredient are allowed to be listed only if efficacy data supporting such claims are submitted or referenced. If such data are provided, the related chemicals must be listed separately and not as a portion of the active ingredient.

Item 6D. INERT INGREDIENTS RECLASSIFIED AS ACTIVE INGREDIENTS - If EPA has reclassified chemicals from inert ingredient status to active ingredient status, registrants of affected products must change the ingredient statement accordingly (See 52 FR 13307-8, April 22, 1987). If such pesticides have food uses, tolerances must either be established for such uses, or an exemption from the requirement for tolerances must be obtained.

Item 6E. NOMINAL CONCENTRATION - The amount of active ingredient declared in the ingredient statement must be the nominal concentration of the product as defined in 40 CFR 158.153(i) and described in P.R. Notice 91-2.

Item 7. WARNINGS AND PRECAUTIONARY STATEMENTS - Front panel precautionary statements must be grouped together, preferably within a block outline. The table below shows the minimum type size requirements for various size labels.

Size of Label on Front Panel in Square Inches	Signal Word Minimum Type Size All Capitals	"Keep Out of Reach of Children" Minimum Type Size
5 and under	6 point	6 point
above 5 to 10	10 point	6 point
above 10 to 15	12 point	8 point
above 15 to 30	14 point	10 point
over 30	18 point	12 point

Item 7A. CHILD HAZARD WARNING STATEMENT - The statement "Keep Out of Reach of Children" must be located on the front panel above the signal word except where contact with children during distribution or use is unlikely. [40 CFR 156.10(h)(1)(ii)]

Item 7B. SIGNAL WORD - The signal word (DANGER, WARNING, or CAUTION) is required on the front panel immediately below the child hazard warning statement. [40 CFR 156.10(h)(1)(i)].

Item 7C. SKULL & CROSSBONES AND WORD "POISON" - On products assigned a toxicity Category I on the basis of oral, dermal, or inhalation toxicity, the word "Poison" shall appear on the label in red on a background of distinctly contrasting color and the skull and crossbones shall appear in immediate proximity to the word POISON. [40 CFR 156.10(h)(1)(i)].

Item 7D. STATEMENT OF PRACTICAL TREATMENT - A statement of practical treatment (first aid or other) shall appear on the label of pesticide products in toxicity Categories I, II, and III. [40 CFR 156.10(h)(1)(iii)]

Item 7E. REFERRAL STATEMENT - The statement "see Side (or Back) Panel for Additional Precautionary Statements" is required on the front panel for all products, unless all required precautionary statements appear on the front panel. [40 CFR 156.10(h)(1)(iii)].

Item 8. SIDE/BACK PANEL PRECAUTIONARY LABELING - The precautionary statements listed below must appear together on the label under the heading "PRECAUTIONARY STATEMENTS." The preferred location is at the top of the side or back panel preceding the directions for use, and it is preferred that these statements be surrounded by a block outline. Each of the three hazard warning statements must be headed by the appropriate hazard title. [40 CFR 156.10(h)(2)]

Item 8A. HAZARD TO HUMANS AND DOMESTIC ANIMALS - Where a hazard exists to humans or domestic animals, precautionary statements are required indicating the particular hazard, the route(s) of exposure and the precautions to be taken to avoid accident, injury or damage. [40 CFR 156.10(h)(2)(i)]

Item 8B. ENVIRONMENTAL HAZARD - Where a hazard exists to non-target organisms excluding humans and domestic animals, precautionary statements are required stating the nature of the hazard and the appropriate precautions to avoid potential accident, injury, or damage. [40 CFR 156.10(h)(2)(ii)]

Item 8C. PHYSICAL OR CHEMICAL HAZARD - FLAMMABILITY Precautionary statements relating to flammability of a product are required to appear on the label if it meets the criteria in the PHYS/CHEM Labeling Appendix. The requirement is based on the results of the flashpoint determinations and flame extension tests required to be submitted for all products. These statements are to be located in the side/back panel precautionary statements section, preceded by the heading "Physical/Chemical Hazards." Note that no signal word is used in conjunction with the flammability statements.

Item 9A. RESTRICTED USE CLASSIFICATION - FIFRA sec. 3(d) requires that all pesticide formulations/uses be classified for either general or restricted use. Products classified for restricted use may be limited to use by certified applicators or persons under their direct supervision (or may be subject to other restrictions that may be imposed by regulation). If your product has been classified for restricted use, then these requirements apply:

1. **All uses restricted.** The following statements must be placed in a black box at the top of the front panel of the label and labeling:

   a. The statement "Restricted Use Pesticide" must appear at the top of the front panel of the label. The statement must be set in type of the same minimum size as required for human hazard signal word [see table in 40 CFR 156.10(h)(1)(iv)]. No statements of any kind may appear above this RUP statement.

   b. The reason for the the restricted use classification must appear below the RUP statement. The RED will prescribe this statement.

   c. A summary statement of the terms of restriction must appear directly below this reason statement on the front panel. If use is restricted to certified applicators, the following statement is required: "For retail sale to and use only by Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicator's Certification." The RED will specify what statement must be used.

2. **Some but not all uses restricted.** If the RED states that some uses are classified for restricted use, and some are unclassified, several courses of action are available:

   a. You may label the product for Restricted use. If you do so, you may include on the label uses that are unrestricted, but you may not distinguish them on the label as being unrestricted.

   b. You may delete all restricted uses from your label and submit draft labeling bearing only unrestricted uses.

   c. You may "split" your registration, i.e., register two separate products with identical formulations, one bearing only unrestricted uses, and the other bearing restricted uses. To do so, submit two applications for reregistration, each containing all forms and necessary labels. Both applications should be submitted simultaneously. Note that the products will be assigned separate registration numbers.

Item 9B. MISUSE STATEMENT - All products must bear the misuse statement, "It is a violation of Federal law to use this product in a manner inconsistent with its labeling." This statement appears at the beginning of the directions for use, directly beneath the heading of that section.

Item 10A. REENTRY STATEMENT - If a restricted entry interval (REI) has been established by the Agency, it must be included on the label. Additional worker protection statements may be required in

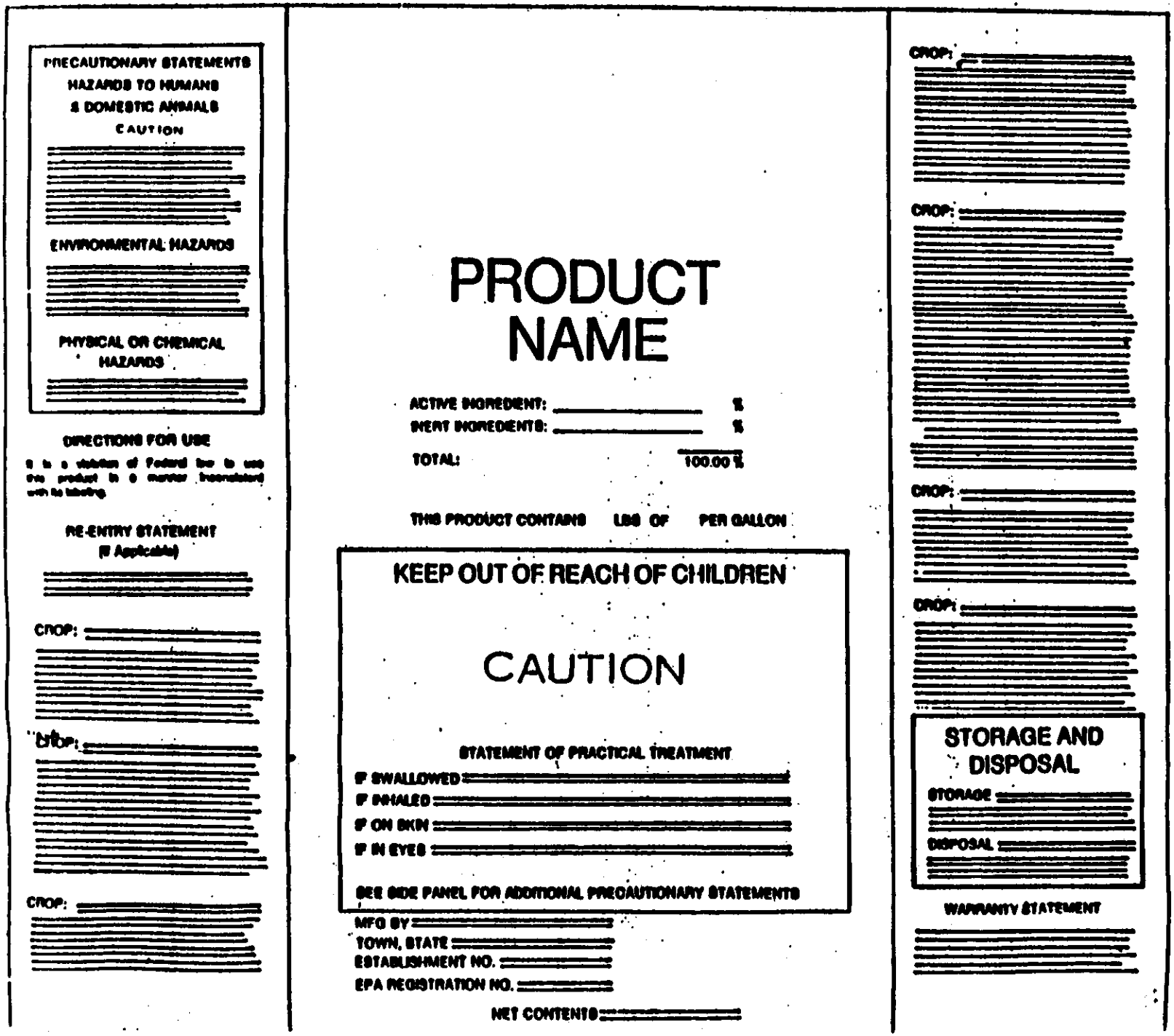
accordance with PR Notice 83-2, March 29, 1983.

Item 10B. STORAGE AND DISPOSAL BLOCK - All labels are required to bear storage and disposal statements. These statements are developed for specific containers, sizes, and chemical content. These instructions must be grouped and appear under the heading "Storage and Disposal" in the directions for use. This heading must be set in the same type sizes as required for the child hazard warning. Refer to P.R. Notices 83-3 and 84-1 to determine the storage and disposal instructions appropriate for your products.

Item 10C. DIRECTIONS FOR USE - Directions for use must be stated in terms which can be easily read and understood by the average person likely to use or to supervise the use of the pesticide. When followed, directions must be adequate to protect the public from fraud and from personal injury and to prevent unreasonable adverse effects on the environment. [40 CFR 156.10(i)(2)]

COLLATERAL LABELING

Bulletins, leaflets, circulars, brochures, data sheets, flyers, or other written or graphic printed matter which is referred to on the label or which is to accompany the product are termed collateral labeling. Such labeling may not bear claims or representations that differ in substance from those accepted in connection with registration of the product. Collateral labeling must be made part of the response to the RED and submitted for review.



LABEL FORMAT FOR UNCLASSIFIED PRODUCTS

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS & DOMESTIC ANIMALS

DANGER

ENVIRONMENTAL HAZARDS

PHYSICAL OR CHEMICAL HAZARDS

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

RE-ENTRY STATEMENT
(If Applicable)

STORAGE AND DISPOSAL

STORAGE

DISPOSAL

CROP:

RESTRICTED USE PESTICIDE

Due to (insert reason*)

FOR RETAIL SALE TO AND USE ONLY BY CERTIFIED APPLICATORS OR PERSONS UNDER THEIR DIRECT SUPERVISION AND ONLY FOR THOSE USES COVERED BY THE CERTIFIED APPLICATOR'S CERTIFICATION

(*for example, "Due to high acute toxicity.")

PRODUCT NAME

ACTIVE INGREDIENT: _____ %

INERT INGREDIENTS: _____ %

TOTAL: 100.00 %

THIS PRODUCT CONTAINS LBS OF PER GALLON

KEEP OUT OF REACH OF CHILDREN

DANGER—POISON

STATEMENT OF PRACTICAL TREATMENT

IF SWALLOWED

IF INHALED

IF ON SKIN

IF IN EYES

SEE SIDE PANEL FOR ADDITIONAL PRECAUTIONARY STATEMENTS

MFG BY

TOWN, STATE

ESTABLISHMENT NO.

EPA REGISTRATION NO.

NET CONTENTS

CROP:

CROP:

CROP:

CROP:

CROP:

WARRANTY STATEMENT

LABEL FORMAT FOR PRODUCTS CLASSIFIED FOR RESTRICTED USE

submitter has asserted a confidential business information claim concerning the material).

(5) A copy of each document, proposal, or other item of written material concerning the Registration Standard provided by the Agency to any person or party outside of government (within 15 working days after the item is made available to such person or party).

(6) A copy of the Registration Standard;

(7) With respect to a Registration Standard for which the Agency has determined that a substantially complete chronic health and teratology data base exists, a copy of the FEDERAL REGISTER notice concerning availability of a proposed Registration Standard, and a copy of each comment received in response to that notice (within 10 working days after receipt by the Agency, or 15 working days if the submitter has asserted a confidential business information claim concerning the material).

(8) A copy of the FEDERAL REGISTER notice announcing the issuance of the Registration Standard (within 10 working days after the publication of the notice).

(c) *Index of the docket.* The Agency will establish and keep current an index to the docket for each Registration Standard. The index will include, but is not limited to:

(1) A list of each meeting between the Agency and any person or party outside of government, containing the date and subject of the meeting, the names of participants and the name of the person requesting the meeting.

(2) A list of each document in the docket by title, source or recipient(s), and the date the document was received or provided by the Agency.

(d) *Availability of docket and indices.* (1) The Agency will make available to the public for inspection and copying the docket and index for any Registration Standard.

(2) The Agency will establish and maintain a mailing list of persons who have specifically requested that they receive indices for Registration Standard dockets. On a quarterly basis, EPA will distribute the indices of new materials placed in the public docket to these persons. Annually, EPA will require that persons on the list renew their requests for inclusion on the list.

(3) The Agency will issue annually in the FEDERAL REGISTER (in conjunction with the annual schedule notice specified in § 155.25) a notice announcing the availability of docket indices.

(4) Each FEDERAL REGISTER notice of availability of a Registration Standard will announce the availability of the docket index for that Standard.

§ 155.34 Notice of availability.

(a) The Agency will issue in the FEDERAL REGISTER a notice announcing the issuance and availability of Registration Standard which:

(1) Concerns a previously unregistered active ingredient; or

(2) Concerns a previously registered active ingredient, and the Registration Standard states that registrants will be required (under FIFRA section 3(c)(2)(B)) to submit chronic health (including, but not limited to, chronic feeding, oncogenicity and reproduction) or teratology studies.

(b) Interested persons may submit comments concerning any Registration Standard described by paragraph (a) of this section at any time.

(c) The Agency will issue in the FEDERAL REGISTER a notice announcing the availability of, and providing opportunity for comment on, each proposed Registration Standard which concerns a previously registered active ingredient for which the Agency has determined that a substantially complete chronic health and teratology data base exists. Following the comment period and issuance of the Registration Standard, the Agency will issue in the FEDERAL REGISTER a notice of availability of the Registration Standard.

PART 156—LABELING REQUIREMENTS FOR PESTICIDES AND DEVICES

AUTHORITY: 7 U.S.C. 136–136y.

§ 156.10 Labeling requirements.

(a) *General*—(1) *Contents of the label.* Every pesticide products shall bear a label containing the information specified by the Act and the regu-

lations in this Part. The contents of a label must show clearly and prominently the following:

(i) The name, brand, or trademark under which the product is sold as prescribed in paragraph (b) of this section;

(ii) The name and address of the producer, registrant, or person for whom produced as prescribed in paragraph (c) of this section;

(iii) The net contents as prescribed in paragraph (d) of this section;

(iv) The product registration number as prescribed in paragraph (e) of this section;

(v) The producing establishment number as prescribed in paragraph (f) of this section;

(vi) An ingredient statement as prescribed in paragraph (g) of this section;

(vii) Warning or precautionary statements as prescribed in paragraph (h) of this section;

(viii) The directions for use as prescribed in paragraph (i) of this section; and

(ix) The use classification(s) as prescribed in paragraph (j) of this section.

(2) *Prominence and legibility.* (i) All words, statements, graphic representations, designs or other information required on the labeling by the Act or the regulations in this part must be clearly legible to a person with normal vision, and must be placed with such conspicuousness (as compared with other words, statements, designs, or graphic matter on the labeling) and expressed in such terms as to render it likely to be read and understood by the ordinary individual under customary conditions of purchase and use.

(ii) All required label text must:

(A) Be set in 6-point or larger type;

(B) Appear on a clear contrasting background; and

(C) Not be obscured or crowded.

(3) *Language to be used.* All required label or labeling text shall appear in the English language. However, the Agency may require or the applicant may propose additional text in other languages as is considered necessary to protect the public. When additional text in another language is necessary, all labeling requirements will be applied equally to both the English and other-language versions of the labeling.

(4) *Placement of Label*—(i) *General.* The label shall appear on or be securely attached to the immediate container of the pesticide product. For purposes of this Section, and the misbranding provisions of the Act, "securely attached" shall mean that a label can reasonably be expected to remain affixed during the foreseeable conditions and period of use. If the immediate container is enclosed within a wrapper or outside container through which the label cannot be clearly read, the label must also be securely attached to such outside wrapper or container, if it is a part of the package as customarily distributed or sold.

(ii) *Tank cars and other bulk containers*—(A) *Transportation.* While a pesticide product is in transit, the appropriate provisions of 49 CFR Parts 170-189, concerning the transportation of hazardous materials, and specifically those provisions concerning the labeling, marking and placarding of hazardous materials and the vehicles carrying them, define the basic Federal requirements. In addition, when any registered pesticide product is transported in a tank car, tank truck or other mobile or portable bulk container, a copy of the accepted label must be attached to the shipping papers, and left with the consignee at the time of delivery.

(B) *Storage.* When pesticide products are stored in bulk containers, whether mobile or stationary, which remain in the custody of the user, a copy of the label of labeling, including all appropriate directions for use, shall be securely attached to the container in the immediate vicinity of the discharge control valve.

(5) *False or misleading statements.* Pursuant to section 2(q)(1)(A) of the Act, a pesticide or a device declared subject to the Act pursuant to § 153.240, is misbranded if its labeling is false or misleading in any particular including both pesticidal and non-pesticidal claims. Examples of statements or representations in the labeling which constitute misbranding include:

(i) A false or misleading statement concerning the composition of the product;

(ii) A false or misleading statement concerning the effectiveness of the product as a pesticide or device;

(iii) A false or misleading statement about the value of the product for purposes other than as a pesticide or device;

(iv) A false or misleading comparison with other pesticides or devices;

(v) Any statement directly or indirectly implying that the pesticide or device is recommended or endorsed by any agency of the Federal Government;

(vi) The name of a pesticide which contains two or more principal active ingredients if the name suggests one or more but not all such principal active ingredients even though the names of the other ingredients are stated elsewhere in the labeling;

(vii) A true statement used in such a way as to give a false or misleading impression to the purchaser;

(viii) Label disclaimers which negate or detract from labeling statements required under the Act and these regulations;

(ix) Claims as to the safety of the pesticide or its ingredients, including statements such as "safe," "nonpoisonous," "noninjurious," "harmless" or "nontoxic to humans and pets" with or without such a qualifying phrase as "when used as directed"; and

(x) Non-numerical and/or comparative statements on the safety of the product, including but not limited to:

(A) "Contains all natural ingredients";

(B) "Among the least toxic chemicals known"

(C) "Pollution approved"

(6) *Final printed labeling.* (i) Except as provided in paragraph (a)(6)(ii) of this section, final printed labeling must be submitted and accepted prior to registration. However, final printed labeling need not be submitted until draft label texts have been provisionally accepted by the Agency.

(ii) Clearly legible reproductions or photo reductions will be accepted for unusual labels such as those silk-screened directly onto glass or metal containers or large bag or drum labels. Such reproductions must be of microfilm reproduction quality.

(b) *Name, brand, or trademark.* (1) The name, brand, or trademark under which the pesticide product is sold shall appear on the front panel of the label.

(2) No name, brand, or trademark may appear on the label which:

(i) Is false or misleading, or

(ii) Has not been approved by the Administrator through registration or supplemental registration as an additional name pursuant to § 152.132.

(c) Name and address of producer, registrant, or person for whom produced. An unqualified name and address given on the label shall be considered as the name and address of the producer. If the registrant's name appears on the label and the registrant is not the producer, or if the name of the person for whom the pesticide was produced appears on the label, it must be qualified by appropriate wording such as "Packed for * * *," "Distributed by * * *," or "Sold by * * *" to show that the name is not that of the producer.

(d) *Net weight or measure of contents.* (1) The net weight or measure of content shall be exclusive of wrappers or other materials and shall be the average content unless explicitly stated as a minimum quantity.

(2) If the pesticide is a liquid, the net content statement shall be in terms of liquid measure at 68° F (20°C) and shall be expressed in conventional American units of fluid ounces, pints, quarts, and gallons.

(3) If the pesticide is solid or semi-solid, viscous or pressurized, or is a mixture of liquid and solid, the net content statement shall be in terms of weight expressed as avoirdupois pounds and ounces.

(4) In all cases, net content shall be stated in terms of the largest suitable units, i.e., "1 pound 10 ounces" rather than "26 ounces."

(5) In addition to the required units specified, net content may be expressed in metric units.

(6) Variation above minimum content or around an average is permissible only to the extent that it represents deviation unavoidable in good manufacturing practice. Variation below a stated minimum is not permitted. In no case shall the average con-

tent of the packages in a shipment fall below the stated average content.

(e) *Product registration number.* The registration number assigned to the pesticide product at the time of registration shall appear on the label, preceded by the phrase "EPA Registration No.," or the phrase "EPA Reg. No." The registration number shall be set in type of a size and style similar to other print on that part of the label on which it appears and shall run parallel to it. The registration number and the required identifying phrase shall not appear in such a manner as to suggest or imply recommendation or endorsement of the product by the Agency.

(f) *Producing establishments registration number.* The producing establishment registration number preceded by the phrase "EPA Est.", of the final establishment at which the product was produced may appear in any suitable location on the label or immediate container. It must appear on the wrapper or outside container of the package if the EPA establishment registration number on the immediate container cannot be clearly read through such wrapper or container.

(g) *Ingredient statement*—(1) *General.* The label of each pesticide product must bear a statement which contains the name and percentage by weight of each active ingredient, the total percentage by weight of all inert ingredients; and if the pesticide contains arsenic in any form, a statement of the percentages of total and water-soluble arsenic calculated as elemental arsenic. The active ingredients must be designated by the term "active ingredients" and the inert ingredients by the term "inert ingredients," or the singular forms of these terms when appropriate. Both terms shall be in the same type size, be aligned to the same margin and be equally prominent. The statement "Inert Ingredients, none" is not required for pesticides which contain 100 percent active ingredients. Unless the ingredient statement is a complete analysis of the pesticide, the term "analysis" shall not be used as a heading for the ingredient statement.

(2) *Position of ingredient statement.* (i) The ingredient statement is normally required on the front panel of the label. If there is an outside container or wrapper through which the ingredient statement cannot be clearly read, the ingredient statement must also appear on such outside container or wrapper. If the size or form of the package makes it impracticable to place the ingredient statement on the front panel of the label, permission may be granted for the ingredient statement to appear elsewhere.

(ii) The text of the ingredient statement must run parallel with other text on the panel on which it appears, and must be clearly distinguishable from and must not be placed in the body of other text.

(3) *Names to be used in ingredient statement.* The name used for each ingredient shall be the accepted common name, if there is one, followed by the chemical name. The common name may be used alone only if it is well known. If no common name has been established, the chemical name alone shall be used. In no case will the use of a trademark or proprietary name be permitted unless such name has been accepted as a common name by the Administrator under the authority of section 25(c)(6).

(4) *Statements of percentages.* The percentages of ingredients shall be stated in terms of weight-to-weight. The sum of percentages of the active and the inert ingredients shall be 100. Percentages shall not be expressed by a range of values such as "22-25%." If the uses of the pesticide product are expressed as weight of active ingredient per unit area, a statement of the weight of active ingredient per unit volume of the pesticide formulation shall also appear in the ingredient statement.

(5) *Accuracy of stated percentages.* The percentages given shall be as precise as possible reflecting good manufacturing practice. If there may be unavoidable variation between manufacturing batches, the value stated for each active ingredient shall be the lowest percentage which may be present.

(6) *Deterioration.* Pesticides which change in chemical composition significantly must meet the following labeling requirements:

(i) In cases where it is determined that a pesticide formulation changes chemical composition significantly, the product must bear the following statement in a prominent position on the label: "Not for sale or use after [date]."

(ii) The product must meet all label claims up to the expiration time indicated on the label.

(7) *Inert ingredients.* The Administrator may require the name of any inert ingredient(s) to be listed in the ingredient statement if he determines that such ingredient(s) may pose a hazard to man or the environment.

(h) *Warnings and precautionary statements.* Required warnings and precautionary statements concerning the general areas of toxicological hazard including hazard to children, environmental hazard, and physical or chemical hazard fall into two groups; those required on the front panel of the labeling and those which may appear elsewhere. Specific requirements concerning content, placement, type size, and prominence are given below.

(1) *Required front panel statements.* With the exception of the child hazard warning statement, the text required on the front panel of the label is determined by the Toxicity Category of the pesticide. The category is assigned on the basis of the highest hazard shown by any of the indicators in the table below:

Hazard indicators	Toxicity categories			
	I	II	III	IV
Oral LD_{50}	Up to and including 50 mg/kg.	From 50 thru 500 mg/kg.	From 500 thru 5000 mg/kg.	Greater than 5000 mg/kg.
Inhalation LC_{50}	Up to and including .2 mg/liter.	From .2 thru 2 mg/liter	From 2. thru 20 mg/liter	Greater than 20 mg/liter.
Dermal LD_{50}	Up to and including 200 mg/kg.	From 200 thru 2000	From 2,000 thru 20,000	Greater than 20,000.
Eye effects	Corrosive; corneal opacity not reversible within 7 days.	Corneal opacity reversible within 7 days; irritation persisting for 7 days.	No corneal opacity; irritation reversible within 7 days.	No irritation.
Skin effects	Corrosive	Severe irritation at 72 hours.	Moderate irritation at 72 hours.	Mild or slight irritation at 72 hours.

(i) *Human hazard signal word*—(A) *Toxicity Category I.* All pesticide products meeting the criteria of Toxicity Category I shall bear on the front panel the signal word "Danger." In addition if the product was assigned to Toxicity Category I on the basis of its oral, inhalation or dermal toxicity (as distinct from skin and eye local effects) the word "Poison" shall appear in red on a background of distinctly contrasting color and the skull and crossbones shall appear in immediate proximity to the word "poison."

(B) *Toxicity Category II.* All pesticide products meeting the criteria of Toxicity Category II shall bear on the front panel the signal word "Warning."

(C) *Toxicity Category III.* All pesticide products meeting the criteria of Toxicity Category III shall bear on the front panel the signal word "Caution."

(D) *Toxicity Category IV.* All pesticide products meeting the criteria of Toxicity Category IV shall bear on the front panel the signal word "Caution."

(E) *Use of signal words.* Use of any signal word(s) associated with a higher Toxicity Category is not permitted except when the Agency determines that such labeling is necessary to prevent unreasonable adverse effects on man or the environment. In no case shall more than one human hazard signal word appear on the front panel of a label.

(ii) *Child hazard warning.* Every pesticide product label shall bear on the front panel the statement "keep out of reach of children." Only in cases where the likelihood of contact with children during distribution, marketing, storage or use is demonstrated by the applicant to be extremely remote, or if the nature of the pesticide is such

that it is approved for use on infants or small children, may the Administrator waive this requirement.

(iii) *Statement of practical treatment*—(A) *Toxicity Category I.* A statement of practical treatment (first aid or other) shall appear on the front panel of the label of all pesticides falling into Toxicity Category I on the basis of oral, inhalation or dermal toxicity. The Agency may, however, permit reasonable variations in the placement of the statement of practical treatment is some reference such as "See statement of practical treatment on back panel" appears on the front panel near the word "Poison" and the skull and crossbones.

(B) *Other toxicity categories.* The statement of practical treatment is not required on the front panel except as described in paragraph (h)(1)(iii)(A) of this section. The applicant may, however, include such a front panel statement at his option. Statements of practical treatment are, however, required elsewhere on the label in accord with paragraph (h)(2) of this section if they do not appear on the front panel.

(iv) *Placement and prominence.* All the require front panel warning statements shall be grouped together on the label, and shall appear with sufficient prominence relative to other front panel text and graphic material to make them unlikely to be overlooked under customary conditions of purchase and use. The following table shows the minimum type size requirements for the front panel warning statements on various sizes of labels:

Size of label front panel in square inches	Points	
	Required signal word, all capitals	"Keep out of reach of children"
5 and under	6	6
Above 5 to 10	10	6
Above 10 to 15	12	8
Above 15 to 30	14	10
Over 30	18	12

(2) *Other required warnings and precautionary statements.* The warnings and precautionary statements as required below shall appear together on the label under the general heading "Precautionary Statements" and under appropriate subheadings of "Hazard to Humans and Domestic Animals," "Environmental Hazard" and "Physical or Chemical Hazard."

(i) *Hazard to humans and domestic animals.* (A) Where a hazard exists to humans or domestic animals, precautionary statements are required indicating the particular hazard, the route(s) of exposure and the precautions to be taken to avoid accident, injury or damage. The precautionary paragraph shall be immediately preceded by the appropriate hazard signal word.

(B) The following table depicts typical precautionary statements. These statements must be modified or expanded to reflect specific hazards.

Toxicity category	Precautionary statements by toxicity category	
	Oral, inhalation, or dermal toxicity	Skin and eye local effects
I	Fatal (poisonous) if swallowed [inhaled or absorbed through skin]. Do not breathe vapor [dust or spray mist]. Do not get in eyes, on skin, or on clothing [Front panel statement of practical treatment required.].	Corrosive, causes eye and skin damage [or skin irritation]. Do not get in eyes, on skin, or on clothing. Wear goggles or face shield and rubber gloves when handling. Harmful or fatal if swallowed. [Appropriate first aid statement required.]
II	May be fatal if swallowed [inhaled or absorbed through the skin]. Do not breathe vapors [dust or spray mist]. Do not get in eyes, on skin, or on clothing. [Appropriate first aid statements required.].	Causes eye [and skin] irritation. Do not get in eyes, on skin, or on clothing. Harmful if swallowed. [Appropriate first aid statement required.]
III	Harmful if swallowed [inhaled or absorbed through the skin]. Avoid breathing vapors [dust or spray mist]. Avoid contact with skin [eyes or clothing]. [Appropriate first aid statement required.].	Avoid contact with skin, eyes or clothing. In case of contact immediately flush eyes or skin with plenty of water. Get medical attention if irritation persists.
IV	[No precautionary statements required.]	[No precautionary statements required.]

(ii) *Environmental hazards.* Where a hazard exists to non target organisms excluding humans and domestic animals, precautionary statements are required stating the nature of the hazard and the appropriate precautions to avoid potential accident, injury or damage. Examples of the hazard statements and the circumstances under which they are required follow:

(A) If a pesticide intended for outdoor use contains an active ingredient with a mammalian acute oral LD_{50} of 100 or less, the statement "This Pesticide is Toxic to Wildlife" is required.

(B) If a pesticide intended for outdoor use contains an active ingredient with a fish acute LC_{50} of 1 ppm or less, the statement "This Pesticide is Toxic to Fish" is required.

(C) If a pesticide intended for outdoor use contains an active ingredient with an avian acute oral LD_{50} of 100 mg/kg or less, or a subacute dietary LC_{50} of 500 ppm or less, the statement "This Pesticide is Toxic to Wildlife" is required.

(D) If either accident history or field studies demonstrate that use of the pesticide may result in fatality to birds, fish or mammals, the statement "This pesticide is extremely toxic to wildlife (fish)" is required.

(E) For uses involving foliar application to agricultural crops, forests, or shade trees, or for mosquito abatement treatments, pesticides toxic to pollinating insects must bear appropriate label cautions.

(F) For all outdoor uses other than aquatic applications the label must bear the caution "Keep out of lakes, ponds or streams. Do not contaminate water by cleaning of equipment or disposal of wastes."

(iii) *Physical or chemical hazards.* Warning statements on the flammability or explosive characteristics of the pesticide are required as follows:

Flash point	Required text
(A) Pressurized Containers	
Flash point at or below 20° F; if there is a flashback at any valve opening.	Extremely flammable. Contents under pressure. Keep away from fire, sparks, and heated surfaces. Do not puncture or incinerate container. Exposure to temperatures above 130° F may cause bursting.
Flash point above 20° F and not over 80° F or if the flame extension is more than 18 in long at a distance of 6 in from the flame.	Flammable. Contents under pressure. Keep away from heat, sparks, and open flame. Do not puncture or incinerate container. Exposure to temperatures above 130° F may cause bursting.
All other pressurized containers	Contents under pressure. Do not use or store near heat or open flame. Do not puncture or incinerate container. Exposure to temperatures above 130° F may cause bursting.
(B) Nonpressurized Containers	
At or below 20° F	Extremely flammable. Keep away from fire, sparks, and heated surfaces.
Above 20° F and not over 80° F	Flammable. Keep away from heat and open flame.
Above 80° F and not over 150° F	Do not use or store near heat or open flame.

(i) *Directions for Use—(1) General requirements—(i) Adequacy and clarity of directions.* Directions for use must be stated in terms which can be easily read and understood by the average person likely to use or to supervise the use of the pesticide. When followed, directions must be adequate to protect the public from fraud and from personal injury and to prevent unreasonable adverse effects on the environment.

(ii) *Placement of directions for use.* Directions may appear on any portion of the label provided that they are conspicuous enough to be easily read by the user of the pesticide product. Directions for use may appear on printed or graphic matter which accompanies the pesticide provided that:

(A) If required by the Agency, such printed or graphic matter is securely attached to each package of the pesticide, or placed within the outside wrapper or bag;

(B) The label bears a reference to the directions for use in accompanying leaflets or circulars, such as "See directions in the enclosed circular:" and

(C) The Administrator determines that it is not necessary for such directions to appear on the label.

(iii) *Exceptions to requirement for direction for use*—(A) Detailed directions for use may be omitted from labeling of pesticides which are intended for use only by manufacturers of products other than pesticide products in their regular manufacturing processes, provided that:

(*1*) The label clearly shows that the product is intended for use only in manufacturing processes and specifies the type(s) of products involved.

(*2*) Adequate information such as technical data sheets or bulletins, is available to the trade specifying the type of product involved and its proper use in manufacturing processes;

(*3*) The product will not come into the hands of the general public except after incorporation into finished products; and

(*4*) The Administrator determines that such directions are not necessary to prevent unreasonable adverse effects on man or the environment.

(B) Detailed directions for use may be omitted from the labeling of pesticide products for which sale is limited to physicians, veterinarians, or druggists, provided that:

(*1*) The label clearly states that the product is for use only by physicians or veterinarians;

(*2*) The Administrator determines that such directions are not necessary to prevent unreasonable adverse effects on man or the environment; and

(*3*) The product is also a drug and regulated under the provisions of the Federal Food, Drug and Cosmetic Act.

(C) Detailed directions for use may be omitted from the labeling of pesticide products which are intended for use only by formulators in preparing pesticides for sale to the public, provided that:

(*1*) There is information readily available to the formulators on the composition, toxicity, methods of use, applicable restrictions or limitations, and effectiveness of the product for pesticide purposes;

(*2*) The label clearly states that the product is intended for use only in manufacturing, formulating, mixing, or repacking for use as a pesticide and specifies the type(s) of pesticide products involved;

(*3*) The product as finally manufactured, formulated, mixed, or repackaged is registered; and

(*4*) The Administrator determines that such directions are not necessary to prevent unreasonable adverse effects on man or the environment.

(2) *Contents of Directions for Use.* The directions for use shall include the following, under the headings "Directions for Use":

(i) The statement of use classification as prescribed in paragraph (j) of this section immediately under the heading "Directions for Use."

(ii) Immediately below the statement of use classification, the statement "It is a violation of Federal law to use this product in a manner inconsistent with its labeling."

(iii) The site(s) of application, as for example the crops, animals, areas, or objects to be treated.

(iv) The target pest(s) associated with each site.

(v) The dosage rate associated with each site and pest.

(vi) The method of application, including instructions for dilution, if required, and type(s) of application apparatus or equipment required.

(vii) The frequency and timing of applications necessary to obtain effective results without causing unreasonable adverse effects on the environment.

(viii) Specific limitations on reentry to areas where the pesticide has been applied, meeting the requirements concerning reentry provided by 40 CFR Part 170.

(ix) Specific directions concerning the storage and disposal of the pesticide and its container, meeting the requirements of 40 CFR Part 165. These instructions shall be grouped and appear under the heading "Storage and Disposal." This heading must be set in type of the same minimum sizes as required for the child hazard warning. (See Table in § 162.10(h)(1)(iv))

(x) Any limitations or restrictions on use required to prevent unreasonable adverse effects, such as:

(A) Required intervals between application and harvest of food or feed crops.

(B) Rotational crop restrictions.

(C) Warnings as required against use on certain crops, animals, objects, or in or adjacent to certain areas.

(D) [Reserved]

(E) For restricted use pesticides, a statement that the pesticide may be applied under the direct supervision of a certified applicator who is not physically present at the site of application but nonetheless available to the person applying the pesticide, unless the Agency has determined that the pesticide may only be applied under the direct supervision of a certified applicator who is physically present.

(F) Other pertinent information which the Administrator determines to be necessary for the protection of man and the environment.

(j) *Statement of Use Classification.* By October 22, 1976, all pesticide products must bear on their labels a statement of use classification as described in paragraphs (j) (1) and (2) of this section. Any pesticide product for which some uses are classified for general use and others for restricted use shall be separately labeled according to the labeling standards set forth in this subsection, and shall be marketed as separate products with different registration numbers, one bearing directions only for general use(s) and the other bearing directions for restricted use(s) except that, if a product has both restricted use(s) and general use(s), both of these uses may appear on a product labeled for restricted use. Such products shall be subject to the provisions of paragraph (j)(2) of this section.

(1) *General Use Classification.* Pesticide products bearing directions for use(s) classified general shall be labeled with the exact words "General Classification" immediately below the heading "Directions for Use." And reference to the general classification that suggests or implies that the general utility of the pesticide extends beyond those purposes and uses contained in the Directions for Use will be considered a false or misleading statement under the statutory definitions of misbranding.

(2) *Restricted Use Classification.* Pesticide products bearing direction for use(s) classified restricted shall bear statements of restricted use classification on the front panel as described below:

(i) *Front panel statement of restricted use classification.* (A) At the top of the front panel of the label, set in type of the same minimum sizes as required for human hazard signal words (see table in paragraph (h)(1)(iv) of this section), and appearing with sufficient prominence relative to other text and graphic material on the front panel to make it unlikely to be overlooked under customary conditions of purchase and use, the statement "Restricted Use Pesticide" shall appear.

(B) Directly below this statement on the front panel, a summary statement of the terms of restriction imposed as a precondition to registration shall appear. If use is restricted to certified applicators, the following statement is required: "For retail sale to and use only by Certified Applicators or persons under their direct supervision and only for those uses covered by the Certified Applicator's certification." If, however, other regulatory restrictions are imposed, the Administrator will define the appropriate wording for the terms of restriction by regulation.

[40 FR 28268, July 3, 1975; 40 FR 32329, Aug. 1, 1975; 40 FR 36571, Aug. 21, 1975, as amended at 43 FR 5786, Feb. 9, 1978. Redesignated and amended at 53 FR 15991, 15999, May 4, 1988]

Appendix F

Generic and Product-Specific Data Call-In

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

GENERIC AND PRODUCT SPECIFIC
DATA CALL-IN NOTICE

FEB 16 1994

CERTIFIED MAIL

Dear Sir or Madam:

This Notice requires you and other registrants of pesticide products containing the active ingredient identified in Attachment A of this Notice, the Data Call-In Chemical Status Sheet, to submit certain data as noted herein to the U.S. Environmental Protection Agency (EPA, the Agency). These data are necessary to maintain the continued registration of your product(s) containing this active ingredient. Within 90 days after you receive this Notice you must respond as set forth in Section III below. Your response must state:

1. How you will comply with the requirements set forth in this Notice and its Attachments 1 through 7; or

2. Why you believe you are exempt from the requirements listed in this Notice and in Attachment 3 (for both generic and product specific data), the Requirements Status and Registrant's Response Form, (see section III-B); or

3. Why you believe EPA should not require your submission of data in the manner specified by this Notice (see section III-D).

If you do not respond to this Notice, or if you do not satisfy EPA that you will comply with its requirements or should be exempt or excused from doing so, then the registration of your product(s) subject to this Notice will be subject to suspension. We have provided a list of all of your products subject to this Notice in Attachment 2. All products are listed on both the generic and product specific Data Call-In Response Forms. Also included is a list of all registrants who were sent this Notice (Attachment 6).

The authority for this Notice is section 3(c)(2)(B) of the Federal Insecticide, Fungicide and Rodenticide Act as amended (FIFRA), 7 U.S.C. section 136a(c)(2)(B). Collection of this

information is authorized under the Paperwork Reduction Act by OMB Approval No. 2070-0107 and 2070-0057 (expiration date 3-31-96).

This Notice is divided into six sections and seven Attachments. The Notice itself contains information and instructions applicable to all Data Call-In Notices. The Attachments contain specific chemical information and instructions. The six sections of the Notice are:

Section I	-	Why You are Receiving this Notice
Section II	-	Data Required by this Notice
Section III	-	Compliance with Requirements of this Notice
Section IV	-	Consequences of Failure to Comply with this Notice
Section V	-	Registrants' Obligation to Report Possible Unreasonable Adverse Effects
Section VI	-	Inquiries and Responses to this Notice

The Attachments to this Notice are:

1 - Data Call-In Chemical Status Sheet
2 - Generic Data Call-In and Product Specific Data Call-In Response Forms with Instructions
3 - Generic Data Call-In and Product Specific Data Call-In Requirements Status and Registrant's Response Forms with Instructions
4 - EPA Grouping of End-Use Products for Meeting Acute Toxicology Data Requirements for Reregistration
5 - EPA Acceptance Criteria
6 - List of Registrants Receiving This Notice
7 - Cost Share and Data Compensation Forms

SECTION I. WHY YOU ARE RECEIVING THIS NOTICE

The Agency has reviewed existing data for this active ingredient(s) and reevaluated the data needed to support continued registration of the subject active ingredient(s). This reevaluation identified additional data necessary to assess the health and safety of the continued use of products containing this active ingredient(s). You have been sent this Notice because you have product(s) containing the subject active ingredients.

SECTION II. DATA REQUIRED BY THIS NOTICE

II-A. DATA REQUIRED

The data required by this Notice are specified in the Requirements Status and Registrant's Response Forms: Attachment 3

(for both generic and product specific data requirements). Depending on the results of the studies required in this Notice, additional studies/testing may be required.

II-B. SCHEDULE FOR SUBMISSION OF DATA

You are required to submit the data or otherwise satisfy the data requirements specified in the Requirements Status and Registrant's Response Forms (Attachment 3) within the timeframes provided.

II-C. TESTING PROTOCOL

All studies required under this Notice must be conducted in accordance with test standards outlined in the Pesticide Assessment Guidelines for those studies for which guidelines have been established.

These EPA Guidelines are available from the National Technical Information Service (NTIS), Attn: Order Desk, 5285 Port Royal Road, Springfield, Va 22161 (Telephone number: 703-487-4650).

Protocols approved by the Organization for Economic Cooperation and Development (OECD) are also acceptable if the OECD recommended test standards conform to those specified in the Pesticide Data Requirements regulation (40 CFR § 158.70). When using the OECD protocols, they should be modified as appropriate so that the data generated by the study will satisfy the requirements of 40 CFR § 158. Normally, the Agency will not extend deadlines for complying with data requirements when the studies were not conducted in accordance with acceptable standards. The OECD protocols are available from OECD, 2001 L Street, N.W., Washington, D.C. 20036 (Telephone number 202-785-6323; Fax telephone number 202-785-0350).

All new studies and proposed protocols submitted in response to this Data Call-In Notice must be in accordance with Good Laboratory Practices [40 CFR Part 160].

II-D. REGISTRANTS RECEIVING PREVIOUS SECTION 3(c)(2)(B) NOTICES ISSUED BY THE AGENCY

Unless otherwise noted herein, this Data Call-In does not in any way supersede or change the requirements of any previous Data Call-In(s), or any other agreements entered into with the Agency pertaining to such prior Notice. Registrants must comply with the requirements of all Notices to avoid issuance of a Notice of Intent to Suspend their affected products.

SECTION III. COMPLIANCE WITH REQUIREMENTS OF THIS NOTICE

You must use the correct forms and instructions when completing your response to this Notice. The type of Data Call-In you must comply with (Generic or Product Specific) is specified in item number 3 on the four Data Call-In forms (Attachments 2 and 3).

III-A. SCHEDULE FOR RESPONDING TO THE AGENCY

The appropriate responses initially required by this Notice for generic and product specific data must be submitted to the Agency within 90 days after your receipt of this Notice. Failure to adequately respond to this Notice within 90 days of your receipt will be a basis for issuing a Notice of Intent to Suspend (NOIS) affecting your products. This and other bases for issuance of NOIS due to failure to comply with this Notice are presented in Section IV-A and IV-B.

III-B. OPTIONS FOR RESPONDING TO THE AGENCY

1. Generic Data Requirements

The options for responding to this Notice for generic data requirements are: (a) voluntary cancellation, (b) delete use(s), (c) claim generic data exemption, (d) agree to satisfy the generic data requirements imposed by this Notice or (e) request a data waiver(s).

A discussion of how to respond if you choose the Voluntary Cancellation option, the Delete Use(s) option or the Generic Data Exemption option is presented below. A discussion of the various options available for satisfying the generic data requirements of this Notice is contained in Section III-C. A discussion of options relating to requests for data waivers is contained in Section III-D.

Two forms apply to generic data requirements, one or both of which must be used in responding to the Agency, depending upon your response. These two forms are the Data-Call-In Response Form, and the Requirements Status and Registrant's Response Form, (contained in Attachments 2 and 3, respectively).

The Data Call-In Response Forms must be submitted as part of every response to this Notice. The Requirements Status and Registrant's Response Forms also must be submitted if you do not qualify for a Generic Data Exemption or are not requesting voluntary cancellation of your registration(s). Please note that the company's authorized representative is required to sign the first page of both Data Call-In Response Forms and the Requirements Status and Registrant's Response Forms (if this form

is required) and initial any subsequent pages. The forms contain separate detailed instructions on the response options. Do not alter the printed material. If you have questions or need assistance in preparing your response, call or write the contact person(s) identified in Attachment 1.

a. Voluntary Cancellation -

You may avoid the requirements of this Notice by requesting voluntary cancellation of your product(s) containing the active ingredient that is the subject of this Notice. If you wish to voluntarily cancel your product, you must submit completed Generic and Product Specific Data Call-In Response Forms (Attachment 2), indicating your election of this option. Voluntary cancellation is item number 5 on both Data Call-In Response Form(s). If you choose this option, these are the only forms that you are required to complete.

If you chose to voluntarily cancel your product, further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provisions of this Notice, which are contained in Section IV-C.

b. Use Deletion -

You may avoid the requirements of this Notice by eliminating the uses of your product to which the requirements apply. If you wish to amend your registration to delete uses, you must submit the Requirements Status and Registrant's Response Form (Attachment 3), a completed application for amendment, a copy of your proposed amended labeling, and all other information required for processing the application. Use deletion is option number 7 under item 9 in the instructions for the Requirements Status and Registrant's Response Forms. You must also complete a Data Call-In Response Form by signing the certification, item number 8. Application forms for amending registrations may be obtained from the Registration Support Branch, Registration Division, Office of Pesticide Programs, EPA, by calling (703) 308-8358.

If you choose to delete the use(s) subject to this Notice or uses subject to specific data requirements, further sale, distribution, or use of your product after one year from the due date of your 90 day response, is allowed only if the product bears an amended label.

c. Generic Data Exemption -

Under section 3(c)(2)(D) of FIFRA, an applicant for registration of a product is exempt from the requirement to submit or cite generic data concerning an active ingredient if the active ingredient in the product is derived exclusively from

purchased, registered pesticide products containing the active ingredient. EPA has concluded, as an exercise of its discretion, that it normally will not suspend the registration of a product which would qualify and continue to qualify for the generic data exemption in section 3(c)(2)(D) of FIFRA. To qualify, all of the following requirements must be met:

(i). The active ingredient in your registered product must be present solely because of incorporation of another registered product which contains the subject active ingredient and is purchased from a source not connected with you;

(ii). Every registrant who is the ultimate source of the active ingredient in your product subject to this DCI must be in compliance with the requirements of this Notice and must remain in compliance; and

(iii). You must have provided to EPA an accurate and current "Confidential Statement of Formula" for each of your products to which this Notice applies.

To apply for the Generic Data Exemption you must submit a completed Data Call-In Response Form, Attachment 2 and all supporting documentation. The Generic Data Exemption is item number 6a on the Data Call-In Response Form. If you claim a generic data exemption you are not required to complete the Requirements Status and Registrant's Response Form. Generic Data Exemption cannot be selected as an option for responding to product specific data requirements.

If you are granted a Generic Data Exemption, you rely on the efforts of other persons to provide the Agency with the required data. If the registrant(s) who have committed to generate and submit the required data fail to take appropriate steps to meet requirements or are no longer in compliance with this Data Call-In Notice, the Agency will consider that both they and you are not compliance and will normally initiate proceedings to suspend the registrations of both your and their product(s), unless you commit to submit and do submit the required data within the specified time. In such cases the Agency generally will not grant a time extension for submitting the data.

d. Satisfying the Generic Data Requirements of this Notice

There are various options available to satisfy the generic data requirements of this Notice. These options are discussed in Section III-C.1. of this Notice and comprise options 1 through 6 of item 9 in the instructions for the Requirements Status and Registrant's Response Form and item 6b on the Data Call-In Response Form. If you choose item 6b (agree to satisfy the

generic data requirements), you must submit the Data Call-In Response Form and the Requirements Status and Registrant's Response Form as well as any other information/data pertaining to the option chosen to address the data requirement. Your response must be on the forms marked "GENERIC" in item number 3.

e. Request for Generic Data Waivers.

Waivers for generic data are discussed in Section III-D.1. of this Notice and are covered by options 8 and 9 of item 9 in the instructions for the Requirements Status and Registrant's Response Form. If you choose one of these options, you must submit both forms as well as any other information/data pertaining to the option chosen to address the data requirement.

2. Product Specific Data Requirements

The options for responding to this Notice for product specific data are: (a) voluntary cancellation, (b) agree to satisfy the product specific data requirements imposed by this Notice or (c) request a data waiver(s).

A discussion of how to respond if you choose the Voluntary Cancellation option is presented below. A discussion of the various options available for satisfying the product specific data requirements of this Notice is contained in Section III-C.2. A discussion of options relating to requests for data waivers is contained in Section III-D.2.

Two forms apply to the product specific data requirements one or both of which must be used in responding to the Agency, depending upon your response. These forms are the Data-Call-In Response Form, and the Requirements Status and Registrant's Response Form, for product specific data (contained in Attachments 2 and 3, respectively). The Data Call-In Response Form must be submitted as part of every response to this Notice. In addition, one copy of the Requirements Status and Registrant's Response Form also must be submitted for each product listed on the Data Call-In Response Form unless the voluntary cancellation option is selected. Please note that the company's authorized representative is required to sign the first page of the Data Call-In Response Form and Requirements Status and Registrant's Response Form (if this form is required) and initial any subsequent pages. The forms contain separate detailed instructions on the response options. Do not alter the printed material. If you have questions or need assistance in preparing your response, call or write the contact person(s) identified in Attachment 1.

a. Voluntary Cancellation

You may avoid the requirements of this Notice by requesting voluntary cancellation of your product(s) containing the active ingredient that is the subject of this Notice. If you wish to voluntarily cancel your product, you must submit a completed Data Call-In Response Form, indicating your election of this option. Voluntary cancellation is item number 5 on both the Generic and Product Specific Data Call-In Response Forms. If you choose this option, you must complete both Data Call-In response forms. These are the only forms that you are required to complete.

If you choose to voluntarily cancel your product, further sale and distribution of your product after the effective date of cancellation must be in accordance with the Existing Stocks provisions of this Notice which are contained in Section IV-C.

b. Satisfying the Product Specific Data Requirements of this Notice.

There are various options available to satisfy the product specific data requirements of this Notice. These options are discussed in Section III-C.2. of this Notice and comprise options 1 through 6 of item 9 in the instructions for the product specific Requirements Status and Registrant's Response Form and item numbers 7a and 7b (agree to satisfy the product specific data requirements for an MUP or EUP as applicable) on the product specific Data Call-In Response Form. Note that the options available for addressing product specific data requirements differ slightly from those options for fulfilling generic data requirements. Deletion of a use(s) and the low volume/minor use option are not valid options for fulfilling product specific data requirements. It is important to ensure that you are using the correct forms and instructions when completing your response to the Reregistration Eligibility Decision document.

c. Request for Product Specific Data Waivers.

Waivers for product specific data are discussed in Section III-D.2. of this Notice and are covered by option 7 of item 9 in the instructions for the Requirements Status and Registrant's Response Form. If you choose this option, you must submit the Data Call-In Response Form and the Requirements Status and Registrant's Response Form as well as any other information/data pertaining to the option chosen to address the data requirement. Your response must be on the forms marked "PRODUCT SPECIFIC" in item number 3.

III-C SATISFYING THE DATA REQUIREMENTS OF THIS NOTICE

1. Generic Data

If you acknowledge on the Generic Data Call-In Response Form that you agree to satisfy the generic data requirements (i.e. you select item number 6b), then you must select one of the six options on the Generic Requirements Status and Registrant's Response Form related to data production for each data requirement. Your option selection should be entered under item number 9, "Registrant Response." The six options related to data production are the first six options discussed under item 9 in the instructions for completing the Requirements Status and Registrant's Response Form. These six options are listed immediately below with information in parentheses to guide you to additional instructions provided in this Section. The options are:

(1) I will generate and submit data within the specified timeframe (Developing Data)
(2) I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing)
(3) I have made offers to cost-share (Offers to Cost Share)
(4) I am submitting an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study)
(5) I am submitting or citing data to upgrade a study classified by EPA as partially acceptable and upgradeable (Upgrading a Study)
(6) I am citing an existing study that EPA has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study)

Option 1. Developing Data

If you choose to develop the required data it must be in conformance with Agency deadlines and with other Agency requirements as referenced herein and in the attachments. All data generated and submitted must comply with the Good Laboratory Practice (GLP) rule (40 CFR Part 160), be conducted according to the Pesticide Assessment Guidelines (PAG) and be in conformance with the requirements of PR Notice 86-5. In addition, certain studies require Agency approval of test protocols in advance of study initiation. Those studies for which a protocol must be submitted have been identified in the Requirements Status and Registrant's Response Form and/or footnotes to the form. If you wish to use a protocol which differs from the options discussed in Section II-C of this Notice, you must submit a detailed description of the proposed protocol and your reason for wishing to use it. The Agency may choose to reject a protocol not specified in Section II-C. If the Agency rejects your protocol you will be notified in writing, however, you should be aware

that rejection of a proposed protocol will not be a basis for extending the deadline for submission of data.

A progress report must be submitted for each study within 90 days from the date you are required to commit to generate or undertake some other means to address that study requirement, such as making an offer to cost share or agreeing to share in the cost of developing that study. This 90-day progress report must include the date the study was or will be initiated and, for studies to be started within 12 months of commitment, the name and address of the laboratory(ies) or individuals who are or will be conducting the study.

In addition, if the time frame for submission of a final report is more than 1 year, interim reports must be submitted at 12 month intervals from the date you are required to commit to generate or otherwise address the requirement for the study. In addition to the other information specified in the preceding paragraph, at a minimum, a brief description of current activity on and the status of the study must be included as well as a full description of any problems encountered since the last progress report.

The time frames in the Requirements Status and Registrant's Response Form are the time frames that the Agency is allowing for the submission of completed study reports or protocols. The noted deadlines run from the date of the receipt of this Notice by the registrant. If the data are not submitted by the deadline, each registrant is subject to receipt of a Notice of Intent to Suspend the affected registration(s).

If you cannot submit the data/reports to the Agency in the time required by this Notice and intend to seek additional time to meet the requirements(s), you must submit a request to the Agency which includes: (1) a detailed description of the expected difficulty and (2) a proposed schedule including alternative dates for meeting such requirements on a step-by-step basis. You must explain any technical or laboratory difficulties and provide documentation from the laboratory performing the testing. While EPA is considering your request, the original deadline remains. The Agency will respond to your request in writing. If EPA does not grant your request, the original deadline remains. Normally, extensions can be requested only in cases of extraordinary testing problems beyond the expectation or control of the registrant. Extensions will not be given in submitting the 90-day responses. Extensions will not be considered if the request for extension is not made in a timely fashion; in no event shall an extension request be considered if it is submitted at or after the lapse of the subject deadline.

Option 2. Agreement to Share in Cost to Develop Data

If you choose to enter into an agreement to share in the cost of producing the required data but will not be submitting the data yourself, you must provide the name of the registrant who will be submitting the data. You must also provide EPA with documentary evidence that an agreement has been formed. Such evidence may be your letter offering to join in an agreement and the other registrant's acceptance of your offer, or a written statement by the parties that an agreement exists. The agreement to produce the data need not specify all of the terms of the final arrangement between the parties or the mechanism to resolve the terms. Section 3(c)(2)(B) provides that if the parties cannot resolve the terms of the agreement they may resolve their differences through binding arbitration.

Option 3. Offer to Share in the Cost of Data Development

If you have made an offer to pay in an attempt to enter into an agreement or amend an existing agreement to meet the requirements of this Notice and have been unsuccessful, you may request EPA (by selecting this option) to exercise its discretion not to suspend your registration(s), although you do not comply with the data submission requirements of this Notice. EPA has determined that as a general policy, absent other relevant considerations, it will not suspend the registration of a product

of a registrant who has in good faith sought and continues to seek to enter into a joint data development/cost sharing program, but the other registrant(s) developing the data has refused to accept the offer. To qualify for this option, you must submit documentation to the Agency proving that you have made an offer to another registrant (who has an obligation to submit data) to share in the burden of developing that data. You must also submit to the Agency a completed EPA Form 8570-32, Certification of Offer to Cost Share in the Development of Data, Attachment 7. In addition, you must demonstrate that the other registrant to whom the offer was made has not accepted your offer to enter into a cost-sharing agreement by including a copy of your offer and proof of the other registrant's receipt of that offer (such as a certified mail receipt). Your offer must, in addition to anything else, offer to share in the burden of producing the data upon terms to be agreed to or, failing agreement, to be bound by binding arbitration as provided by FIFRA section 3(c)(2)(B)(iii) and must not qualify this offer. The other registrant must also inform EPA of its election of an option to develop and submit the data required by this Notice by submitting a Data Call-In Response Form and a Requirements Status and Registrant's Response Form committing to develop and submit the data required by this Notice.

In order for you to avoid suspension under this option, you may not withdraw your offer to share in the burden of developing the data. In addition, the other registrant must fulfill its

commitment to develop and submit the data as required by this Notice. If the other registrant fails to develop the data or for some other reason is subject to suspension, your registration as well as that of the other registrant normally will be subject to initiation of suspension proceedings, unless you commit to submit, and do submit, the required data in the specified time frame. In such cases, the Agency generally will not grant a time extension for submitting the data.

Option 4. Submitting an Existing Study

If you choose to submit an existing study in response to this Notice, you must determine that the study satisfies the requirements imposed by this Notice. You may only submit a study that has not been previously submitted to the Agency or previously cited by anyone. Existing studies are studies which predate issuance of this Notice. Do not use this option if you are submitting data to upgrade a study. (See Option 5).

You should be aware that if the Agency determines that the study is not acceptable, the Agency will require you to comply with this Notice, normally without an extension of the required date of submission. The Agency may determine at any time that a study is not valid and needs to be repeated.

To meet the requirements of the DCI Notice for submitting an existing study, all of the following three criteria must be clearly Met:

a. You must certify at the time that the existing study is submitted that the raw data and specimens from the study are available for audit and review and you must identify where they are available. This must be done in accordance with the requirements of the Good Laboratory Practice (GLP) regulation, 40 CFR Part 160. As stated in 40 CFR 160.3 "'[r]aw data' means any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study. In the event that exact transcripts of raw data have been prepared (e.g., tapes which have been transcribed verbatim, dated, and verified accurate by signature), the exact copy or exact transcript may be substituted for the original source as raw data. 'Raw data' may include photographs, microfilm or microfiche copies, computer printouts, magnetic media, including dictated observations, and recorded data from automated instruments." The term "specimens", according to 40 CFR

160.3, means "any material derived from a test system for examination or analysis."

b. Health and safety studies completed after May 1984 also must also contain all GLP-required quality assurance and quality control information, pursuant to the requirements of 40 CFR Part 160. Registrants also must certify at the time of submitting the existing study that such GLP information is available for post May 1984 studies by including an appropriate statement on or attached to the study signed by an authorized official or representative of the registrant.

c. You must certify that each study fulfills the acceptance criteria for the Guideline relevant to the study provided in the FIFRA Accelerated Reregistration Phase 3 Technical Guidance and that the study has been conducted according to the Pesticide Assessment Guidelines (PAG) or meets the purpose of the PAG (both available from NTIS). A study not conducted according to the PAG may be submitted to the Agency for consideration if the registrant believes that the study clearly meets the purpose of the PAG. The registrant is referred to 40 CFR 158.70 which states the Agency's policy regarding acceptable protocols. If you wish to submit the study, you must, in addition to certifying that the purposes of the PAG are met by the study, clearly articulate the rationale why you believe the study meets the purpose of the PAG, including copies of any supporting information or data. It has been the Agency's experience that studies completed prior to January 1970 rarely satisfied the purpose of the PAG and that necessary raw data usually are not available for such studies.

If you submit an existing study, you must certify that the study meets all requirements of the criteria outlined above.

If EPA has previously reviewed a protocol for a study you are submitting, you must identify any action taken by the Agency on the protocol and must indicate, as part of your certification, the manner in which all Agency comments, concerns, or issues were addressed in the final protocol and study.

If you know of a study pertaining to any requirement in this Notice which does not meet the criteria outlined above but does contain factual information regarding unreasonable adverse effects, you must notify the Agency of such a study. If such study is in the Agency's files, you need only cite it along with the notification. If not in the Agency's files, you must submit a summary and copies as required by PR Notice 86-5.

Option 5. Upgrading a Study

If a study has been classified as partially acceptable and upgradeable, you may submit data to upgrade that study. The Agency will review the data submitted and determine if the requirement is satisfied. If the Agency decides the requirement is not satisfied, you may still be required to submit new data normally without any time extension. Deficient, but upgradeable studies will normally be classified as supplemental. However, it is important to note that not all studies classified as supplemental are upgradeable. If you have questions regarding the classification of a study or whether a study may be upgraded, call or write the contact person listed in Attachment 1. If you submit data to upgrade an existing study you must satisfy or supply information to correct all deficiencies in the study identified by EPA. You must provide a clearly articulated rationale of how the deficiencies have been remedied or corrected and why the study should be rated as acceptable to EPA. Your submission must also specify the MRID number(s) of the study which you are attempting to upgrade and must be in conformance with PR Notice 86-5.

Do not submit additional data for the purpose of upgrading a study classified as unacceptable and determined by the Agency as not capable of being upgraded.

This option also should be used to cite data that has been previously submitted to upgrade a study, but has not yet been reviewed by the Agency. You must provide the MRID number of the data submission as well as the MRID number of the study being upgraded.

The criteria for submitting an existing study, as specified in Option 4 above, apply to all data submissions intended to upgrade studies. Additionally, your submission of data intended to upgrade studies must be accompanied by a certification that you comply with each of those criteria, as well as a certification regarding protocol compliance with Agency requirements.

Option 6. Citing Existing Studies

If you choose to cite a study that has been previously submitted to EPA, that study must have been previously classified by EPA as acceptable, or it must be a study which has not yet been reviewed by the Agency. Acceptable toxicology studies generally will have been classified as "core-guideline" or "core-minimum." For ecological effects studies, the classification generally would be a rating of "core." For all other disciplines the classification would be "acceptable." With respect to any studies for which you wish to select this option, you must

provide the MRID number of the study you are citing and, if the study has been reviewed by the Agency, you must provide the Agency's classification of the study.

If you are citing a study of which you are not the original data submitter, you must submit a completed copy of EPA Form 8570-31, Certification with Respect to Data Compensation Requirements.

2. Product Specific Data

If you acknowledge on the product specific Data Call-In Response Form that you agree to satisfy the product specific data requirements (i.e. you select option 7a or 7b), then you must select one of the six options on the Requirements Status and Registrant's Response Form related to data production for each data requirement. Your option selection should be entered under item number 9, "Registrant Response." The six options related to data production are the first six options discussed under item 9 in the instructions for completing the Requirements Status and Registrant's Response Form. These six options are listed immediately below with information in parentheses to guide registrants to additional instructions provided in this Section. The options are:

(1) I will generate and submit data within the specified time-frame (Developing Data)
(2) I have entered into an agreement with one or more registrants to develop data jointly (Cost Sharing)
(3) I have made offers to cost-share (Offers to Cost Share)
(4) I am submitting an existing study that has not been submitted previously to the Agency by anyone (Submitting an Existing Study)

(5) I am submitting or citing data to upgrade a study classified by EPA as partially acceptable and upgradeable (Upgrading a Study)

(6) I am citing an existing study that EPA has classified as acceptable or an existing study that has been submitted but not reviewed by the Agency (Citing an Existing Study)

Option 1. Developing Data -- The requirements for developing product specific data are the same as those described for generic data (see Section III.C.1, Option 1) except that normally no protocols or progress reports are required.

Option 2. Agree to Share in Cost to Develop Data -- If you enter into an agreement to cost share, the same requirements apply to product specific data as to generic data (see Section III.C.1, Option 2). However, registrants may only choose this option for

acute toxicity data and certain efficacy data and only if EPA has indicated in the attached data tables that your product and at least one other product are similar for purposes of depending on the same data. If this is the case, data may be generated for just one of the products in the group. The registration number of the product for which data will be submitted must be noted in the agreement to cost share by the registrant selecting this option.

Option 3. Offer to Share in the Cost of Data Development --The same requirements for generic data (Section III.C.I., Option 3) apply to this option. This option only applies to acute toxicity and certain efficacy data as described in option 2 above.

Option 4. Submitting an Existing Study -- The same requirements described for generic data (see Section III.C.1., Option 4) apply to this option for product specific data.

Option 5. Upgrading a Study -- The same requirements described for generic data (see Section III.C.1., Option 5) apply to this option for product specific data.

Option 6. Citing Existing Studies -- The same requirements described for generic data (see Section III.C.1., Option 6) apply to this option for product specific data.

Registrants who select one of the above 6 options must meet all of the requirements described in the instructions for completing the Data Call-In Response Form and the Requirements Status and Registrant's Response Form, and in the generic data requirements section (III.C.1.), as appropriate.

III-D REQUESTS FOR DATA WAIVERS

1. Generic Data

There are two types of data waiver responses to this Notice. The first is a request for a low volume/minor use waiver and the second is a waiver request based on your belief that the data requirement(s) are not appropriate for your product.

a. Low Volume/Minor Use Waiver

Option 8 under item 9 on the Requirements Status and Registrant's Response Form. Section 3(c)(2)(A) of FIFRA requires EPA to consider the appropriateness of requiring data for low volume, minor use pesticides. In implementing

this provision, EPA considers low volume pesticides to be only those active ingredients whose total production volume for all pesticide registrants is small. In determining whether to grant a low volume, minor use waiver, the Agency will consider the extent, pattern and volume of use, the economic incentive to conduct the testing, the importance of the pesticide, and the exposure and risk from use of the pesticide. If an active ingredient is used for both high volume and low volume uses, a low volume exemption will not be approved. If all uses of an active ingredient are low volume and the combined volumes for all uses are also low, then an exemption may be granted, depending on review of other information outlined below. An exemption will not be granted if any registrant of the active ingredient elects to conduct the testing. Any registrant receiving a low volume minor use waiver must remain within the sales figures in their forecast supporting the waiver request in order to remain qualified for such waiver. If granted a waiver, a registrant will be required, as a condition of the waiver, to submit annual sales reports. The Agency will respond to requests for waivers in writing.

To apply for a low volume, minor use waiver, you must submit the following information, as applicable to your product(s), as part of your 90-day response to this Notice:

(i). Total company sales (pounds and dollars) of all registered product(s) containing the active ingredient. If applicable to the active ingredient, include foreign sales for those products that are not registered in this country but are applied to sugar (cane or beet), coffee, bananas, cocoa, and other such crops. Present the above information by year for each of the past five years.

(ii) Provide an estimate of the sales (pounds and dollars) of the active ingredient for each major use site. Present the above information by year for each of the past five years.

(iii) Total direct production cost of product(s) containing the active ingredient by year for the past five years. Include information on raw material cost, direct labor cost, advertising, sales and marketing, and any other significant costs listed separately.

(iv) Total indirect production cost (e.g. plant overhead, amortized plant and equipment) charged to product(s) containing the active ingredient by year for the past five years. Exclude all non-recurring costs that were directly related to the active ingredient, such as costs of initial registration and any data development.

(v) A list of each data requirement for which you seek a waiver. Indicate the type of waiver sought and the estimated cost to you (listed separately for each data requirement and associated test) of conducting the testing needed to fulfill each of these data requirements.

(vi) A list of each data requirement for which you are not seeking any waiver and the estimated cost to you (listed separately for each data requirement and associated test) of conducting the testing needed to fulfill each of these data requirements.

(vii) For each of the next ten years, a year-by-year forecast of company sales (pounds and dollars) of the active ingredient, direct production costs of product(s) containing the active ingredient (following the parameters in item 2 above), indirect production costs of product(s) containing the active ingredient (following the parameters in item 3 above), and costs of data development pertaining to the active ingredient.

(viii) A description of the importance and unique benefits of the active ingredient to users. Discuss the use patterns and the effectiveness of the active ingredient relative to registered alternative chemicals and non-chemical control strategies. Focus on benefits unique to the active ingredient, providing information that is as quantitative as possible. If you do not have quantitative data upon which to base your estimates, then present the reasoning used to derive your estimates. To assist the Agency in determining the degree of importance of the active ingredient in terms of its benefits, you should provide information on any of the following factors, as applicable to your product(s): (a) documentation of the usefulness of the active ingredient in Integrated Pest Management, (b) description of the beneficial impacts on the environment of use of the active ingredient, as opposed to its registered alternatives, (c) information on the breakdown of the active ingredient after use and on its persistence in the environment, and (d) description of its usefulness against a pest(s) of public health significance.

Failure to submit sufficient information for the Agency to make a determination regarding a request for a low volume/minor use waiver will result in denial of the request for a waiver.

b. Request for Waiver of Data

Option 9, under Item 9, on the Requirements Status and Registrant's Response Form. This option may be used if you believe that a particular data requirement should not apply because the requirement is inappropriate. You must submit a

rationale explaining why you believe the data requirements should not apply. You also must submit the current label(s) of your product(s) and, if a current copy of your Confidential Statement of Formula is not already on file you must submit a current copy.

You will be informed of the Agency's decision in writing. If the Agency determines that the data requirements of this Notice are not appropriate to your product(s), you will not be required to supply the data pursuant to section 3(c)(2)(B). If EPA determines that the data are required for your product(s), you must choose a method of meeting the requirements of this Notice within the time frame provided by this Notice. Within 30 days of your receipt of the Agency's written decision, you must submit a revised Requirements Status and Registrant's Response Form indicating the option chosen.

2. Product Specific Data

If you request a waiver for product specific data because you believe it is inappropriate, you must attach a complete justification for the request including technical reasons, data and references to relevant EPA regulations, guidelines or policies. (Note: any supplemental data must be submitted in the format required by PR Notice 86-5). This will be the only opportunity to state the reasons or provide information in support of your request. If the Agency approves your waiver request, you will not be required to supply the data pursuant to section 3(c)(2)(B) of FIFRA. If the Agency denies your waiver request, you must choose an option for meeting the data requirements of this Notice within 30 days of the receipt of the Agency's decision. You must indicate and submit the option chosen on the product specific Requirements Status and Registrant's Response Form. Product specific data requirements for product chemistry, acute toxicity and efficacy (where appropriate) are required for all products and the Agency would grant a waiver only under extraordinary circumstances. You should also be aware that submitting a waiver request will not automatically extend the due date for the study in question. Waiver requests submitted without adequate supporting rationale will be denied and the original due date will remain in force.

SECTION IV. CONSEQUENCES OF FAILURE TO COMPLY WITH THIS NOTICE

IV-A NOTICE OF INTENT TO SUSPEND

The Agency may issue a Notice of Intent to Suspend products subject to this Notice due to failure by a registrant to comply with the requirements of this Data Call-In Notice, pursuant to

FIFRA section 3(c)(2)(B). Events which may be the basis for issuance of a Notice of Intent to Suspend include, but are not limited to, the following:

1. Failure to respond as required by this Notice within 90 days of your receipt of this Notice.

2. Failure to submit on the required schedule an acceptable proposed or final protocol when such is required to be submitted to the Agency for review.

3. Failure to submit on the required schedule an adequate progress report on a study as required by this Notice.

4. Failure to submit on the required schedule acceptable data as required by this Notice.

5. Failure to take a required action or submit adequate information pertaining to any option chosen to address the data requirements (e.g., any required action or information pertaining to submission or citation of existing studies or offers, arrangements, or arbitration on the sharing of costs or the formation of Task Forces, failure to comply with the terms of an agreement or arbitration concerning joint data development or failure to comply with any terms of a data waiver).

6. Failure to submit supportable certifications as to the conditions of submitted studies, as required by Section III-C of this Notice.

7. Withdrawal of an offer to share in the cost of developing required data.

8. Failure of the registrant to whom you have tendered an offer to share in the cost of developing data and provided proof of the registrant's receipt of such offer or failure of a registrant on whom you rely for a generic data exemption either to:

   i. Inform EPA of intent to develop and submit the data required by this Notice on a Data Call-In Response Form and a Requirements Status and Registrant's Response Form.

   ii. Fulfill the commitment to develop and submit the data as required by this Notice; or

   iii. Otherwise take appropriate steps to meet the requirements stated in this Notice,

unless you commit to submit and do submit the required data in the specified time frame.

9. Failure to take any required or appropriate steps, not mentioned above, at any time following the issuance of this Notice.

IV-B. BASIS FOR DETERMINATION THAT SUBMITTED STUDY IS UNACCEPTABLE

The Agency may determine that a study (even if submitted within the required time) is unacceptable and constitutes a basis for issuance of a Notice of Intent to Suspend. The grounds for suspension include, but are not limited to, failure to meet any of the following:

1) EPA requirements specified in the Data Call-In Notice or other documents incorporated by reference (including, as applicable, EPA Pesticide Assessment Guidelines, Data Reporting Guidelines, and GeneTox Health Effects Test Guidelines) regarding the design, conduct, and reporting of required studies. Such requirements include, but are not limited to, those relating to test material, test procedures, selection of species, number of animals, sex and distribution of animals, dose and effect levels to be tested or attained, duration of test, and, as applicable, Good Laboratory Practices.

2) EPA requirements regarding the submission of protocols, including the incorporation of any changes required by the Agency following review.

3) EPA requirements regarding the reporting of data, including the manner of reporting, the completeness of results, and the adequacy of any required supporting (or raw) data, including, but not limited to, requirements referenced or included in this Notice or contained in PR 86-5. All studies must be submitted in the form of a final report; a preliminary report will not be considered to fulfill the submission requirement.

IV-C EXISTING STOCKS OF SUSPENDED OR CANCELLED PRODUCTS

EPA has statutory authority to permit continued sale, distribution and use of existing stocks of a pesticide product which has been suspended or cancelled if doing so would be consistent with the purposes of the Act.

The Agency has determined that such disposition by registrants of existing stocks for a suspended registration when

a section 3(c)(2)(B) data request is outstanding generally would not be consistent with the Act's purposes. Accordingly, the Agency anticipates granting registrants permission to sell, distribute, or use existing stocks of suspended product(s) only in exceptional circumstances. If you believe such disposition of existing stocks of your product(s) which may be suspended for failure to comply with this Notice should be permitted, you have the burden of clearly demonstrating to EPA that granting such permission would be consistent with the Act. You also must explain why an "existing stocks" provision is necessary, including a statement of the quantity of existing stocks and your estimate of the time required for their sale, distribution, and use. Unless you meet this burden, the Agency will not consider any request pertaining to the continued sale, distribution, or use of your existing stocks after suspension.

If you request a voluntary cancellation of your product(s) as a response to this Notice and your product is in full compliance with all Agency requirements, you will have, under most circumstances, one year from the date your 90 day response to this Notice is due, to sell, distribute, or use existing stocks. Normally, the Agency will allow persons other than the registrant such as independent distributors, retailers and end users to sell, distribute or use such existing stocks until the stocks are exhausted. Any sale, distribution or use of stocks of voluntarily cancelled products containing an active ingredient for which the Agency has particular risk concerns will be determined on a case-by-case basis.

Requests for voluntary cancellation received <u>after</u> the 90 day response period required by this Notice will not result in the agency granting any additional time to sell, distribute, or use existing stocks beyond a year from the date the 90 day response was due, <u>unless</u> you demonstrate to the Agency that you are in full compliance with all Agency requirements, including the requirements of this Notice. For example, if you decide to voluntarily cancel your registration six months before a 3-year study is scheduled to be submitted, all progress reports and other information necessary to establish that you have been conducting the study in an acceptable and good faith manner must have been submitted to the Agency, before EPA will consider granting an existing stocks provision.

SECTION V. <u>REGISTRANTS' OBLIGATION TO REPORT POSSIBLE UNREASONABLE ADVERSE EFFECTS</u>

Registrants are reminded that FIFRA section 6(a)(2) states that if at any time after a pesticide is registered a registrant has additional factual information regarding unreasonable adverse effects on the environment by the pesticide, the registrant shall submit the information to the Agency. Registrants must notify the

Agency of any factual information they have, from whatever source, including but not limited to interim or preliminary results of studies, regarding unreasonable adverse effects on man or the environment. This requirement continues as long as the products are registered by the Agency.

SECTION VI. INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the requirements and procedures established by this Notice, call the contact person(s) listed in Attachment 1, the Data Call-In Chemical Status Sheet.

All responses to this Notice must include completed Data Call-In Response Forms (Attachment 2)and completed Requirements Status and Registrant's Response Forms (Attachment 3), for both (generic and product specific data) and any other documents required by this Notice, and should be submitted to the contact person(s) identified in Attachment 1. If the voluntary cancellation or generic data exemption option is chosen, only the Generic and Product Specific Data Call-In Response Forms need be submitted.

The Office of Compliance Monitoring (OCM) of the Office of Prevention, Pesticides and Toxic Substances (OPPTS), EPA, will be monitoring the data being generated in response to this Notice.

Sincerely yours,

Daniel M. Barolo, Director
Special Review and
Reregistration Division

Attachments

The Attachments to this Notice are:

1 - Data Call-In Chemical Status Sheet
2 - Generic Data Call-In and Product Specific Data Call-In Response Forms with Instructions
3 - Generic Data Call-In and Product Specific Data Call-In Requirements Status and Registrant's Response Forms with Instructions
4 - EPA Grouping of End-Use Products for Meeting Acute Toxicology Data Requirements for Reregistration
5 - EPA Acceptance Criteria
6 - List of Registrants Receiving This Notice
7 - Cost Share and Data Compensation Forms

Attachment 1

Chemical Status Sheet

GLYPHOSATE: DATA CALL-IN CHEMICAL STATUS SHEET

DATA REQUIRED BY THIS NOTICE

The additional data requirements needed to complete the data base for glyphosate are contained in Generic DCI and Product Specific DCI Requirements Status and Registrant's Response forms (Attachment 3).

INQUIRIES AND RESPONSES TO THIS NOTICE

If you have any questions regarding the generic data base for glyphosate, please contact Eric Feris, the Review Manager for this chemical through the Virginia Relay (1-800-828-1140) at (703) 308-8048.

If you have any questions regarding the product specific data requirements and procedures established by this Notice, please contact Frank Rubis at (703) 308-8184.

All responses to this Notice should be submitted to:

Eric Feris
Special Review and Reregistration Division (7508W)
Office of Pesticide Programs
U.S. Environmental Protection Agency
Washington, D.C. 20460

RE: Glyphosate

Attachment 2

Generic DCI and Product Specific DCI Response Forms with Instructions

Instructions For Completing
The
"Data Call-In Response Forms"
For The Generic And Product Specific Data Call-In

INTRODUCTION

These instructions apply to the Generic and Product Specific "Data Call-In Response Forms" and are to be used by registrants to respond to generic and product specific Data Call-Ins as part of EPA's Reregistration Program under the Federal Insecticide Fungicide and Rodenticide Act. **The type of data call-in (generic or product specific) is indicated in item number 3 ("Date and Type of DCI") on each form. BOTH** "Data Call-In Response" forms must be completed.

Although the form is the same for both generic and product specific data, instructions for completing these forms are different. Please read these instructions carefully before filling out the forms.

EPA has developed these forms individually for each registrant, and has preprinted these forms with a number of items. DO NOT use these forms for any other active ingredient.

Items 1 through 4 have been preprinted on the form. Items 5 through 7 must be completed by the registrant as appropriate. Items 8 through 11 must be completed by the registrant before submitting a response to the Agency.

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggesting for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, D.C. 20460; and to the Office of Management and Budget, Paperwork Reduction Project 2070-0107, Washington, D.C. 20503.

INSTRUCTIONS FOR COMPLETING THE DATA CALL-IN RESPONSE FORMS
Generic and Product Specific Data Call-In

Item 1. **ON BOTH FORMS:** This item identifies your company name, number and address.

Item 2. **ON BOTH FORMS:** This item identifies the case number, case name, EPA chemical number and chemical name.

Item 3. **ON BOTH FORMS:** This item identifies the type of Data Call-In. The date of issuance is date stamped.

Item 4. **ON BOTH FORMS:** This item identifies the EPA product registrations relevant to the data call-in. Please note that you are also responsible for informing the Agency of your response regarding any product that you believe may be covered by this Data Call-In but that is not listed by the Agency in Item 4. You must bring any such apparent omission to the Agency's attention within the period required for submission of this response form.

Item 5. **ON BOTH FORMS:** Check this item for each product registration you wish to cancel voluntarily. If a registration number is listed for a product for which you previously requested voluntary cancellation, indicate in Item 5 the date of that request. Since this Data Call-In requires both generic and product specific data, you must complete item 5 on both Data Call-In response forms. You do not need to complete any item on the Requirements Status and Registrant's Response Forms.

Item 6a. **ON THE GENERIC DATA FORM:** Check this Item if the Data Call-In is for generic data as indicated in Item 3 and you are eligible for a Generic Data Exemption for the chemical listed in Item 2 and used in the subject product. By electing this exemption, you agree to the terms and conditions of a Generic Data Exemption as explained in the Data Call-In Notice.

If you are eligible for or claim a Generic Data Exemption, enter the EPA registration Number of each registered source of that active ingredient that you use in your product.

Typically, if you purchase an EPA-registered product from one or more other producers (who, with respect to the incorporated product, are in compliance with this and any other outstanding Data Call-In Notice), and

incorporate that product into all your products, you may complete this item for all products listed on this form. If, however, you produce the active ingredient yourself, or use any unregistered product (regardless of the fact that some of your sources are registered), you may not claim a Generic Data Exemption and you may not select this item.

Item 6b. **ON THE GENERIC DATA FORM:** Check this Item if the Data Call-In is for generic data as indicated in Item 3 and if you are agreeing to satisfy the generic data requirements of this Data Call-In. Attach the Requirements Status and Registrant's Response Form that indicates how you will satisfy those requirements.

NOTE: **Item 6a and 6b are not applicable for Product Specific Data.**

Item 7a. **ON THE PRODUCT SPECIFIC DATA FORM:** For each manufacturing use product (MUP) for which you wish to maintain registration, you must agree to satisfy the data requirements by responding "yes."

Item 7b. For each end use product (EUP) for which you wish to maintain registration, you must agree to satisfy the data requirements by responding "yes."

FOR BOTH MUP and EUP products

You should also respond "yes" to this item (7a for MUP's and 7b for EUP's) if your product is identical to another product and you qualify for a data exemption. You must provide the EPA registration numbers of your source(s); do not complete the Requirements Status and Registrant's Response form. Examples of such products include repackaged products and Special Local Needs (Section 24c) products which are identical to federally registered products.

If you are requesting a data waiver, answer "yes" here; in addition, on the "Requirements Status and Registrant's Response" form under Item 9, you must respond with option 7 (Waiver Request) for each study for which you are requesting a waiver.

NOTE: **Item 7a and 7b are not applicable for Generic Data.**

INSTRUCTIONS FOR COMPLETING THE DATA CALL-IN RESPONSE FORMS
Generic and Product Specific Data Call-In

Item 8. **ON BOTH FORMS:** This certification statement must be signed by an authorized representative of your company and the person signing must include his/her title. Additional pages used in your response must be initialled and dated in the space provided for the certification.

Item 9. **ON BOTH FORMS:** Enter the date of signature.

Item 10. **ON BOTH FORMS:** Enter the name of the person EPA should contact with questions regarding your response.

Item 11. **ON BOTH FORMS:** Enter the phone number of your company contact.

Note: You may provide additional information that does not fit on this form in a signed letter that accompanies your response. For example, you may wish to report that your product has already been transferred to another company or that you have already voluntarily cancelled this product. For these cases, please supply all relevant details so that EPA can ensure that its records are correct.

United States Environmental Protection Agency
Washington, D. C. 20460

DATA CALL-IN RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY 1234 MAIN STREET ANYWHERE, USA 54321	0178 Glyphosate	GENERIC FEB 16 1994

4. EPA Product Registration	5. I wish to cancel this product registration voluntarily.	6. Generic Data		7. Product Specific Data	
		6a. I am claiming a Generic Data Exemption because I obtain the active ingredient from the source EPA registration number listed below.	6b. I agree to satisfy Generic Data requirements as indicated on the attached form entitled "Requirements Status and Registrant's Response."	7a. My product is a MUP and I agree to satisfy the MUP requirements on the attached form entitled "Requirements Status and Registrant's Response."	7b. My product is an EUP and I agree to satisfy the EUP requirements on the attached form entitled "Requirements Status and Registrant's Response."
		N.A.	N.A.		

8. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

9. Date

10. Name of Company Contact

11. Phone Number

United States Environmental Protection Agency
Washington, D. C. 20460

DATA CALL-IN RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY 1234 MAIN STREET ANYWHERE, USA 54321	0178 Glyphosate	PRODUCT SPECIFIC FEB 16 1994

4. EPA Product Registration	5. I wish to cancel this product registration voluntarily.	6. Generic Data		7. Product Specific Data	
		6a. I am claiming a Generic Data Exemption because I obtain the active ingredient from the source EPA registration number listed below.	6b. I agree to satisfy Generic Data requirements as indicated on the attached form entitled "Requirements Status and Registrant's Response."	7a. My product is a MUP and I agree to satisfy the MUP requirements on the attached form entitled "Requirements Status and Registrant's Response."	7b. My product is an EUP and I agree to satisfy the EUP requirements on the attached form entitled "Requirements Status and Registrant's Response."
		N.A.	N.A.		

8. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

9. Date

10. Name of Company Contact

11. Phone Number

Attachment 3

Generic DCI and Product Specific DCI Requirements Status and Registrants' Response Forms with Instructions

Instructions For Completing
The
"Requirements Status and Registrant's Response Forms"
For The Generic and Product Specific Data Call-In

INTRODUCTION

These instructions apply to the Generic and Product Specific "Requirements Status and Registrant's Response Forms" and are to be used by registrants to respond to generic and product specific Data Call-In's as part of EPA's reregistration program under the Federal Insecticide Fungicide and Rodenticide Act. **The type of Data Call-In (generic or product specific) is indicated in item number 3 ("Date and Type of DCI") on each form.** Both "Requirements Status and Registrant's Response" forms must be completed.

Although the form is the same for both product specific and generic data, instructions for completing the forms differ slightly. Specifically, options for satisfying product specific data requirements do not include (1) deletion of uses or (2) request for a low volume/minor use waiver. Please read these instructions carefully before filling out the forms.

EPA has developed these forms individually for each registrant, and has preprinted these forms with a number of items. DO NOT use these forms for any other active ingredient.

Items 1 through 8 have been preprinted on the form. Item 9 must be completed by the registrant as appropriate. Items 10 through 13 must be completed by the registrant before submitting a response to the Agency.

INSTRUCTIONS FOR COMPLETING THE "REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE FORMS"
Generic and Product Specific Data Call-In

Item 1. **ON BOTH FORMS:** This item identifies your company name, number and address.

Item 2. **ON THE GENERIC DATA FORM:** This item identifies the case number, case name, EPA chemical number and chemical name.

ON THE PRODUCT SPECIFIC DATA FORM: This item identifies the case number, case name, and the EPA Registration Number of the product for which the Agency is requesting product specific data.

Item 3. **ON THE GENERIC DATA FORM:** This item identifies the type of Data Call-In. The date of issuance is date stamped.

ON THE PRODUCT SPECIFIC DATA FORM: This item identifies the type of Data Call-In. The date of issuance is also date stamped. Note the unique identifier number (ID#) assigned by the Agency. This ID number must be used in the transmittal document for any data submissions in response to this Data Call-In Notice.

Item 4. **ON BOTH FORMS:** This item identifies the guideline reference number of studies required. These guidelines, in addition to the requirements specified in the Data Call-In Notice, govern the conduct of the required studies. Note that series 61 and 62 in product chemistry are now listed under 40 CFR 158.155 through 158.180, Subpart c.

Item 5. **ON BOTH FORMS:** This item identifies the study title associated with the guideline reference number and whether protocols and 1, 2, or 3-year progress reports are required to be submitted in connection with the study. As noted in Section III of the Data Call-In Notice, 90-day progress reports are required for all studies.

If an asterisk appears in Item 5, EPA has attached information relevant to this guideline reference number to the Requirements Status and Registrant's Response Form.

INSTRUCTIONS FOR COMPLETING THE "REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE FORMS"
Generic and Product Specific Data Call-In

Item 6. **ON BOTH FORMS:** This item identifies the code associated with the use pattern of the pesticide. In the case of efficacy data (product specific requirement), the required study only pertains to products which have the use sites and/or pests indicated. A brief description of each code follows:

A	Terrestrial food
B	Terrestrial feed
C	Terrestrial non-food
D	Aquatic food
E	Aquatic non-food outdoor
F	Aquatic non-food industrial
G	Aquatic non-food residential
H	Greenhouse food
I	Greenhouse non-food crop
J	Forestry
K	Residential
L	Indoor food
M	Indoor non-food
N	Indoor medical
O	Indoor residential

Item 7. **ON BOTH FORMS:** This item identifies the code assigned to the substance that must be used for testing. A brief description of each code follows:

EUP	End-Use Product
MP	Manufacturing-Use Product
MP/TGAI	Manufacturing-Use Product and Technical Grade Active Ingredient
PAI	Pure Active Ingredient
PAI/M	Pure Active Ingredient and Metabolites
PAI/PAIRA	Pure Active Indredient or Pute Active Ingredient Radiolabelled
PAIRA	Pure Active Ingredient Radiolabelled
PAIRA/M	Pure Active Ingredient Radiolabelled and Metabolites
PAIRA/PM	Pure Active Ingredient Radiolabelled and Plant Metabolites
TEP	Typical End-Use Product
TEP ___%	Typical End-Use Product, Percent Active Ingredient Specified
TEP/MET	Typical End-Use Product and Metabolites
TEP/PAI/M	Typical End-Use Product or Pure Active Ingredient and Metabolites

INSTRUCTIONS FOR COMPLETING THE "REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE FORMS"
Generic and Product Specific Data Call-In

TGAI	Technical Grade Active Ingredient
TGAI/PAI	Technical Grade Active Ingredient or Pure Active Ingredient
TGAI/PAIRA	Technical Grade Active Ingredient or Pure Active Ingredient Radiolabelled
TGAI/TEP	Technical Grade Active Ingredient or Typical End-Use Product
MET	Metabolites
IMP	Impurities
DEGR	Degradates
*	See: guideline comment

Item 8. This item completed by the Agency identifies the time frame allowed for submission of the study or protocol identified in item 5.

ON THE GENERIC DATA FORM: The time frame runs from the date of your receipt of the Data Call-In notice.

ON THE PRODUCT SPECIFIC DATA FORM: The due date for submission of product specific studies begins from the date stamped on the letter transmitting the Reregistration Eligibility Decision document, and not from the date of receipt. However, your response to the Data Call-In itself is due 90 days from the date of receipt.

Item 9. **ON BOTH FORMS:** Enter the appropriate Response Code or Codes to show how you intend to comply with each data requirement. Brief descriptions of each code follow. The Data Call-In Notice contains a fuller description of each of these options.

Option 1. **ON BOTH FORMS:** (Developing Data) I will conduct a new study and submit it within the time frames specified in item 8 above. By indicating that I have chosen this option, I certify that I will comply with all the requirements pertaining to the conditions for submittal of this study as outlined in the Data Call-In Notice and that I will provide the protocols and progress reports required in item 5 above.

Option 2. **ON BOTH FORMS:** (Agreement to Cost Share) I have entered into an agreement with one or more registrants to develop data jointly. By indicating

that I have chosen this option, I certify that I will comply with all the requirements pertaining to sharing in the cost of developing data as outlined in the Data Call-In Notice.

However, for Product Specific Data, I understand that this option is available for acute toxicity or certain efficacy data **ONLY** if the Agency indicates in an attachment to this notice that my product is similar enough to another product to qualify for this option. I certify that another party in the agreement is committing to submit or provide the required data; if the required study is not submitted on time, my product may be subject to suspension.

Option 3. **ON BOTH FORMS:** (Offer to Cost Share) I have made an offer to enter into an agreement with one or more registrants to develop data jointly. I am also submitting a completed "Certification of offer to Cost Share in the Development of Data" form. I am submitting evidence that I have made an offer to another registrant (who has an obligation to submit data) to share in the cost of that data. I am including a copy of my offer and proof of the other registrant's receipt of that offer. I am identifying the party which is committing to submit or provide the required data; if the required study is not submitted on time, my product may be subject to suspension. I understand that other terms under Option 3 in the Data Call-In Notice apply as well.

However, for Product Specific Data, I understand that this option is available only for acute toxicity or certain efficacy data and only if the Agency indicates in an attachment to this Data Call-In Notice that my product is similar enough to another product to qualify for this option.

Option 4. **ON BOTH FORMS:** (Submitting Existing Data) I will submit an existing study by the specified due date that has never before been submitted to EPA. By indicating that I have chosen this option, I certify that this study meets all the requirements pertaining to the conditions for submittal of

existing data outlined in the Data Call-In Notice and I have attached the needed supporting information along with this response.

Option 5. **ON BOTH FORMS:** (Upgrading a Study) I will submit by the specified due date, or will cite data to

upgrade a study that EPA has classified as partially acceptable and potentially upgradeable. By indicating that I have chosen this option, I certify that I have met all the requirements pertaining to the conditions for submitting or citing existing data to upgrade a study described in the Data Call-In Notice. I am indicating on attached correspondence the Master Record Identification Number (MRID) that EPA has assigned to the data that I am citing as well as the MRID of the study I am attempting to upgrade.

Option 6. **ON BOTH FORMS:** (Citing a Study) I am citing an existing study that has been previously classified by EPA as acceptable, core, core minimum, or a study that has not yet been reviewed by the Agency. If reviewed, I am providing the Agency's classification of the study.

However, for Product Specific Data, I am citing another registrant's study. I understand that this option is available **ONLY** for acute toxicity or certain efficacy data and **ONLY** if the cited study was conducted on my product, an identical product or a product which the Agency has "grouped" with one or more other products for purposes of depending on the same data. I may also choose this option if I am citing my own data. In either case, I will provide the MRID or Accession number (s). If I cite another registrant's data, I will submit a completed "Certification With Respect To Data Compensation Requirements" form.

FOR THE GENERIC DATA FORM ONLY: The following three options (Numbers 7, 8, and 9) are responses that apply only to the "Requirements Status and Registrant's Response Form" for generic data.

Option 7. (Deleting Uses) I am attaching an application for amendment to my registration deleting the uses for which the data are required.

INSTRUCTIONS FOR COMPLETING THE "REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE FORMS"
Generic and Product Specific Data Call-In

Option 8. (Low Volume/Minor Use Waiver Request) I have read the statements concerning low volume-minor use data waivers in the Data Call-In Notice and I request a low-volume minor use waiver of the data requirement. I am attaching a detailed justification to support this waiver request including, among other things, all information required to support the request. I understand that, unless modified by the Agency in writing, the data requirement as stated in the Notice governs.

Option 9. (Request for Waiver of Data) I have read the statements concerning data waivers other than low-volume minor-use data waivers in the Data Call-In Notice and I request a waiver of the data requirement. I am attaching a rationale explaining why I believe the data requirements do not apply. I am also submitting a copy of my current labels. (You must also submit a copy of your Confidential Statement of Formula if not already on file with EPA). I understand that, unless modified by the Agency in writing, the data requirement as stated in the Notice governs.

FOR PRODUCT SPECIFIC DATA: The following option (number 7) is a response that applies to the "Requirements Status and Registrant's Response Form" for product specific data.

Option 7. (Waiver Request) I request a waiver for this study because it is inappropriate for my product. I am attaching a complete justification for this request, including technical reasons, data and references to relevant EPA regulations, guidelines or policies. [Note: any supplemental data must be submitted in the format required by P.R. Notice 86-5]. I understand that this is my only opportunity to state the reasons or provide information in support of my request. If the Agency approves my waiver request, I will not be required to supply the data pursuant to Section 3(c) (2) (B) of FIFRA. If the Agency denies my waiver request, I must choose a method of meetingthe data requirements of this Notice by the due date stated by this Notice. In this case, I must, within 30 days-of my receipt of the Agency's written decision, submit a revised "Requirements Status" form specifying the option chosen. I also

understand that the deadline for submission of data as specified by the original Data Call-In notice will not change.

Item 10. **ON BOTH FORMS:** This item must be signed by an authorized representative of your company. The person signing must include his/her title, and must initial and date all other pages of this form.

Item 11. **ON BOTH FORMS:** Enter the date of signature.

Item 12. **ON BOTH FORMS:** Enter the name of the person EPA should contact with questions regarding your response.

Item 13. **ON BOTH FORMS:** Enter the phone number of your company contact.

NOTE: You may provide additional information that does not fit on this form in a signed letter that accompanies this your response. For example, you may wish to report that your product has already been transferred to another company or that you have already voluntarily cancelled this product. For these cases, please supply all relevant details so that the Agency can ensure that its records are correct.

United States Environmental Protection Agency
Washington, D.C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary

1. Company name and Address	2. Case # and Name 0178 Glyphosate Chemical # and Name 103601 Isopropylamine glyphosate	3. Date and Type of DCI GENERIC FEB 16 1994

4. Guideline Requirement Number	5. Study Title	DCI	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
123-1(b)	Vegetative vigor					ABCDEFJK	TGAI	12 mos.	
201-1	Droplet size spectrum					ABCDEJ	TEP	12 mos.	
202-1	Drift field evaluation		Y			ABCDEJ	TEP	24 mos.	

10. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

11. Date

12. Name of Company Contact

13. Phone Number

United States Environmental Protection Agency
Washington, D. C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY 1234 MAIN STREET ANYWHERE, USA 54321	0178 Glyphosate EPA Reg. No. 70-269	PRODUCT SPECIFIC ID# 70-RD-3263 FEB 16 1994

4. Guideline Requirement Number	5. Study Title	PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
	Prod Chem - Regular Chemical								
61-1	Product identity & composition (1)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
61-2(a)	Descriptn starting materials, productn & formulatn process (1,2)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
61-2(b)	Discussion of formation of impurities (1,3)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
62-1	Preliminary analysis (1,4)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
62-2	Certification of limits (1,5)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
62-3	Analytical method (1)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-3	Physical state					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-5	Melting point (6)					ABCDEFGHIJKLMNO	TGAI	8 mos.	
63-6	Boiling point (7)					ABCDEFGHIJKLMNO	TGAI	8 mos.	
63-7	Density					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-8	Solubility					ABCDEFGHIJKLMNO	TGAI/PAI	8 mos.	
63-9	Vapor pressure					ABCDEFGHIJKLMNO	TGAI/PAI	8 mos.	

10. Certification

I certify that the statements made on this form and all attachments are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine, imprisonment or both under applicable law.

Signature and Title of Company's Authorized Representative_____

11. Date

12. Name of Company Contact

13. Phone Number

United States Environmental Protection Agency
Washington, D. C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address
SAMPLE COMPANY
1234 MAIN STREET
ANYWHERE, USA 54321

2. Case # and Name
0178 Glyphosate

EPA Reg. No. 70-269

3. Date and Type of DCI
PRODUCT SPECIFIC
ID# 70-RD-3263
FEB 16 1994

4. Guideline Requirement Number	5. Study Title		PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
63-10	Dissociation constant						ABCDEFGHIJKLMNO	TGAI/PAI	8 mos.	
63-11	Octanol/water partition coefficient	(8)					ABCDEFGHIJKLMNO	PAI	8 mos.	
63-12	pH	(9)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
63-13	Stability						ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-14	Oxidizing or reducing action	(10)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-15	Flammability	(11)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-16	Explodability	(12)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-17	Storage stability						ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-18	Viscosity	(13)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-19	Miscibility	(14)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-20	Corrosion characteristics						ABCDEFGHIJKLMNO	MP/EP	8 mos.	
63-21	Dielectric breakdown voltage	(15)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
	Acute Toxic - Regular Chemical									
81-1	Acute oral toxicity-rat	(1,36,37)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
81-2	Acute dermal toxicity-rabbit/rat	(1,2,37)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
81-3	Acute inhalation toxicity-rat	(3)					ABCDEFGHIJKLMNO	MP/EP and TGAI	8 mos.	
81-4	Primary eye irritation-rabbit	(2)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	
81-5	Primary dermal irritation	(1,2)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	

Initial to indicate certification as to information on this page (full text of certification is on page one).

Date

United States Environmental Protection Agency
Washington, D. C. 20460

REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 03-31-96

INSTRUCTIONS: Please type or print in ink. Please read carefully the attached instructions and supply the information requested on this form. Use additional sheet(s) if necessary.

1. Company name and Address	2. Case # and Name	3. Date and Type of DCI
SAMPLE COMPANY 1234 MAIN STREET ANYWHERE, USA 54321	0178 Glyphosate EPA Reg. No. 70-269	PRODUCT SPECIFIC ID# 70-RD-3263 FEB 16 1994

4. Guideline Requirement Number	5. Study Title	PROTOCOL	Progress Reports 1	2	3	6. Use Pattern	7. Test Substance	8. Time Frame	9. Registrant Response
81-6	Dermal sensitization (4)					ABCDEFGHIJKLMNO	MP/EP	8 mos.	

Initial to indicate certification as to information on this page (full text of certification is on page one).

Date

United States Environmental Protection Agency
Washington, D. C. 20460

FOOTNOTES AND KEY DEFINATIONS FOR GUIDELINE REQUIREMENTS

Case # and Name: 0178 Glyphosate

Key: MP = manufacturing-use product; EP = end-use product; provided formulators purchase their active ingredient(s) from a registered source, they need not submit or cite data pertaining to the purchased product. [NOTE: If a product is a 100 percent repackage of another registered product that is purchased, and any use for the product does not differ from those of the purchased and registered source, users are not subject to any data requirements identified in the tables.]; TEP = typical end-use product; TGAI = technical grade of the active ingredient; PAI = "pure" active ingredient; PAIRA = "pure" active ingredient, radiolabeled.

Use Categories Key:

A - Terrestrial food crop	B - Terrestrial food feed crop	C - Terrestrial nonfood crop	D - Aquatic food crop	E - Aquatic nonfood outdoor
F - Aquatic nonfood Industrial	G - Aquatic nonfood residential	H - Greenhouse food crop	I - Greenhouse nonfood crop	J - Forestry
K - Residential outdoor	L - Indoor food	M - Indoor nonfood	N - Indoor Medical	O - Indoor residential

Footnotes: [The following notes are referenced in column two (5. Study Title) of the REQUIREMENTS STATUS AND REGISTRANT'S RESPONSE form.]

Prod Chem - Regular Chemical

1 Requirements pertaining to product identity, composition, analysis, and certification of ingredients are detailed further in the following sections: *158.155 for product identity and composition (61-1); *158.160, 158.162, and 158.165 for description of starting materials and manufacturing process (61-2); *158.167 for discussion of formation of impurities (61-3); *158.170 for preliminary analysis (62-1); *158.175 for certification of limits (62-2); and *158.180 for enforcement analytical methods (62-3).
2 A schematic diagram and/or brief description of the production process will suffice if the pesticide is not already under full scale production and an experimental use permit is being sought.
3 If the pesticide is not already under full scale production and an experimental use permit is sought, a discussion of unintentional ingredients shall be submitted to the extent this information is available.
4 To support registration of an MP or EP, whether produced by an integrated system or not, the technical grade of Active Ingredient must be analyzed. If the technical grade of Active Ingredient cannot be isolated, a statement of composition of the practical equivalent of the technical grade of Active Ingredient must be submitted. Data on EPs or MPs will be required on a case-by-case basis.
5 Certified limits are not required for inert ingredients in products proposed for experimental use.
6 Required if technical chemical is solid at room temperature.
7 Required if technical chemical is liquid at room temperature.
8 Required if technical chemical is organic and non-polar.
9 Required if test substances are dispersible with water.
10 Required if product contains an oxidizing or reducing agent.
11 Required if product contains combustible liquids.
12 Required if product is potentially explosive.
13 Required if product is a liquid.
14 Required if product is an emulsifiable liquid and is to be diluted with petroleum solvents.
15 Required if end-use product is liquid and is to be used around electrical equipment.

Acute Toxic - Regular Chemical

1 Not required if test material is a gas or highly volatile.
2 Not required if test material is corrosive to skin or has pH less than 2 or greater than 11.5; such a product will be classified as Toxicity Category I on the basis of potential eye and dermal irritation effects.

United States Environmental Protection Agency
Washington, D. C. 20460

FOOTNOTES AND KEY DEFINATIONS FOR GUIDELINE REQUIREMENTS

Case # and Name: 0178 Glyphosate

Footnotes (cont.):

3 Required if the product consists of, or under conditions of use will result in, an inhalable material (e. g., gas, volatile substances, or aerosol/particulate).
4 Required unless repeated dermal exposure does not occur under conditions of use.
36 Special testing (acute, subchronic, and/or chronic) is required for organophospates, and may be required for other cholinesterase inhibitors and other pesticides which have demonstrated a potential to adversely affect the visual system. Registrants should consult with the agency for development of protocols and methodology prior to initiation of studies.
37 Testing of the EP dilution is required if it can be reasonably anticipated that the results of such testing may meet the criteria for restriction to use by certified applicators specified in 40 CFR 152.170(b) or the criteria for initiation of special review specified in 40 CFR 154.7 (a)(1).

Attachment 4

EPA Grouping of End Use Products for meeting Acute Toxicology Data Requirements

EPA'S BATCHING OF GLYPHOSATE PRODUCTS FOR MEETING ACUTE TOXICITY DATA REQUIREMENTS FOR REREGISTRATION

In an effort to reduce the time, resources and number of animals needed to fulfill the acute toxicity data requirements for reregistration of products containing the active ingredient glyphosate, the Agency has batched products which can be considered similar for purposes of acute toxicity. Factors considered in the sorting process include each product's active and inert ingredients (identity, percent composition and biological activity), type of formulation (e.g., emulsifiable concentrate, aerosol, wettable powder, granular, etc.), and labeling (e.g., signal word, use classification, precautionary labeling, etc.). Note that the Agency is not describing batched products as "substantially similar" since some products within a batch may not be considered chemically similar or have identical use patterns.

Batching has been accomplished using the readily available information described above, and frequently acute toxicity data on individual products has been found to be incomplete. Notwithstanding the batching process, the Agency reserves the right to require, at any time, acute toxicity data for an individual product should the need arise.

Registrants of products within a batch may choose to cooperatively generate, submit or cite a single battery of six acute toxicological studies to represent all the products within that batch. It is the registrants' option to participate in the process with all other registrants, only some of the other registrants, or only their own products within a batch, or to generate all the required acute toxicological studies for each of their own products. If a registrant chooses to generate the data for a batch, he/she must use one of the products within the batch as the test material. If a registrant chooses to rely upon previously submitted acute toxicity data, he/she may do so provided that the data base is complete and valid by today's standards (see acceptance criteria attached), the formulation tested is considered by EPA to be similar for acute toxicity, and the formulation has not been significantly altered since submission and acceptance of the acute toxicity data. Regardless of whether new data is generated or existing data is referenced, registrants must clearly identify the test material by EPA Registration Number.

In deciding how to meet the product specific data requirements, registrants must follow the directions given in the Data Call-In Notice and its attachments appended to the RED. The DCI Notice contains two response forms which are to be completed and submitted to the Agency within 90 days of receipt. The first form, "Data Call-In Response," asks whether the registrant will meet the data requirements for each product. The second form, "Requirements Status and Registrant's Response," lists the

product specific data required for each product, including the standard six acute toxicity tests. A registrant who wishes to participate in a batch must decide whether he/she will provide the data or depend on someone else to do so. If a registrant supplies the data to support a batch of products, he/she must select one of the following options: Developing Data (Option 1), Submitting an Existing Study (Option 4), Upgrading an Existing Study (Option 5) or Citing an Existing Study (Option 6). If a registrant depends on another's data, he/she must choose among: Cost Sharing (Option 2), Offers to Cost Share (Option 3) or Citing an Existing Study (Option 6). If a registrant does not want to participate in a batch, the choices are Options 1, 4, 5 or 6. However, a registrant should know that choosing not to participate in a batch does not preclude other registrants in the batch from citing his/her studies and offering to cost share (Option 3) those studies.

Fifty-six products were found which contain glyphosate as the active ingredient. The products have been placed into five batches and a "no batch" category in accordance with the active and inert ingredients, type of formulation and current labeling. Table 1 identifies the products in each batch. Table 2 lists the twenty-seven products which have been placed in the "no batch" category.

The Agency requires that products in batch four include separate primary eye irritation studies for each product within these batches. The remaining acute toxicity requirements for the products in batch four may be satisfied by one of the procedures described above.

Table 1

Batch	EPA Reg. No.	% Glyphosate	Formulation Type
1	70-269	0.96	Liq
	239-2467	0.5	Liq
	524-330	0.96	Liq
	7401-304	0.5	Liq
	7401-307	0.5	Liq
	7401-357	1.0	Liq
	7401-400	1.0	Liq
	7401-401	0.5	Liq
	7401-402	0.5	Liq
	7401-403	0.5	Liq
	10370-282	0.96	Liq
	10583-14	0.96	Liq
	46515-5	0.96	Liq
	56644-64	0.96	Liq
2	19713-320	0.96	Aerosol
	46515-7	0.96	Aerosol
3	70-284	5.0	Liq
	7401-306	5.0	Liq
	7401-404	5.0	Liq
	34911-25	5.0	Liq
	46515-3	5.0	Liq
	56644-48	5.0	Liq
4	524-339	41.0	Liq
	524-454	41.0	Liq
5	524-318	53.5	Liq
	524-343	53.8	Liq
	524-350	53.8	Liq
	19713-364	53.8	Liq

Table II lists products that were either considered not to be similar or the Agency lacked sufficient information for decision making and were not placed in any batch. Registrants of these products are responsible for meeting the acute toxicity data requirements separately for each product.

Table 2 (No batch)

EPA Reg. No.	% Glyphosate and other actives	Formulation Type
239-2469	Glyphosate 5.0	Liq
239-2509	Glyphosate 0.5, Acifluorfen 0.12	Liq
239-2516	Glyphosate 0.25, Oxyfluorfen 0.25	Liq
239-2596	Glyphosate 0.75	Aerosol
524-308	Glyphosate 41.0	Liq
524-326	Glyphosate 41.5	Liq
524-332	Glyphosate 75.0	Solid
524-333	Glyphosate 62.0	Liq
524-341	Glyphosate 14.8, Alachlor 27.6	Liq
524-370	Glyphosate 18.0	Liq
524-376	Glyphosate 13.3, 2,4-D 11.1	Liq
524-382	Glyphosate 28.6	Liq
524-390	Glyphosate 16.5, Dicamba 7.0	Liq
524-420	Glyphosate 96.3	Solid
524-421	Glyphosate 76.0	Solid
524-435	Glyphosate 83.5	Capsular
524-439	Glyphosate 7.7, Oxadiazon 14.9	Liq
524-440	Glyphosate 25.1	Liq
524-445	Glyphosate 41.0	Liq
524-449	Glyphosate 12.4, Oryzalin 11.8	Liq
524-450	Glyphosate 15.8	Liq
524-451	Glyphosate 0.96	Liq
524-452	Glyphosate 60.0	Solid
524-432	Glyphosate 18.3	Liq
7401-405	Glyphosate 10.0	Liq
935-48	Glyphosate 12.9, 2,4-D 20.6	Liq
10370-283	Glyphosate 10.0	Liq
10583-15	Glyphosate 8.2	Liq

Attachment 5

EPA Acceptance Criteria

SUBDIVISION D

Guideline	Study Title
Series 61	Product Identity and Composition
Series 62	Analysis and Certification of Product Ingredients
Series 63	Physical and Chemical Characteristics

61 Product Identity and Composition

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Name of technical material tested (include product name and trade name, if appropriate).

2.____ Name, nominal concentration, and certified limits (upper and lower) for each active ingredient and each intentionally-added inert ingredient.

3.____ Name and upper certified limit for each impurity or each group of impurities present at $\geq$ 0.1% by weight and for certain toxicologically significant impurities (e.g., dioxins, nitrosamines) present at <0.1%.

4.____ Purpose of each active ingredient and each intentionally-added inert.

5.____ Chemical name from Chemical Abstracts index of Nomenclature and Chemical Abstracts Service (CAS) Registry Number for each active ingredient and, if available, for each intentionally-added inert.

6.____ Molecular, structural, and empirical formulas, molecular weight or weight range, and any company assigned experimental or internal code numbers for each active ingredient.

7.____ Description of each beginning material in the manufacturing process.
- ____ EPA Registration Number if registered; for other beginning materials, the following:
- ____ Name and address of manufacturer or supplier.
- ____ Brand name, trade name or commercial designation.
- ____ Technical specifications or data sheets by which manufacturer or supplier describes composition, properties or toxicity.

8.____Description of manufacturing process.
- ____ Statement of whether batch or continuous process.
- ____ Relative amounts of beginning materials and order in which they are added.
- ____ Description of equipment.
- ____ Description of physical conditions (temperature, pressure, humidity) controlled in each step and the parameters that are maintained.
- ____ Statement of whether process involves intended chemical reactions.
- ____ Flow chart with chemical equations for each intended chemical reaction.
- ____ Duration of each step of process.
- ____ Description of purification procedures.
- ____ Description of measures taken to assure quality of final product.

9.____ Discussion of formation of impurities based on established chemical theory addressing (1) each impurity which may be present at $\geq$ 0.1% or was found at $\geq$ 0.1% by product analyses and (2) certain toxicologically significant impurities (see #3).

62 Analysis and Certification of Product Ingredients

ACCEPTANCE CRITERIA

The following criteria apply to the technical grade of the active ingredient being reregistered. Use a table to present the information in items 6, 7, and 8.

Does your study meet the following acceptance criteria?

1.____ Five or more representative samples (batches in case of batch process) analyzed for each active ingredient and all impurities present at $\geq$ 0.1%.
2.____ Degree of accountability or closure $\geq$ ca 98%.
3.____ Analyses conducted for certain trace toxic impurities at lower than 0.1% (examples, nitrosamines in the case of products containing dinitroanilines or containing secondary or tertiary amines/alkanolamines plus nitrites; polyhalogenated dibenzodioxins and dibenzofurans). [Note that in the case of nitrosamines both fresh and stored samples must be analyzed.].
4.____ Complete and detailed description of each step in analytical method used to analyze above samples.
5.____ Statement of precision and accuracy of analytical method used to analyze above samples.
6.____ Identities and quantities (including mean and standard deviation) provided for each analyzed ingredient.
7.____ Upper and lower certified limits proposed for each active ingredient and intentionally added inert along with explanation of how the limits were determined.
8.____ Upper certified limit proposed for each impurity present at $\geq$ 0.1% and for certain toxicologically significant impurities at <0.1% along with explanation of how limit determined.
9.____ Analytical methods to verify certified limits of each active ingredient and impurities (latter not required if exempt from requirement of tolerance or if generally recognized as safe by FDA) are fully described.
10.____ Analytical methods (as discussed in #9) to verify certified limits validated as to their precision and accuracy.

63 Physical and Chemical Characteristics

ACCEPTANCE CRITERIA

The following criteria apply to the technical grade of the active ingredient being reregistered.

Does your study meet the following acceptance criteria?

63-2 Color

____ Verbal description of coloration (or lack of it)
____ Any intentional coloration also reported in terms of Munsell color system

63-3 Physical State

____ Verbal description of physical state provided using terms such as "solid, granular, volatile liquid"
____ Based on visual inspection at about 20-25° C

63-4 Odor

____ Verbal description of odor (or lack of it) using terms such as "garlic-like, characteristic of aromatic compounds"
____ Observed at room temperature

63-5 Melting Point

____ Reported in °C
____ Any observed decomposition reported

63-6 Boiling Point

____ Reported in °C
____ Pressure under which B.P. measured reported
____ Any observed decomposition reported

63-7 Density, Bulk Density, Specific Gravity

____ Measured at about 20-25° C
____ Density of technical grade active ingredient reported in g/ml or the specific gravity of liquids reported with reference to water at 20° C. [Note: Bulk density of registered products may be reported in lbs/ft^3 or lbs/gallon.]

63-8 Solubility

____ Determined in distilled water and representative polar and non-polar solvents, including those used in formulations and analytical methods for the pesticide
____ Measured at about 20-25° C
____ Reported in g/100 ml (other units like ppm acceptable if sparingly soluble)

63-9 Vapor Pressure

____ Measured at 25° C (or calculated by extrapolation from measurements made at higher temperature if pressure too low to measure at 25° C)
____ Experimental procedure described
____ Reported in mm Hg (torr) or other conventional units

63-10 Dissociation Constant

____ Experimental method described
____ Temperature of measurement specified (preferably about 20-25°C)

63-11 Octanol/water Partition Coefficient

____ Measured at about 20-25° C

____ Experimentally determined and description of procedure provided (preferred method-45 Fed. Register 77350)

____ Data supporting reported value provided

63-12 pH

____ Measured at about 20-25° C

____ Measured following dilution or dispersion in distilled water

63-13 Stability

____ Sensitivity to metal ions and metal determined

____ Stability at normal and elevated temperatures

____ Sensitivity to sunlight determined

SUBDIVISION F

Guideline	Study Title
81-1	Acute Oral Toxicity in the Rat
81-2	Acute Dermal Toxicity in the Rat, Rabbit or Guinea Pig
81-3	Acute Inhalation Toxicity in the Rat
81-4	Primary Eye Irritation in the Rabbit
81-5	Primary Dermal Irritation Study
81-6	Dermal Sensitization in the Guinea Pig

81-1 Acute Oral Toxicity in the Rat

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ At least 5 young adult rats/sex/group.
3.____ Dosing, single oral may be administered over 24 hrs.
4.____ Vehicle control if other than water.
5.____ Doses tested, sufficient to determine a toxicity category or a limit dose (5000 mg/kg).
6.____ Individual observations at least once a day.
7.____ Observation period to last at least 14 days, or until all test animals appear normal whichever is longer.
8.____ Individual daily observations.
9.____ Individual body weights.
10.____ Gross necropsy on all animals.

Criteria marked with an * are supplemental and may not be required for every study.

81-2 Acute Dermal toxicity in the Rat, Rabbit or Guinea Pig

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1._____ Identify material tested (technical, end-use product, etc).
2._____ At least 5 animals/sex/group.
3.*____ Rats 200-300 gm, rabbits 2.0-3.0 kg or guinea pigs 350-450 gm.
4._____ Dosing, single dermal.
5._____ Dosing duration at least 24 hours.
6.*____ Vehicle control, only if toxicity of vehicle is unknown.
7._____ Doses tested, sufficient to determine a toxicity category or a limit dose (2000 mg/kg).
8._____ Application site clipped or shaved at least 24 hours before dosing.
9._____ Application site at least 10% of body surface area.
10._____ Application site covered with a porous nonirritating cover to retain test material and to prevent ingestion.
11._____ Individual observations at least once a day.
12._____ Observation period to last at least 14 days.
13._____ Individual body weights.
14._____ Gross necropsy on all animals.

Criteria marked with an * are supplemental and may not be required for every study.

81-3 Acute Inhalation Toxicity in the Rat

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ Product is a gas, a solid which may produce a significant vapor hazard based on toxicity and expected use or contains particles of inhalable size for man (aerodynamic diameter 15 μm or less).
3.____ At least 5 young adult rats/sex/group.
4.____ Dosing, at least 4 hours by inhalation.
5.____ Chamber air flow dynamic, at least 10 air changes/hour, at least 19% oxygen content.
6.____ Chamber temperature, 22° C ($\pm$2°), relative humidity 40-60%.
7.____ Monitor rate of air flow.
8.____ Monitor actual concentrations of test material in breathing zone.
9.____ Monitor aerodynamic particle size for aerosols.
10.___ Doses tested, sufficient to determine a toxicity category or a limit dose (5 mg/L actual concentration of respirable substance).
11.___ Individual observations at least once a day.
12.___ Observation period to last at least 14 days.
13.___ Individual body weights.
14.___ Gross necropsy on all animals.

81-4 Primary Eye Irritation in the Rabbit

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ Study not required if material is corrosive, causes severe dermal irritation or has a pH of ≤ 2 or ≥ 11.5.
3.____ 6 adult rabbits.
4.____ Dosing, instillation into the conjunctival sac of one eye per animal.
5.____ Dose, 0.1 ml if a liquid; 0.1 ml or not more than 100 mg if a solid, paste or particulate substance.
6.____ Solid or granular test material ground to a fine dust.
7.____ Eyes not washed for at least 24 hours.
8.____ Eyes examined and graded for irritation before dosing and at 1, 24, 48 and 72 hr, then daily until eyes are normal or 21 days (whichever is shorter).
9.*___ Individual daily observations.

Criteria marked with an * are supplemental and may not be required for every study.

81-5 Primary Dermal Irritation Study

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.____ Identify material tested (technical, end-use product, etc).
2.____ Study not required if material is corrosive or has a pH of ≤ 2 or ≥ 11.5.
3.____ 6 adult animals.
4.____ Dosing, single dermal.
5.____ Dosing duration 4 hours.
6.____ Application site shaved or clipped at least 24 hours prior to dosing.
7.____ Application site approximately 6 cm^2.
8.____ Application site covered with a gauze patch held in place with nonirritating tape.
9.____ Material removed, washed with water, without trauma to application site.
10.___ Application site examined and graded for irritation at 1, 24, 48 and 72 hr, then daily until normal or 14 days (whichever is shorter).
11.*__ Individual daily observations.

Criteria marked with an * are supplemental and may not be required for every study.

81-6 Dermal Sensitization in the Guinea Pig

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.___ Identify material tested (technical, end-use product, etc).
2.___ Study not required if material is corrosive or has a pH of ≤ 2 or ≥ 11.5.
3.___ One of the following methods is utilized:
   _____ Freund's complete adjuvant test
   _____ Guinea pig maximization test
   _____ Split adjuvant technique
   _____ Buehler test
   _____ Open epicutaneous test
   _____ Mauer optimization test
   _____ Footpad technique in guinea pig.
4.___ Complete description of test.
5.*__ Reference for test.
6.___ Test followed essentially as described in reference document.
7.___ Positive control included (may provide historical data conducted within the last 6 months).

Criteria marked with an * are supplemental and may not be required for every study.

Attachment 6

List of all Registrants sent this DCI

List of All Registrants Sent This Data Call-In Notice

Case # and Name
0178 Glyphosate
Chemical # and Name
103601 Isopropylamine glyphosate (N-(phosphonomethyl)gly

Company Number	Company Name	Additional Name	Address	City & State	Zip
000070	WILBUR-ELLIS COMPANY		BOX 16458	FRESNO CA	93755
000239	CHEVRON CHEMICAL CO	ORTHO CONSUMER PRODUCTS DIVISION	940 HENSLEY ST	RICHMOND CA	94804
000524	MONSANTO CO	AGENT FOR: MONSANTO AGRICULTURAL C	700 14TH ST, N.W. SUITE 1100	WASHINGTON DC	20005
000935	OCCIDENTAL CHEMICAL CORPORATION		DEVELOPMENT CENTER, V-81 BOX 344	NIAGARA FALLS NY	14302
007401	VOLUNTARY PURCHASING GROUP, INC.		P. O. BOX 460	BONHAM TX	75418
010370	ROUSSEL UCLAF CORP		95 CHESTNUT RIDGE RD	MONTVALE NJ	07645
010583	LUNDAL ASSOCIATES INC		7493 E TIMBERLANE COURT.	SCOTTSDALE AZ	85258
019713	DREXEL CHEMICAL CO		BOX 9306	MEMPHIS TN	38109
034911	HI-YIELD CHEMICAL COMPANY		BOX 460	BONHAM TX	75418
046515	CELEX CORPORATION		377 AMELIA ST.	PLYMOUTH MI	48170
056644	SECURITY PRODUCTS COMPANY OF DELAW		BOX 59084	MINNEAPOLIS MN	55459
066459	KAUAI TARO GROWERS ASSOCIATION		BOX 427	HANALEI HI	96714

Attachment 7

Cost Share/Data Compensation Forms

United States Environmental Protection Agency
Washington, DC 20460

CERTIFICATION WITH RESPECT TO DATA COMPENSATION REQUIREMENTS

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 3-31-9

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, DC 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070-0106), Washington, DC 20503.

Please fill in blanks below.

Company Name	Company Number
Chemical Name	EPA Chemical Number

I Certify that:

1. For each study cited in support of registration or reregistration under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) that is an exclusive use study, I am the original data submitter, or I have obtained the written permission of the original data submitter to cite that study.

2. That for each study cited in support of registration or reregistration under FIFRA that is NOT an exclusive use study, I am the original data submitter, or I have obtained the written permission of the original data submitter, or I have notified in writing the company(ies) that submitted data I have cited and have offered to: (a) Pay compensation for those data in accordance with sections 3(c)(1)(D) and 3(c)(2)(D) of FIFRA; and (b) Commence negotiation to determine which data are subject to the compensation requirement of FIFRA and the amount of compensation due, if any. The companies I have notified are: (check one)

   [ ] All companies on the data submitters' list for the active ingredient listed on this form (Cite-All Method or Cite-All Option under the Selective Method). (Also sign the General Offer to Pay below.)

   [ ] The companies who have submitted the studies listed on the back of this form or attached sheets, or indicated on the attached "Requirements Status and Registrants' Response Form."

3. That I have previously complied with section 3(c)(1)(D) of FIFRA for the studies I have cited in support of registration or reregistration under FIFRA.

Signature	Date
Name and Title (Please Type or Print)	

GENERAL OFFER TO PAY: I hereby offer and agree to pay compensation to other persons, with regard to the registration or reregistration of my products, to the extent required by FIFRA sections 3(c)(1)(D) and 3(c)(2)(D).

Signature	Date
Name and Title (Please Type or Print)	

United States Environmental Protection Agency
Washington, DC 20460

CERTIFICATION OF OFFER TO COST SHARE IN THE DEVELOPMENT OF DATA

Form Approved
OMB No. 2070-0107
2070-0057
Approval Expires 3-31-96

Public reporting burden for this collection of information is estimated to average 15 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M St., S.W., Washington, DC 20460; and to the Office of Management and Budget, Paperwork Reduction Project (2070-0106), Washington, DC 20503.

Please fill in blanks below.

Company Name	Company Number
Chemical Name	**EPA Chemical Number**

I Certify that:

My company is willing to develp and submit the data required by EPA under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), if necessary. However, my company would prefer to enter into an agreement with one or more registrants to develop jointly or share in the cost of developing data.

My firm has offered in writing to enter into such an agreement. That offer was irrevocable and included an offer to be bound by arbitration decision under section 3(c)(2)(B)(iii) of FIFRA if final agreement on all terms could not be reached otherwise. This offer was made to the following firm(s) on the following date(s):

Name of Firm(s)	Date of Offer

Certification:

I certify that I am duly authorized to represent the company name above, and that the statements that I have made on this form and all attachments therein are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment or both under applicable law.

Signature of Company's Authorized Representative	Date
Name and Title (Please Type or Print)	

U.S. Environmental Protection Agency (EPA)

Second Peer Review of Glyphosate

October 1991

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 30 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: SECOND Peer Review of Glyphosate

CAS No. 1071-83-6
EPA Chem. Code 417300
40 CFR 180.364
TOX Chem. No.: 661A
Reg Group: List A (6B)

FROM: William Dykstra, Ph.D.
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

and

George Z. Ghali, Ph.D. G. Ghali 8/22/91
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Robert Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

and

Lois Rossi, Chief
Reregistration Branch
Special Review and Reregistration Division (H7508W)

The Health Effects Division Carcinogenicity Peer Review Committee convened on June 26, 1991 to discuss and evaluate the weight of the evidence on Glyphosate with particular emphasis on its carcinogenic potential. The Committee concluded that Glyphosate should be classified as a Group E (evidence of non-carcinogenicity for humans), based upon lack of convincing carcinogenicity evidence in adequate studies in two animal species.

It should be emphasized, however, that designation of an agent in Group E is based on the available evidence at the time of evaluation and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

A. Individual in Attendance

1. Peer Review Committee (Signature indicates concurrence with the peer review unless otherwise stated.)

Penny Fenner-Crisp	Penelope G. Fenner-Crisp
William L. Burnam	Wm L Burnam
Karl Baetcke	Karl A. Baetcke
Marcia Van Gemert	Marcia van Gemert
Esther Rinde	E. Rinde
Hugh Pettigrew	Hugh M. Pettigrew
Marion Copley	Marion P. Copley
Lucas Brennecke	Lucas H. Brennecke
George Ghali	G. Ghali

2. Peer Review Members in Absentia (Committee members who were unable to attend the discussion; signature indicates concurrence with the overall conclusions of the Committee.)

Reto Engler	Reto Engler
Richard Hill	—
John Quest	John A. Quest
Kerry Dearfield	Kerry Dearfield
Yin-Tak Woo	Yin Tak Woo
Jean Parker	— NONCONCUR
William Sette	William Sette
Robert Beliles	DO NOT CONCUR
Julie Du	Julie T. D.

3. Scientific Reviewers (Committee or noncommittee members responsible for data presentation; signature indicates technical accuracy of panel report.)

William Dykstra	William Dykstra
Roger Gardner	Roger Gardner 9-5-91

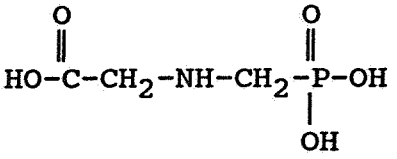
B. Background Information

Glyphosate is the isopropylamine (IPA) or sodium salt of N-(phosphonomethyl) glycine, marketed under the trade names of Roundup, Rodeo, Shackle, and Polado. Glyphosate is a wide spectrum plant growth regulator herbicide which is used to control grasses, sedges, and broadleaf weeds. It acts by the inhibition of amino acid synthesis.

Tolerances established for glyphosate and its aminomethyl phosphonic acid (AMPA) metabolite in 40 CFR 180.364 include the following:

IPA salt of glyphosate: soybeans, cotton, corn, sorghum, wheat, rice, vegetables, citrus fruits, pome fruits, stone fruits, tropical fruits, pastures, and alfalfa.

Sodium salt of glyphosate: sugarcane.



Glyphosate

On February 11, 1985, the carcinogenic potential of glyphosate was first considered by a panel (then called the Toxicology Branch Ad Hoc Committee) comprised of members of the Toxicology Branch of the Hazard Evaluation Division. The Committee, in a consensus review dated March 4, 1985, classified glyphosate as a Group C carcinogen based on an increased incidence of renal tubular adenomas in male mice. According to the consensus review, the tumor is rare, it occurred in a dose-related manner, and the incidence was outside the reported historical control range. The Committee also concluded that dose levels tested in a 26-month rat feeding study were not adequate for the assessment of glyphosate's carcinogenic potential in this species.

The kidney slides from the long-term mouse feeding study were subsequently reexamined, and one pathologist diagnosed an additional kidney tumor in control males. These findings were presented to the FIFRA Scientific Advisory Panel (SAP) which proposed that glyphosate be classified into Group D (inadequate animal evidence of carcinogenic potential). The SAP, in their meeting of February 11-12, 1986 (report dated February 24, 1986), concluded that, after adjusting for the greater survival in the high-dose mice compared to concurrent controls, no statistically significant pairwise differences existed, although the trend was significant. The SAP further noted that, although comparison of

these findings to historical control incidences yielded a statistically significant result, this finding did not override the lack of pairwise significance of comparisons to concurrent controls.

The SAP determined that the carcinogenic potential of glyphosate could not be determined from existing data and proposed that rat and/or mouse studies be repeated in order to clarify these equivocal findings.

HED deferred a decision on the repeat of an additional mouse oncogenicity study until the 1990 rat feeding study had been evaluated by the Peer Review Committee.

C. Material Evaluated

The material available for review consisted of a document prepared by Dr. William Dykstra summarizing major scientific and regulatory issues and relevant toxicology information, data evaluation records of a combined chronic toxicity/carcinogenicity study in rats and a carcinogenicity study in mice, the FIFRA Scientific Advisory Panel report dated Feb 24, 1986, a review of historical control data on mouse kidney tumors, a toxicology one-liner for the glyphosate data base and an OPP peer review report entitled "Consensus Review of Glyphosate" dated March 4, 1985.

D. Evaluation of Carcinogenicity Data

1. **Lankas, G. P. December 23, 1981. A Lifetime Study of Glyphosate in Rats. Unpublished report No. 77-2062 prepared by BioDynamics, Inc. EPA Acc. Nos. 247617 - 247621. MRID 00093879.**

a. Experimental Design

The lifetime feeding study in Sprague-Dawley rats at 50/sex/dose was conducted at dietary concentrations of glyphosate of 0, 30, 100, and 300 ppm. These concentrations were adjusted during the course of the study so that actual doses of 0, 3, 10, and 31 mg/kg/day in males and 0, 3, 11, and 34 mg/kg/day in female rats were maintained.

b. Discussion of Tumor Data

An increase in the incidence of interstitial cell tumors of the testes was observed in male rats. Because of the absence of a dose-response relationship, the lack of preneoplastic changes, the wide variability in the spontaneous incidence of this tumor, the similarity in incidences between the high-dose

group and the historical controls, and lack of any evidence of genotoxicity, it was concluded by the previous Peer Review Committee that the observed incidence did not reflect a carcinogenic response.

Additionally, there was the question of possible thyroid carcinomas in high-dose females. After a review of the slides by a consulting pathologist, and a reassessment of all relevant data, including the fact that no effect of treatment on tumor latency or the combined incidences of adenoma and carcinoma was apparent, the earlier Peer Review Committee concluded that the data did not demonstrate a carcinogenic response in the thyroid.

c. Nonneoplastic Lesions and Adequacy of Dosing Considerations

No effect of treatment on the incidence of nonneoplastic lesions was noted. No effects of treatment on survival, body weight gain, clinical pathology, or findings at necropsy were noted. Therefore, there is no evidence that the highest dose tested was adequate to evaluate the carcinogenic potential of glyphosate.

2. **Stout, L. D. and Ruecker, F. A. (1990). Chronic Study of glyphosate Administered in Feed to Albino Rats. Laboratory Project No. MSL-10495; Sept. 26, 1990. MRID No. 416438-01; Historical Controls; MRID No. 417287-00.**

a. Experimental Design

This chronic toxicity/carcinogenicity study in the rat was submitted to the Agency as a replacement study for the 26-month 1981 chronic toxicity/carcinogenicity study in the rat. In this study, randomized groups of 60 male and 60 female young (8 weeks old) Sprague-Dawley rats were fed dietary levels of 0, 2000, 8000, or 20,000 ppm or the equivalent of 0, 100, 400, and 1000 mg/kg/day of technical glyphosate for 2 years. At 12 months, 10 animals/sex/group were sacrificed.

b. Discussion of Tumor Data

Age-adjusted, statistical analyses of the tumor data are presented. The most frequently observed tumors in this study were pancreatic islet cell adenomas in males, thyroid C-cell adenomas and/or carcinomas in males and females, and hepatocellular adenomas and carcinomas in males. The following is a discussion of each type of tumor.

i. Pancreas (Tables 1 - 3)

Low-dose and high-dose males had a statistically significant increased incidence of pancreatic islet cell adenomas.

Table 1: Glyphosate - Sprague-Dawley Male Rats, Pancreatic Islet Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values).

	Dose (ppm)			
Tumors	0	2000	8000	20,000
Carcinomas	1/43[a]	0/45	0/49	0/48
(%)	(2)	(0)	(0)	(0)
p =	0.159	0.409(n)	0.467(n)	0.472(n)
Adenomas	1/43	8/45	5/49	7/48[b]
(%)	(2)	(18) *	(10)	(15) *
p =	0.170	0.018*	0.135	0.042*
Adenomas/carcinomas	2/43	8/45	5/49	7/48
(%)	(5)	(18)	(10)	(15)
p =	0.241	0.052	0.275	0.108
Hyperplasia only	2/43	0/45	3/49	2/48[c]
(%)	(5)	(0)	(6)	(4)
p =	0.323	0.236	0.526	0.649

[+] Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

[a] First carcinoma observed at week 105, dose 0 ppm.

[b] First adenoma observed at week 81, dose 20000 ppm.

[c] First hyperplasia observed at week 91, dose 20000 ppm.

[d] $p \leq 0.05$; Fisher's Exact test with Bonferoni correction.

Note:
Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. If * then $p < 0.05$.

Historical control data on the incidence of pancreatic islet cell adenomas from Monsanto's EHL are shown in Table 2 below.

Table 2: EHL 87122 - Historical Control Information for Histopathological Findings (All Deaths)

Terminal Necropsy Study	Months of Date	Study Length (Months)	No. Observed	No. Affected	% Affected
1	07/83	24	68	2	2.9
2	02/85	23	59	5	8.5
3	10/85	24	69	4	5.8
4	06/85	24	57	1	1.8
5	09/88	24	60	5	8.3
6	01/89	24	60	3	5.0
7	03/89	24	59	3	5.1

Committee's interpretation: Although the incidences of the pancreatic islet cell adenomas at the low-, mid- and high-dose groups exceeded the historical control range of 1.8 to 8.5 percent in male rats, there was no statistically significant positive dose-related trend in the occurrence of these tumors in males, no progression to carcinoma, and the incidence of hyperplasia was not dose-related. Therefore, the pancreatic islet cell tumors were not considered to be compound-related. It was also noted that the incidence of this lesion in the concurrent control for males was at the low end of the historical control range. The Committee concluded that the apparent statistical significance of the pairwise comparisons of the treated male groups with the concurrent control might have been attributable to this factor and not to actual carcinogenic response.

The incidences of islet cell pancreatic tumors in the earlier rat study (Bio/dynamics Project No. 77-2062) are shown in Table 3. The incidence of pancreatic islet cell tumors for the two studies does not show a dose-related increase in adenomas or adenoma/carcinoma combined and is within the range of open literature control data for male Sprague-Dawley rats (0 to 17%) for unadjusted data.

Table 3: Incidence of Pancreatic Islet Cell Tumors in Male Sprague-Dawley Rats Given Diets Containing Glyphosate for 26 Months (first rat feeding study).

Tumors	Dose (mg/kg/day) 0	3	10	30
Hyperplasia	3/50	2/49	1/50	0/50
(%)	(6)	(4)	(2)	(0)
Adenomas	0/50	5/49	2/50	2/50
(%)	(0)	(10)	(4)	(4)
Carcinomas	0/50	0/49	0/50	1/50
(%)	(0)	(0)	(0)	(2)
Adenoma/carcinoma	0/50	5/49	2/50	3/50
(%)	(0)	(10)	(4)	(6)

ii. Thyroid (Tables 4 - 6)

C-cell adenomas were slightly increased in male and female mid- and high-dose groups as shown in Tables 4 and 5. Historical control ranges for the thyroid tumors in Sprague-Dawley rats were reported as shown in Table 6.

Committee's interpretation: Although C-cell adenomas slightly exceeded the historical control range for both sexes, there was no statistically significant trend or pairwise comparison with controls in males. In females, the incidence of C-cell adenomas was not statistically significant in the pairwise comparison with controls but had a statistically significant positive dose-related trend. However, there was no progression to carcinoma in a dose-related manner, and no significant dose-related increase in severity of grade or incidence of hyperplasia in either sex. Therefore, the C-cell adenomas in males and females are not considered compound-related.

Table 4: Glyphosate - Sprague-Dawley Male Rats, Thyroid C-Cell Tumor Rates[+] and Cochran-Armitage Trend and Fisher's Exact Test Results (p values).

	Dose (ppm)			
Tumors	0	2000	8000	20,000
Carcinomas	0/54	2/55[a]	0/58	1/58
(%)	(0)	(4)	(0)	(2)
p =	0.452	0.252	1.000	0.518
Adenomas	2/54[b]	4/55	8/58	7/58
(%)	(4)	(7)	(14)	(12)
p =	0.069	0.348	0.060	0.099
Adenoma/carcinoma	2/54	6/55	8/58	8/58
(%)	(4)	(11)	(14)	(14)
p =	0.077	0.141	0.060	0.060
Hyperplasia only	4/54	1/55	5/58[c]	4/58
(%)	(7)	(2)	(9)	(7)
p =	0.312	0.176	0.546	0.601

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[a] First carcinoma observed at week 93 at 8000 ppm.

[b] First adenoma observed at week 54 at 0 ppm.

[c] First hyperplasia observed at week 54 at 8000 ppm.

[+] Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. If * then $p < 0.05$.

Table 5: Glyphosate - Sprague-Dawley Female Rats, Thyroid C-Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Tests Results (p values).

	Dose (ppm)			
Tumors	0	2000	8000	20,000
Carcinomas	0/57	0/60	1/59[a]	0/55
(%)	(0)	(0)	(2)	(0)
p =	0.445	1.000	0.509	1.000
Adenomas	2/57	2/60	6/59[b]	6/55
(%)	(4)	(3)	(10)	(11)
p =	0.031*	0.671(n)	0.147	0.124
Adenoma/carcinoma	2/57	2/60	7/59	6/55
(%)	(4)	(3)	(12)	(11)
p =	0.033*	0.671(n)	0.090	0.124
Hyperplasia only	10/57[c]	5/60	7/59	4/55
(%)	(18)	(8)	(12)	(7)
p =	0.113	0.112	0.274	0.086(n)

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[a] First carcinoma observed at week 93 at 8000 ppm.

[b] First adenoma observed at week 72 at 0 ppm.

[c] First hyperplasia observed at week 54 at 8000 ppm.

[+] Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

(n) Negative change from control.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. If * then $p < 0.05$.

Table 6: Historical Control Data for the Incidence of Thyroid C-Cell Tumors in Sprague-Dawley Strain Rats.

	Range (%)	
Tumor	Males	Females
Carcinomas	0.0 - 5.2	0.0 - 2.9
Adenomas	1.8 - 10.6	3.3 - 10.0
Hyperplasia	4.3 - 20.0	4.3 - 16.9

iii. Liver (Table 7)

There was a slight dose-related increase in hepatocellular adenomas in males but the incidence was within the range of historical controls from Monsanto's EHL. The reported historical control incidence of hepatocellular carcinomas ranged from 0 to 6.7%, and that for hepatocellular adenomas ranged from 1.4 to 18.3%. There were no dose-related increases in the incidences of other hepatocellular lesions.

Table 7: Glyphosate - Sprague-Dawley Male Rats, Hepatocellular Tumor Rates[+] and Cochran-Armitage Trend and Fisher's Exact Test Results (p values).

	Dose (ppm)			
Tumors	0	2000	8000	20,000
Carcinomas	3/44	2/45	1/49	2/48[a]
(%)	(7)	(4)	(2)	(4)
p =	0.324	0.489(n)	0.269(n)	0.458(n)
Adenomas	2/44	2/45	3/49	7/48[b]
(%)	(5)	(4)	(6)	(15)
p =	0.016*	0.683(n)	0.551	0.101
Adenoma/carcinoma	5/44	4/45	4/49	9/48
(%)	(11)	(9)	(8)	(19)
p =	0.073	0.486(n)	0.431(n)	0.245
Hyperplasia only	0/44	0/45	1/49[c]	0/48
(%)	(0)	(0)	(2)	(0)
p =	0.462	1.000	0.527	1.000

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[a] First carcinoma observed at week 85 at 20,000 ppm.

[b] First adenoma observed at week 88 at 20,000 ppm.

[c] First hyperplasia observed at week 89 at 8000 ppm.

[+] Number of tumor-bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 55.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. If * then $p < 0.05$.

Committee's interpretation: Despite the slight dose-related increase in hepatocellular adenomas in males, this increase was not significant in the pair-wise comparison with controls and was within the historical control range. Furthermore, there was no progression from adenoma to carcinoma and incidences of hyperplasia were not compound-related. Therefore, the slightly increased occurrence of hepatocellular adenomas in males is not considered compound-related.

c. Nonneoplastic lesions

There were no compound-related nonneoplastic lesions.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The HDT was 20,000 ppm which is the limit dose for carcinogenicity testing in rats. However, it appears that animals could have tolerated higher doses.

3. **Hogan, G. K. (1983). A chronic feeding study of glyphosate in mice. Unpublished report prepared by Bio/Dynamics Inc., dated July 21, 1983. Report No. 77-2061. EPA Acc. Nos. 251007 - 251009, and 251014.**

a. Experimental Design

Groups of 50 male and 50 female CD-1 mice were administered glyphosate in the diet at concentrations of 1000, 5000, or 30,000 ppm for 18 months.

b. Discussion of Tumor Data

Glyphosate produced an equivocal carcinogenic response in males characterized by an incidence of renal tubular neoplasms of 1/49, 0/49, 1/50, and 3/50 in the control, low-, mid-, and high-dose groups, respectively. No kidney tumors were found in females. Historical control data from 16 studies terminated between 1978 and 1982 provided by the testing laboratory indicated that the incidence of this type of tumor was found in 2/19 control groups (1/54 and 2/60, or a total of 3/1286).

The Toxicology Branch Ad Hoc Oncogenicity Peer Review Committee, in their meeting of February 11, 1985, tentatively classified glyphosate as a "Class C" carcinogen (report dated March 4, 1985). The kidney slides were reexamined by a consulting pathologist, and data were submitted indicating that an additional kidney tumor had been found in control males (the incidence in the control group was originally reported as 0/49 before the reexamination of the slides).

The Agency then requested that additional kidney sections from the mouse study be prepared and examined. The resultant microslides were examined by a number of pathologists. These examinations revealed no additional tumors, but confirmed the presence of the tumors identified in the original study report. The tumor in the control kidney was not present in any of the additional sections.

Because of the equivocal nature of the findings, the Toxicology Branch Ad Hoc Oncogenicity Peer Review Committee asked the expert assistance of the FIFRA Scientific Advisory Panel (SAP) in determining the proper Weight-of-the-Evidence classification of the study. After reviewing all the available evidence, the SAP, in their meeting of February 11-12, 1986, proposed that glyphosate be classified as "Class D," or having "inadequate animal evidence of oncogenicity." The principal reason for this assessment by SAP was their determination that, after adjusting for the greater survival in the high-dose mice compared to concurrent controls, no statistically significant pairwise differences existed, although the trend was significant. The SAP further noted that, although comparison of these findings to historical control incidences yielded a statistically significant result, this finding did not override the lack of pairwise significance of comparisons to concurrent controls.

The SAP determined that the carcinogenic potential of glyphosate could not be determined from existing data and proposed that rat and/or mouse studies be repeated in order to clarify these equivocal findings.

Committee's interpretation: In their meeting of June 26, 1991, the Health Effects Carcinogenicity Peer Review Committee concluded that despite the fact that the incidence of renal tubular neoplasm in the high dose males exceeded that of historical controls, the biological significance of the findings was questionable because of: a) lack of significance in pairwise comparison with concurrent controls, b) there was no concurrent increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/regeneration, hyperplasia, hypertrophy ..etc), c) the examination of multiple sections of kidneys from all groups resulted in no additional neoplasms; this fact is particularly important since not only were the original sections closely scrutinized by more than one pathologist, but additional sections as well, and d) increased incidence in high dose group was very small compared to control considering the very high concentration which produced highly significant reduction in body weight gain in males. Furthermore, the increased incidence of chronic interstitial nephritis in males is not relevant to the tubular neoplasms. There was actually a decrease in renal tubular epithelial changes (basophilia and hyperplasia) in males, and although there was a dose-related increase in these changes in female mice, no tubular neoplasms were observed in females.

c. Nonneoplastic lesions:

Other nonneoplastic changes noted in high-dose male mice included centrilobular hypertrophy and necrosis of hepatocytes, chronic interstitial nephritis, and proximal tubule epithelial cell basophilia and hypertrophy in the kidneys of females. The no-observable-effect level (NOEL) for nonneoplastic chronic effects was the mid-dose level, 5000 ppm.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Glyphosate was tested in this study at levels higher than the limit dose. Body weight gain in males of the high dose was 13, 17 and 27% less than the controls at 3, 12 and 24 months respectively. The decrease in body weight gains was statistically significant ($p < 0.01$). This effect was less obvious in females. The doses tested were considered adequate for the carcinogenic potential assessment of glyphosate.

E. Additional Toxicology Data on Glyphosate

1. Metabolism

When Sprague-Dawley rats were given a single oral dose of C-14 glyphosate, 30 to 36 percent of orally administered glyphosate was absorbed.

Data showed that less than 0.27 percent of the dose was expired as CO_2 within 24 hours. Glyphosate, per se, was the highest radiolabeled material found in the urine and feces. The minimum level of glyphosate extracted from urine and feces was 97.5 percent. Amino methyl phosphonic acid (AMPA) was found in the excreta of animals at levels of 0.2 to 0.3 percent and 0.2 to 0.4 percent in urine and feces, respectively. No detectable AMPA metabolite was found in intravenously dosed rats and high dose, orally dosed rats. There were no other metabolites of glyphosate found.

Based on analysis of radioactivity in urine and feces and using the "sigma-minus" plotting method, males and females had alpha half-lives of 2.11 and 7.52 hours and 5.00 to 6.44 hours, respectively. The beta half-lives of males and females in these groups ranged from 69.0 to 181 hours for males and 79.9 to 337 hours for females.

Less than 1 percent of the absorbed dose remains in tissues and organs, primarily bone. Repeated dosing with glyphosate

does not significantly change the metabolism, distribution, or excretion of glyphosate.

N-Nitrosoglyphosate (NNG)

The Agency has determined that carcinogenicity testing of nitroso contaminants will normally be required only in those cases in which the level of nitroso compounds exceeds 1.0 ppm [see "Pesticide Contaminated with N-nitroso Compounds, proposed policy 45 FR 42854 (June 25, 1980)"]. The levels of NNG in technical glyphosate have been examined by HED. The overall NNG content in individual samples of technical glyphosate analyzed at production plants is shown below:

Samples Analyzed		NNG Observed
No. Samples	Per cent	(ppb)
2035		
124		
24		
13		
2		

The overall data show that 92.6 percent of the individual glyphosate samples analyzed contain less than 1.0 ppm (1000 ppb) of NNG. TB concluded that the NNG content of glyphosate technical is not toxicologically significant.

2. Mutagenicity

Glyphosate has been tested in several mutagenicity assays and found to be negative in each of the three categories recommended for evaluating genotoxic potential. The acceptable studies include the following: Salmonella assay, both with and without S-9, up to toxicity or 5000 ug/plate, in vivo cytogenetic assay in rat bone marrow up to 1000 mg/kg, mammalian gene HGPRT mutation assay in CHO cells in vitro both with and without S-9 up to toxic levels (10 mg/mL) and rec assay with B. subtilis up to 2000 ug/disk.

Unacceptable studies which were also negative included DNA repair in rat hepatocytes between 0.0000135 and 0.125 mg/ml, and a dominant lethal assay in mice up to 2000 mg/kg.

3. Developmental and Reproductive Toxicity

In rats, doses up to 3500 mg/kg/day showed no evidence of malformations. Evidence of developmental toxicity in the form of unossified sternebrae and decreased fetal body weight was noted in fetuses from the high dose (3500 mg/kg/day). This dose was also toxic to dams as evidenced by weight gain

deficits, altered physical appearance, and mortality during treatment. The developmental and maternal toxic NOEL for this study was 1000 mg/kg/day.

In rabbits, doses up to 350 mg/kg/day showed no evidence of malformations. The highest dose tested was toxic to does as evidenced by altered physical appearance and mortality. No treatment-related developmental effects were noted. The NOEL for maternal toxicity is 175 mg/kg/day and the NOEL for developmental toxicity is 350 mg/kg/day.

In a three-generation reproduction study in the rat, the only toxicologically significant finding was focal renal tubular dilation in the kidneys of male pups from the F_{3b} generation of high-dose dams (30 mg/kg/day). The NOEL for this effect was 10 mg/kg/day. No effects on fertility, reproductive, or other study parameters were noted.

4. Structure - Activity Relationships

Currently there are no structurally related pesticides registered by the Agency which resemble glyphosate. A nonregistered pesticide, sulfosate, has been reviewed for carcinogenic potential in mice and rats and reported to be negative.

5. Acute, Subchronic and Chronic Feeding/ Oncogenicity Data

Glyphosate is not considered to be toxic to mammals (rat oral LD_{50} of 4320 mg/kg (both sexes), and a dermal LD_{50} greater than 7940 mg/kg in rabbits).

A 1-year chronic feeding study in dogs at 6/sex/dose was conducted using doses of 0, 20, 100, and 500 mg/kg/day, administered by capsule. The NOEL for the study was 500 mg/kg/day (HDT).

F. Weight of the Evidence Considerations

The Committee considered the following findings to be of significance regarding the weight-of-the-evidence determination of the carcinogenic potential of glyphosate.

1. Glyphosate was associated with increased incidences of pancreatic islet cell adenomas in male Sprague-Dawley rats at all treatment levels in comparison to the concurrent control group (Table 1). Although the low- (18%), mid- (10%) and high-dose group (15%) incidences exceeded the 1.8 to 8.5% range of historical controls from Monsanto's EHL data base, the pancreatic islet cell adenomas were not considered

compound-related for the following reasons: a) there was no statistically significant positive dose-related trend in the occurrence of these tumors or in the incidence of hyperplasia in males over the wide range of dosing (2000 to 20000 ppm), and b) there was no progression to carcinoma. Tertiary evidence from the open literature cited by the registrant showed a range of 0 to 17% for pancreatic islet cell adenomas in Sprague-Dawley male rats for unadjusted data. The incidence of pancreatic islet cell tumors for the two rat studies does not show a dose-related increase in adenomas or adenoma/carcinoma combined and is within the range of open literature control data for male Sprague-Dawley rats (0 to 17%) for unadjusted data.

No increased incidence of these tumors was observed in female rats in comparision to concurrent controls.

2. C-cell adenomas were slightly increased in male and female mid- and high-dose groups in the rat (Tables 4 and 5). Although C-cell adenomas slightly exceeded the historical control range for both sexes, there was no statistically significant trend or pairwise comparison with controls in males. In females, the incidence of C-cell adenomas was not statistically significant in the pairwise comparison with controls but had a statistically significant positive dose-related trend. However, there was no progression to carcinoma in a dose-related manner, and no significant dose-related increase in severity of grade or incidence of hyperplasia in either sex. Therefore, the C-cell adenomas in males and females are not considered compound-related.

3. There was a slight dose-related increase in hepatocellular adenomas in male rats (Table 7), but the incidence was within the range of historical controls from Monsanto's EHL. This increase was not significant in the pair-wise comparison with controls and there was no progression from adenoma to carcinoma. The incidence of hyperplasia was not compound-related. There were no dose-related increases in the incidences of other hepatocellular lesions. Therefore, the increased incidence of hepatocellular adenomas in males was not considered compound-related.

4. Glyphosate produced an equivocal carcinogenic response in male mice characterized by an incidence of renal tubular neoplasms of 1/49, 0/49, 1/50, and 3/50 in the control, low-, mid-, and high-dose groups, respectively. No kidney tumors were found in females. Historical control data from 16 studies terminated between 1978 and 1982 provided by the testing laboratory indicated that the incidence of this type of tumor was found in 2/19 control groups (1/54 and 2/60, or a total of 3/1286).

Despite the fact that the incidence of renal tubular neoplasm in the high dose males exceeded that of historical controls, the biological significance of the findings was questionable because of: a) lack of significance in pairwise comparison with concurrent controls, b) there was no concurrent increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/regeneration, hyperplasia, hypertrophy ..etc), c) the examination of multiple sections of kidneys from all groups resulted in no additional neoplasms; this fact is particularly important since not only were the original sections closely scrutinized by more than one pathologist, but additional sections as well, and d) increased incidence in high dose group was very small compared to control considering the very high concentration which produced highly significant reduction in body weight gain in males. Furthermore, the increased incidence of chronic interstitial nephritis in males is not relevant to the tubular neoplasms. There was actually a decrease in renal tubular epithelial changes (basophilia and hyperplasia) in males, and although there was a dose-related increase in these changes in female mice, no tubular neoplasms were observed in females. Overall, the Peer Review Committee did not feel that this lesion was compound-related.

5. Glyphosate was tested up to the limit dose in the rat, and up to levels higher than the limit dose in mice.

6. There was no evidence of genotoxicity for glyphosate.

7. Currently there are no structurally related pesticides registered by the Agency which resemble glyphosate. A nonregistered pesticide, sulfosate, has been reviewed for carcinogenic potential in mice and rats and was reported to be negative.

G. <u>Classification</u>:

Considering criteria contained in EPA Guidelines (FR 51:33992-34003, 1986] for classifying a carcinogen, the Committee concluded that Glyphosate should be classified as a Group E (evidence of non-carcinogenicity for humans), based on lack of convincing carcinogenicity evidence in adequate studies in two animal species.

It should be emphasized, however, that designation of an agent in Group E is based on the available evidence at the time of evaluation and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

Glyphosate

1987

4.17 Glyphosate (T,R)

TOXICOLOGY

Glyphosate was evaluated toxicologically by the 1986 JMPR, which allocated an ADI of 0-0.3 mg/kg bw.

The primary degradation product of glyphosate in plants, soil, and water, is aminomethylphosphonic acid (AMPA), whose chemical structure is very similar to that of glyphosate. AMPA itself has no commercial use. On the basis of the low residual levels of AMPA in crops which are susceptible to glyphosate the 1986 Joint Meeting concluded that AMPA could be omitted from the definition of the residue when considering recommendations for MRLs, but recent supervised trials on the application of glyphosate to crops genetically modified to be glyphosate-resistant have shown that AMPA can be the main residue. As residues of AMPA may therefore be of toxicological concern, the compound was evaluated by the present Meeting.

After oral administration of AMPA to rats, 20% of the dose was absorbed and excreted unmetabolized in the urine within 120 h (17% of the dose within 24 h), and 73% of the dose was eliminated in the faeces. Only 0.07% of the dose was excreted as expired carbon dioxide within 24 h, and 0.06% was recovered from tissues after 120 h. Minor amounts (1-6 μ g/kg) were found in tissues after 120 h.

AMPA is slightly hazardous to rats given a single oral dose, with an LD_{50} of 8300 mg/kg bw.

In a 90-day study of toxicity, rats received AMPA in the diet at 0, 400, 1200, or 4800 mg/kg bw per day. A significant, dose-related decrease in body-weight gain was seen in males at the two highest doses and in females at the highest dose. The two highest doses also resulted in significantly increased lactate dehydrogenase activity, whereas aspartate aminotransferase activity and cholesterol levels were significantly increased only at the highest dose. Urinalysis showed a significant decrease in urinary pH and increased amounts of calcium oxalate crystals in the urine of animals at the highest dose. Dose-related irritation of the mucosal and submucosal layers of the urinary tract, corresponding to hyperplasia of the urinary bladder, was seen in rats at 1200 and 4800 mg/kg bw per day, the effect being more marked in males than in females. In addition, epithelial hyperplasia in the renal pelvis was observed at the highest dose. The NOAEL was 400 mg/kg bw per day.

In a 90-day study of toxicity in dogs receiving AMPA at 0, 10, 30, 100, or 300 mg/kg bw per day in gelatin capsules, no statistically significant treatment-related changes were observed. The NOAEL was thus the highest dose, 300 mg/kg bw per day. It should be noted that in a one-month range-finding study with groups of only two male and two female dogs, changes in some haematological parameters (e.g. decreased haemoglobin and PCVs, decreased erythrocyte counts) were seen in animals at 300 or 1000 mg/kg bw per day. These effects were not reproduced in the 90-day study.

No indication of genotoxic activity was seen in studies of gene mutation in bacteria, of DNA repair in bacteria and mammalian cells *in vitro,* or of micronucleus formation *in vivo.* No assays for gene mutation were performed in mammalian cells *in vitro,* but the structural similarity of AMPA to glyphosate and the negative results of genotoxicity assays of glyphosate, including one for gene mutation in mammalian cells *in vitro,* indicate that such an assay with AMPA would be redundant.

In a study of developmental toxicity, rats received AMPA at 0, 150, 400, or 1000 mg/kg bw per day in corn oil by gavage. Dose-related increases in the incidences of soft stools, mucoid faeces, and hair loss were seen in dams at the two higher doses. Dams at the highest dose also had short periods of decreased body-weight gain and food consumption.

Fetal body weight was decreased at 1000 mg/kg bw per day. No teratogenic effects were observed. Dams at 150 mg/kg bw per day also had an increased incidence of soft stools; however in the absence of any associated effects, such as hair loss or mucoid faeces, the Meeting considered this dose to be the NOAEL for maternal toxicity. The NOAEL for developmental toxicity was 400 mg/kg bw per day.

AMPA did not induce dermal or ocular irritation in rabbits.

No long-term study of the toxicity or carcinogenicity of AMPA has been carried out, but in the more recent of two such studies with technical-grade glyphosate in rats at dietary levels of 0.2, 0.8, or 2%, the AMPA content of the test compound was given, namely 0.68%. At the highest dose of 2% glyphosate in the diet, females showed decreased body-weight gain and males showed an increased incidence of degenerative lenticular changes. The NOAEL for technical-grade glyphosate was 0.8% in the diet, corresponding to 400 mg/kg bw per day for glyphosate and 2.7 mg/kg bw per day for AMPA. No increase in tumour incidence was seen in this study (as evaluated by the International Programme on Chemical Safety (IPCS)[1]).

No multigeneration study of the reproductive toxicity of AMPA has been reported, but in a recent two-generation study in rats with technical-grade glyphosate at dietary levels of 0.2, 1, or 3%, the test compound contained 0.61% AMPA. At the highest dose, soft stools, decreased parental body weights, slightly decreased litter sizes, and decreased pup weights were observed. The NOAEL was 1% in the diet, corresponding to 740 mg/kg bw per day glyphosate and 4.5 mg/kg bw per day AMPA (as evaluated by IPCS[1]).

[1]WHO (1994) *Glyphosate* (Environmental Health Criteria 159), Geneva

Glyphosate and AMPA have very similar chemical structures. Studies of the metabolism of glyphosate in experimental animals indicate that essentially none is biotransformed into AMPA. Toxicological data on the metabolite are therefore essential for risk assessment. The Meeting compared the toxicity profile of AMPA with that of glyphosate and concluded that the major targets of the toxicity of AMPA had been investigated. The results showed little toxicity. The Meeting concluded that the two compounds have similar toxicological profiles and considered that a full database on AMPA is unnecessary. AMPA was considered to be of no greater toxicological concern than its parent compound. The Meeting established a group ADI for AMPA alone or in combination with glyphosate of 0-0.3 mg/kg bw on the basis of the 26-month study of toxicity in rats fed technical-grade glyphosate, using a safety factor of 100 (see 1986 JMPR report and toxicological evaluations, FAO/WHO, 1986d, 1987a).

Since the last JMPR evaluation for toxicity in 1986, new data have become available on glyphosate, some of which are evaluated in EHC 159. The Meeting therefore recommended that glyphosate be re-evaluated by the JMPR.

A toxicological monograph on AMPA was prepared.

TOXICOLOGICAL EVALUATION

Levels that cause no toxic effect

AMPA

Rat: 400 mg/kg bw per day (90-day study of toxicity)
150 mg/kg bw per day (maternal toxicity in a study of developmental toxicity)
400 mg/kg bw per day (fetal toxicity in a study of developmental toxicity)

Dog: 300 mg/kg bw per day (highest dose in 90-day study of toxicity)

Glyphosate (from 1986 JMPR)

Mouse: 0.5% in the diet, equal to 814 mg/kg bw per day (two-year study of toxicity and carcinogenicity)

Rat: 31 mg/kg bw per day (26-month study of toxicity and carcinogenicity)

Dog: 500 mg/kg bw per day (one-year study of toxicity)

Estimate of acceptable daily intake for humans

0-0.3 mg/kg bw (sum of glyphosate and AMPA)

Toxicological criteria for setting guidance values for dietary and non-dietary exposure to aminomethylphosphonic acid (AMPA)

Human exposure	Relevant route, study type, species	Results/remarks
Short-term (1-7 days)	Oral toxicity, rat	LD_{50} = 8300 mg/kg bw
	Skin irritation, rabbit	Not irritating
	Eye irritation, rabbit	Not irritating
	Skin sensitization	No data
Medium-term (1-26 weeks)	Repeated oral, 90 days, toxicity, rat	NOAEL = 400 mg/kg bw per day: urinary tract changes
		NOAEL = 150 mg/kg bw per day: maternal toxicity
	Repeated oral, developmental toxicity, rat	NOAEL = 400 mg/kg bw per day: developmental toxicity.
	Repeated oral, reproductive toxicity	No data
Long-term (> 1 year)	Repeated oral, toxicity	No data

RESIDUE AND ANALYTICAL ASPECTS

Glyphosate was first evaluated in 1986, and residue aspects were reviewed in 1987, 1988 and 1994. Maximum residue levels were estimated for kiwifruit and a range of vegetables, cereals, oilseeds and animal products.

The 1997 JMPR was requested to evaluate the new uses of glyphosate on cotton, maize and sorghum according to GAP. These new uses are (1) pre-harvest topical applications and (2) in-crop applications to cotton and maize crops which have been genetically modified to be resistant to glyphosate. Relevant data on metabolism and residue trials were submitted to the Meeting.

Genetic modification of crops

Glyphosate binds to and blocks the activity of 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), an enzyme of the aromatic amino acid biosynthetic pathway. Glyphosate inhibition of EPSPS prevents the plant from synthesizing the aromatic amino acids essential for protein production, Glyphosate-resistant EPSPS is derived from *Agrobacterium sp.* strain CP4 (CP4 EPSPS), and has been used to develop glyphosate-resistant (i.e. glyphosate-tolerant) crops.

While CP4 EPSPS has been successful in providing glyphosate resistance in cotton, its activity alone has been insufficient to ensure adequate resistance in other crops. In maize, a second mechanism has been developed to ensure sufficient levels of crop resistance to allow applications of glyphosate at rates necessary for effective weed control. The second mechanism is glyphosate inactivation, which effectively reduces cellular levels of glyphosate by converting it to aminomethylphosphonic acid (AMPA). The enzyme

responsible for glyphosate inactivation is glyphosate oxidoreductase (gox). The gene encoding *gox* was isolated from a naturally-occurring bacterium, *Achromobacter sp.,* and has been modified to optimize its expression in plants.

Plant metabolism

Numerous plant metabolism studies with vegetable, orchard tree, nut tree and pasture crops were reported to the 1986 JMPR. The 1986 Meeting concluded that glyphosate applied to the soil was absorbed very slightly or not at all by the crops examined and its conversion to AMPA, the primary metabolite, was not observed.

However, hydroponic administration allows sufficient uptake of glyphosate to elucidate its metabolism in plants. Metabolic studies with glyphosate in hydroponically-grown maize, wheat, cotton and soya beans have shown the conversion of glyphosate to AMPA and further degradation in plant tissues.

Metabolic studies in plants that have been genetically modified to be resistant to glyphosate show that the metabolism is the same as in susceptible plants. Glyphosate is metabolized to AMPA, which is either non-selectively bound to natural plant constituents, further degraded to one-carbon fragments that are incorporated into natural products, or conjugated with naturally-occurring organic acids to give trace-level metabolites. The metabolites are the same in resistant and susceptible crops but their relative distribution depends on the speed and extent of conversion to AMPA.

Methods of residue analysis

Glyphosate and its major metabolite AMPA can be determined by GLC or HPLC after derivatization. In the GLC method evaluated by the 1986 JMPR, clean-up on anion exchange, cation exchange and carbon columns is followed by trifluoroacetylation and methylation. The limit of determination was 0.05 mg/kg in cotton seed and hay and recoveries of glyphosate and AMPA respectively at 0.05-0.4 mg/kg fortification levels were 66.3-89.4% and 66.0-84.9% in cotton hay, and 56.7-74.8% and 63.4-93.2% in cotton seed.

HPLC methods were discussed in the 1986 and 1994 monographs. The preferred method employs two-column switched HPLC with a post-column reactor. The limit of determination was 0.05 mg/kg in all commodities and mean recoveries were 77-88% for glyphosate and 78-90% for AMPA.

Residues of AMPA in or on crops and definition of the residue

The Meeting received data on supervised trials on maize into which the *gox* gene had been introduced, which showed that residue levels of AMPA were much higher than those in normal crops.

The Meeting agreed to recommend two MRLs for residues in maize, one as glyphosate to accommodate uses on glyphosate-susceptible crops and the other as AMPA to accommodate uses on glyphosate-resistant crops. A violation would occur if either MRL were exceeded.

The current definition of the residue is "glyphosate" because residues of AMPA in crops are usually very low or undetectable, except in soya beans.

The Meeting agreed that the definition of the residue for estimations of dietary intake should include AMPA but the definition for enforcement purposes for all commodities, including genetically modified crops, should remain as "glyphosate" for the following reasons.

1. Already many commodities have CXLs based on the residue defined as glyphosate. All existing CXLs would have to be reviewed if the definition of the residue were changed.

2. It is not thought appropriate to establish a separate definition of the residue for maize.

3. The existing definition of the residue has already been incorporated into many national regulations, and a change of the definition would be likely to cause difficulties in international harmonization.

The Meeting also noted the significant residue levels of AMPA that occurred in soya beans, and recommended that their significance should be evaluated in a future periodic review even though they are not believed to pose any risk to consumers.

Supervised trials

In the following text the sum of glyphosate + AMPA expressed as glyphosate is referred to as "total glyphosate". The total glyphosate residue was evaluated to estimate STMRs for the assessment of dietary intake.

Cotton. Twelve supervised trials were carried out on glyphosate-susceptible cotton in the USA with pre-harvest application at 3.4 kg ai/ha. US GAP allows pre-emergence (crop) application (including pre-plant or at-planting applications), post-directed application (post-crop-emergence, directed at weeds), spot treatment and pre-harvest application at 4.2 kg ai/ha as the maximum for each treatment. The total application is restricted to 6.7 kg ai/ha per year.

Six of the trials were with pre-emergence and post-emergence applications before a pre-harvest application. The pre-emergence application rate (6.7 kg ai/ha) and the total applied (10-26 kg ai/ha) exceeded the GAP limits, but the Meeting concluded that these trials were comparable with GAP because the rate of the pre-harvest application (3.4 kg ai/ha), which should be most influential on the residue in the harvested crops, was within the GAP rate of 4.2 kg ai/ha and the studies of plant metabolism indicated that the uptake of glyphosate from soil would be negligible. The other six trials with only one pre-harvest application at 3.4 kg ai/ha were according to GAP.

Sixteen supervised trials, with three different application patterns in each, were carried out on glyphosate-resistant cotton in the USA with 4 or 5 applications which included pre-emergent, post-emergent, post-directed and pre-harvest treatments. Eleven trials were with genotype 1445 cotton and five with genotype 1698 cotton but these have the same basic genetic structure and would be expected to show no differences in glyphosate metabolism.

All the application patterns slightly exceeded US GAP: post-emergence (trials: 0.84-1.26 kg ai/ha, GAP: 0.84 kg ai/ha), post-directed (trials: 1.26 kg ai/ha, GAP: 0.84 kg ai/ha), and total application (trials 7.56-8.8 kg ai/ha, GAP: 6.7 kg ai/ha), but the Meeting again concluded that the trials complied with GAP because the most influential final applications were compatible with GAP and earlier applications would be unlikely to have much effect on the residues.

In susceptible cotton seed the residues of glyphosate were 0.54-5.9 mg/kg at 5-9 days and 0.15-3.6 mg/kg at 10-14 days, and those of AMPA were <0.05-0.20 mg/kg at 5-14 days. The residues of total glyphosate were 0.62-6.0 mg/kg at 5-9 days and 0.23-3.7 mg/kg at 10-14 days, and of total glyphosate after maximum GAP treatments 0.62, 0.71, 2.4, 2.8, 3.0 and 6.0 mg/kg.

In resistant cotton seed the residues of glyphosate were 0.13-5.0 mg/kg at 6-9 days and 0.30-0.50 mg/kg at 17 days, and those of AMPA were <0.05-0.21 mg/kg at 7-9 days. The residues of total glyphosate were 0.21-5.2 mg/kg at 6-9 days and 0.38-0.58 mg/kg at 17 days. Those of total glyphosate after maximum GAP treatments were 0.21, 0.30, 0.42, 0.49, 0.51 (2), 0.52, 0.54, 0.55, 0.66, 0.68, 0.73, 0.75, 0.77 (2), 1.1 (2), 1.3, 1.4, 1.5, 1.8, 1.9, 2.1 (2), 2.2, 2.3, 2.5, 2.6 (3), 2.8, 2.9 (2), 3.2, 3.5, 3.7, 3.8, 4.2 (2), 4.4, 4.7 and 5.2 mg/kg.

Since the differences between both the median and maximum total glyphosate residues in resistant and susceptible crops were not significant, the Meeting based the STMR on the

combined residues from the two sets of trials.

The total glyphosate residues from the 48 individual trials which complied with GAP (six on susceptible cotton and 42 on resistant cotton) in rank order (median underlined) were 0.21, 0.30, 0.42, 0.49, 0.51 (2), 0.52, 0.54, 0.55, 0.62, 0.66, 0.68, 0.71, 0.73, 0.75, 0.77 (2), 1.1 (2), 1.3, 1.4, 1.5, 1.8, 1.9, 2.1 (2), 2.2, 2.3, 2.4, 2.5, 2.6 (3), 2.8 (2), 2.9 (2), 3.0, 3.2, 3.5, 3.7, 3.8, 4.2 (2), 4.4, 4.7, 5.2 and 6.0 mg/kg.

The Meeting estimated an STMR level of 2.0 mg/kg total glyphosate. Taking into account the residues of glyphosate alone in susceptible (0.54-5.9 mg/kg) and resistant (0.13-5.0 mg/kg) crops, the Meeting estimated a maximum residue level of 10 mg/kg glyphosate and recommended the withdrawal of the CXL of 0.5 mg/kg.

The residues of glyphosate in the hay from susceptible cotton were 3.8-33 mg/kg at 5-9 days and 6.3-84 mg/kg at 10-14 days, and those of AMPA were 0.10-0.46 mg/kg at 5-14 days. The residues of total glyphosate were 4.1-33 mg/kg at 5-9 days and 6.4-85 mg/kg at 10-14 days.

The glyphosate residues (3.8-84 mg/kg) were below the existing CXL for the straw and fodder (dry) of cereal grains (100 mg/kg), although cotton hay is not classified within this group of commodities. The Meeting agreed not to recommend an MRL for cotton hay in view of its insignificance in international trade.

The residues of glyphosate in the gin by-product from resistant cotton were 3.7-84 mg/kg at 6-9 days and 0.79-2.2 mg/kg at 17 days, and those of AMPA were <0.05-0.84 mg/kg at 6-9 days and <0.05 mg/kg at 17 days. The residues of total glyphosate were 3.8-85 mg/kg at 6-9 days and 0.87-2.3 mg/kg at 17 days.

The Meeting did not recommend an MRL because the commodity does not figure in international trade.

Maize. Twelve supervised trials on susceptible maize and 66 on resistant maize were carried out in the USA. The 12 trials were with one pre-harvest application (2.5 kg ai/ha). US GAP allows pre-emergence application (0.32-4.2 kg ai/ha), spot treatment (0.32-4.2 kg ai/ha) and pre-harvest application (2.5 kg ai/ha for ground, 0.84 kg ai/ha for aerial) but the Meeting considered that the trials were effectively compatible with the maximum GAP application because the residue from pre-emergence application would be expected to be negligible and spot treatment should not affect crops if carried out according to GAP.

The 66 trials on resistant maize were with 2 to 4 applications which included pre-emergent, post-emergent and pre-harvest applications; 22 of the trials were according to maximum GAP.

Grain. The residues of glyphosate, AMPA and total glyphosate in the susceptible maize were 0.05-0.54 mg/kg, 0.05-0.13 mg/kg and 0.13-0.62 mg/kg respectively at 6-7 days. The residues of total glyphosate after maximum GAP treatments were 0.13 (5), 0.13, 0.14, 0.19, 0.23, 0.25, 0.27 and 0.62 mg/kg.

The residues of glyphosate, AMPA and total glyphosate in the resistant maize were 0.05-0.34 mg/kg, 0.05-1.4 mg/kg and 0.13-2.2 mg/kg respectively at 6-8 days. The residues of total glyphosate after maximum GAP treatments were <0.13 (2), 0.22 (2), 0.23, 0.26, 0.37, 0.38 (2), 0.41, 0.42, 0.51 (2), 0.52, 0.54 (2), 0.60, 0.67, 0.78, 1.0, 1.6 and 2.2 mg/kg.

Since the total glyphosate residues in the susceptible and resistant maize clearly belonged to difference populations, the Meeting estimated an STMR of 0.47 mg/kg total glyphosate, based on the residues in the resistant maize.

On the basis of the residues of glyphosate in susceptible (<0.05-0.54 mg/kg) and resistant (<0.05-0.34 mg/kg) maize, the Meeting recommended an MRL of 1 mg/kg for glyphosate to replace the existing CXL (0.1* mg/kg). The Meeting also estimated a maximum residue level of 2 mg/kg for AMPA in maize on the basis of the residues of AMPA found in resistant maize (<0.05-1.4 mg/kg).

Fodder. The residues of glyphosate, AMPA and total glyphosate in the susceptible maize fodder were 3.7-92 mg/kg, 0.09-0.81 mg/kg and 3.8-93 mg/kg respectively at 6-7 days. The corresponding residues in the fodder from resistant maize were 1.8-41 mg/kg, <0.05-4.7 mg/kg and 2.0-48 mg/kg respectively at 6-8 days. The residues in both susceptible and resistant maize fodder were below the existing CXL for the straw and fodder (dry) of cereal grains (100 mg/kg).

The Meeting estimated a maximum residue level of 5 mg/kg for AMPA in maize fodder from the residues in fodder from resistant maize (<0.05-4.7 mg/kg).

Forage. According to GAP, the forage of susceptible crops should be cut before the pre-harvest application of glyphosate, whereas the forage of resistant crops can be cut after the application before harvest. Trials to determine residues in forage were therefore restricted to resistant maize.

The residues of glyphosate, AMPA and total glyphosate in the maize forage were <0.05-0.52 mg/kg, 0.06-1.1 mg/kg and 0.18-1.9 mg/kg respectively after 48-65 days. Those of total glyphosate from maximum GAP treatments were 0.18, 0.23, 0.26, 0.35, 0.55, 0.61, 0.64, 0.81, 0.86, 0.92, 1.0 (2), 1.1, 1.8 and 1.9 mg/kg.

The Meeting estimated maximum residue levels of 1 mg/kg glyphosate and 2 mg/kg AMPA, which are recommended for use as MRLs, and an STMR of 0.81 mg/kg total glyphosate.

Sorghum (pre-harvest applications to susceptible plants). Eight supervised trials were carried out in the USA with one pre-harvest application at 1.7 kg ai/ha. US GAP allows pre-emergence application at 0.32-4.2 kg ai/ha, spot treatment at 0.32-4.2 kg ai/ha and pre-harvest application at 1.7 kg ai/ha. For the reasons given above, the Meeting considered the trials to be compatible with maximum GAP.

Grain. The residues of glyphosate, AMPA and total glyphosate were 1.4-13, <0.05-0.22 and 1.6-13 mg/kg respectively after 6-8 days. Those of total glyphosate in rank order were 1.6, 1.8, 1.9, 5.4, 6.2, 6.6 and 13(2) mg/kg.

The Meeting recommended an MRL of 20 mg/kg for glyphosate to replace the existing CXL (0.1* mg/kg), and an STMR of 5.8 mg/kg for total glyphosate.

Fodder and hay. Residue data said to be on sorghum hay were submitted, but the Meeting concluded that the commodity analysed in the trial should be classified as sorghum fodder.

The residues of glyphosate, AMPA and total glyphosate in fodder were 2.9-33, <0.05-0.41 and 3.0-34 mg/kg respectively at 6-8 days. The corresponding residues in "hay" were 3.1-37, <0.05-0.45 and 3.2-37 mg/kg at 10-15 days.

The glyphosate residues in both fodder (2.9-33 mg/kg) and hay (3.1-37 mg/kg) were below the existing CXL for the straw and fodder (dry) of cereal grains (100 mg/kg).

Processing

Cotton. Although only one study was available the Meeting agreed to calculate STMR-Ps because the processing adequately simulated industrial practice.

Processing factors from cotton seed to delinted cotton seed, cotton kernels, cotton hulls and cotton meal were 0.19, 0.084, 0.34 and 0.12 respectively. They were 0.034 for processing to crude cotton seed oil, cotton soapstock, refined cotton seed oil and bleached-deodorized cotton seed oil.

The Meeting estimated maximum residue levels of 0.05* mg/kg for crude and edible cotton seed oil, and STMR-Ps of 0.38, 0.17, 0.68 and 0.24 mg/kg for delinted cotton seed, cotton kernels, cotton hulls and cotton meal respectively, by calculation from the cotton seed STMR of 2.0 mg/kg.

Maize. Residues of glyphosate and AMPA were determined in the processed commodities but the residue of glyphosate in the raw grain was below the LOD, although AMPA was detected. Information on the conversion of glyphosate to AMPA during the processing was not available. The Meeting could not use the data to estimate STMR-Ps.

Sorghum. The mean processing factors were 4.7, 1.2, 0.36, 4.7 and 0.49 from sorghum to bran, clean grain, flour, grain dust and grits (medium) respectively and <0.028 or <0.11 for processing to germ and starch.

The Meeting estimated STMR-Ps of 0 for sorghum germ and starch because they contained negligible residues of glyphosate and AMPA individually, and 27, 7.0, 2.1, 27 and 2.8 mg/kg for bran, clean grain, flour, grain dust and grits (medium) respectively, by calculation from the sorghum STMR (5.8 mg/kg).

FURTHER WORK OR INFORMATION

Desirable

Processing studies with both susceptible and resistant maize in which the raw grain contains measurable residues of both glyphosate and AMPA.