Glyphosate

DOCUMENT M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
22nd July 2020	MCA 6.3.1: Table 6.3.1-1 was updated. The use in citrus fruit was added to the orchard use.	110054-MCA6_GRG_Rev 1 Jul_2020 Replaces the Doc ID 110054-MCA6_GRG_Jun_2020 – Changes are given in yellow
22nd July 2020	MCA 6.7.1: Table 6.7.1-15 was corrected and completed.	110054-MCA6_GRG_Rev 1 Jul_2020 Replaces the Doc ID 110054-MCA6_GRG_Jun_2020 – Changes are given in yellow

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

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

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

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

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

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

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

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

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

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

Bayer Agriculture BV[4] submits the dossier on behalf of the Glyphosate Renewal Group (GRG) for the AIR 5 process.

In the frame of the pre-submission meeting held between the GRG and the Assessment Group on Glyphosate (AGG) on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when summarizing studies from past submissions in the June 2020 renewal dossier[5].

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

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

A literature search for the active substance glyphosate and metabolites was performed in accordance with the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009" and according to the updated Appendix to this Guidance document[8]. The scientific literature review was performed for the period of 01st January 2010 until 31st December 2019, and total of 11 relevant and reliable articles were identified for the residues section. The identified relevant and reliable articles are presented as appendix E summaries in this M-CA section. For further detailed information on the Literature Review Report (LRR) and the corresponding evaluation, please refer to M-CA Section 9 "Literature". In the frame of the pre-submission meeting held on 27th September 2019, the AGG provided a reference document to GRG on the process to be considered when presenting literature in the June 2020 submission dossier[9].

During the former EU processes, public literature data was evaluated, listed and reported by the RMS. An annex, containing information about all previously submitted and/or included public literature articles from the former EU process is presented, for sake of completeness, as Annex to this M-CA section 6.

[4] Due to the Bayer-Monsanto acquisition in 2018, the legal entity name Monsanto Europe S.A. / N.V. has been changed to Bayer Agriculture BV.

[5] AGG_Advice to GTF2_Literature search_Final Oct 2019 "HOW TO SUMMARISE STUDIES IN DOSSIERS FROM 1998 AND 2012 IN THE DOSSIER TO BE SUBMITTED JUNE 2020"

[6] Monograph and Addendum to the monograph EU 2001: Glyphosate monograph

[7] In the AIR 5 dossier, in each M document, a category has been assigned to each regulatory study included in the AIR 5 dossier (for details please refer to the Doc ID: 110054-B-GRG_Jun_2020).

[8] Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances approved 27 March 2019 (doi: 10.2903/sp.efsa.2019.EN-1612)

[9] AGG_Advice to GTF2_Literature search_Final Oct 2019 "ADVICE TO GTF2: HOW TO PRESENT THE LITERATURE SEARCH IN THE DOSSIER TO BE SUBMITTED JUNE 2020"

Appendix G – Table for presenting metabolism data and supervised residues trials ('Metabolism_Residues trials_template.xls')

Data format submitted to the EU for the first time

Data point:	CA 6/001
Report author	Anonymous
Report year	2019
Report title	MCA_Sec_6_Residues_EFSA_Apx G_Metabolism_Residue_trials
Report No	Not available
Document No	Not relevant
Guidelines followed in study	EFSA, 2019. Administrative guidance on submission of dossiers and assessment reports for the peer-review of pesticide active substances, EFSA supporting publication 2019:EN-1612, 49 pp. doi:10.2903/sp.efsa.2019.EN-1612
Deviations from current test guideline	None
Previous evaluation	No
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Supportive only

The results of metabolism in primary plants, rotational crops and livestock as well as supervised residue trials are included in the table. The template 'Metabolism_Residues trials_template.xls' (see Appendix G) provided by EFSA with directions for such tables was considered and is submitted as part of the dossier. As Excel files are not allowed within the CADDY itself, the stand-alone Excel file is located on the data medium along with the CADDY.

CA 6.1 Storage stability of Residues

Fifteen storage stability studies presented in this dossier were already evaluated in the EU before, either in the context of the previous renewal dossier for glyphosate and its salts or in the context of the first approval of glyphosate-trimesium. In addition, a new study on the storage stability of glyphosate and AMPA in honey is also presented.

Together these studies provide data on the storage stability of glyphosate and AMPA in a variety of crop commodities belonging to the five matrix groups and in animal matrices.
For *N*-acetyl-glyphosate and *N*-acetly-AMPA data on storage stability are available for high water content, high starch content, high oil content and dry matrices.

An overview on the storage stability studies for glyphosate, AMPA, *N*-acetyl-glyphosate, *N*-acetyl-AMPA is presented in the table below. For each matrix group and each analyte the longest period for which storage stability was demonstrated is highlighted in **bold** print. Data considered as additional/supportive are presented in *italic*.

A new storage stability study is currently being conducted for AMPA in a high protein plant commodity.

Table 6.1-1: Overview of storage stability data for glyphosate and its metabolites at ≤ - 18 °C (unless stated otherwise)

Characteristics of the matrix	Matrix	Acceptable maximum storage duration	Comment
Glyphosate			
Plant products			
High water content	Sugar beet leaves	18 months	[redacted] 2010
	Maize forage	12 months	[redacted] 2007 (6.1/006)
	Maize green plant	12 months	[redacted] 2007 (6.1/004)
	Maize forage	12 months	[redacted] 2007 (6.1/004)
	Soybean forage	12 months	[redacted] 2007 (6.1/005)
	Pasture grass	*12 months*	*At -10°C, [redacted] 1993*
	Banana (whole fruit)	12 months	[redacted] 1996
	Tomato (exogenous)	31 months	[redacted] 1991
	Soybean forage (exogenous)	31 months	[redacted] 1991
	Soybean forage (incurred)	71 months (6 years)	[redacted] 1991
	Clover (exogenous)	31 months	[redacted] 1991
	Clover (incurred)	**75 months (6 years)**	[redacted] 1991
High protein content	Dry beans	**18 months**	[redacted] 1997
High starch content	Maize grain	18 months	[redacted] 2010
	Maize grain	12 months	[redacted] 2007 (6.1/006)
	Maize grain	12 months	[redacted] 2007 (6.1/004)
	Maize grain (exogenous)	31 months	[redacted] 1991
	Maize grain (incurred)	37 months (3 years)	[redacted] 1991
	Barley grain	18 months	[redacted] 2010
	Wheat/rye grain	45 months	[redacted] 1995
	Wheat grain	24 months	[redacted] 1989
	Sorghum grain	**48 months**	[redacted] 1989
	Sugar beet roots	18 months	[redacted] 2010
	Alfalfa seed (incurred)	25 months (2 years)	[redacted] 1991
High oil content	Soybean seeds	12 months	[redacted] 2007 (6.1/005)
	Soybean seeds	**24 months**	[redacted] 1989
	Soybean seed	*6 months*	*At -10°C, [redacted] 1993*
	Oilseed rape seeds/linseeds	18 months	[redacted] 1997
High acid content	Orange	**24 months**	[redacted], [redacted] 2012
Dry	Barley straw	18 months	[redacted] 2010
	Wheat/rye straw	45 months	[redacted] 1995
	Soybean straw	*13 months*	*At -10°C, [redacted] 1993*

Table 6.1-1: Overview of storage stability data for glyphosate and its metabolites at ≤ - 18 °C (unless stated otherwise)

Characteristics of the matrix	Matrix	Acceptable maximum storage duration	Comment
	Soybean straw	24 months	[redacted] 1989
	Soybean hay	12 months	[redacted] 2007 (6.1/005)
	Maize stover	12 months	[redacted] 2007(6.1/006)
	Maize stover	23 months	[redacted] 2007 (6.1/004)
	Sorghum stover (exogenous)	31 months	[redacted] 1991
	Sorghum stover (incurred)	**71 months (6 years)**	[redacted] 1991
Animal products			
Pig	Fat, muscle, liver, kidney	**26 months**	[redacted] 1988
Ruminant	Fat, muscle, liver, kidney	24 months	[redacted] 1988
Ruminant	Milk	**16 months**	[redacted] 1988
Poultry	Fat, muscle, liver	25 months	[redacted] 1988
Poultry	Kidney	13 months	[redacted] 1988
Poultry	Egg	14 months	[redacted] 1988
Poultry	Egg	**23 months**	[redacted] 1987
Bee	Honey	**6 months**	[redacted] 2020
AMPA			
Plant products			
High water content	Sugar beet leaves	18 months	[redacted] 2010
	Maize forage	12 months	[redacted] 2007 (6.1/006)
	Maize green plant	12 months	[redacted] 2007 (6.1/004)
	Maize forage	12 months	[redacted] 2007 (6.1/004)
	Soybean forage	12 months	[redacted] 2007 (6.1/005)
	Pasture grass	*12 months*	*At -10°C,* [redacted] *1993*
	Tomato (exogenous)	**31 months**	[redacted] 1991
	Soybean forage (exogenous)	24 months	[redacted] 1991
	Clover (exogenous)	6 months	[redacted] 1991
High starch content	Maize grain	18 months	[redacted] 2010
	Maize grain	12 months	[redacted] 2007 (6.1/006)
	Maize grain	12 months	[redacted] 2007 (6.1/004)
	Maize grain (exogenous)	31 months	[redacted] 1991
	Barley grain	18 months	[redacted] 2010
	Sugar beet roots	18 months	[redacted] 2010
	Wheat/rye grain	10 months	[redacted] 1995
	Wheat grain	24 months	[redacted] 1989

Table 6.1-1: Overview of storage stability data for glyphosate and its metabolites at ≤ - 18 °C (unless stated otherwise)

Characteristics of the matrix	Matrix	Acceptable maximum storage duration	Comment
	Sorghum grain	**48 months**	[redacted] 1989
High oil content	Soybean seed	12 months	[redacted] 2007 (6.1/005)
	Soybean seed	*6 months*	*At -10°C,* [redacted] *1993*
	Soybean seed	**24 months**	[redacted] 1989
High acid content	Orange	**24 months**	[redacted] 2012
Dry [1]	Barley straw	18 months	Weber 2010
	Maize stover	12 months	[redacted] 2007 (6.1/006)
	Maize stover	23 months	[redacted] 2007 (6.1/004)
	Soybean hay	12 months	[redacted] 2007 (6.1/005)
	Soybean straw	*13 months*	*At -10°C,* [redacted] *1993*
	Soybean straw	**24 months**	[redacted] 1989
	Wheat/rye straw	6 months	[redacted] 1995
	Sorghum stover (exogenous)	9 months	[redacted] 1991
	Sorghum stover (incurred)	11 months **(6 years)**	[redacted] 1991
Animal products			
Pig	Fat, muscle, liver, kidney	**26 months**	[redacted] 1988
Ruminant	Fat, muscle, liver, kidney	24 months	[redacted] 1988
Ruminant	Milk	**16 months**	[redacted], [redacted] 1988
Poultry	Fat, muscle, liver	25 months	[redacted] 1988
Poultry	Kidney	13 months	[redacted] 1988
Poultry	Egg	14 months	[redacted] 1988
Poultry	Egg	**23 months**	[redacted] 1987
Bee	Honey	**6 months**	[redacted] 2020
***N*-acetyl-glyphosate**			
Plant products			
High water content	Maize forage	**12 months**	[redacted] 2007 (6.1/006)
	Maize green plant	**12 months**	[redacted] 2007 (6.1/004)
	Maize forage	**12 months**	[redacted] 2007 (6.1/004)
	Soybean forage	**12 months**	[redacted] 2007 (6.1/005)
High starch content	Maize grain	**12 months**	[redacted] 2007 (6.1/006)
	Maize grain	**12 months**	[redacted] 2007 (6.1/004)
High oil content	Soybean seed	**12 months**	[redacted] 2007 (6.1/005)
Dry [1]	Maize stover	12 months	[redacted] 2007 (6.1/006)
	Maize stover	**23 months**	[redacted] 2007 (6.1/004)

Table 6.1-1: Overview of storage stability data for glyphosate and its metabolites at ≤ - 18 °C (unless stated otherwise)

Characteristics of the matrix	Matrix	Acceptable maximum storage duration	Comment
	Soybean hay	12 months	[redacted] 2007 (6.1/005)
***N*-acetyl-AMPA**			
Plant products			
High water content	Maize green plant	**23 months**	[redacted] 2007 (6.1/004)
	Maize forage	**23 months**	[redacted] 2007 (6.1/004)
	Soybean forage	18 months	[redacted] 2007 (6.1/005)
High starch content	Maize grain	**23 months**	[redacted] 2007 (6.1/004)
High oil content	Soybean seed	**18 months**	[redacted] 2007 (6.1/005)
Dry [1]	Maize stover	**23 months**	[redacted] 2007 (6.1/004)
	Soybean hay	18 months	[redacted] 2007 (6.1/005)

[1] In the OECD guideline 506 these commodities are not allocated to any of the five categories for storage stability. However, according to the draft EU guidance document on pesticide analytical methods for pre- and post-registration purposes, they belong to the group of "dry commodities".

For **glyphosate** maximum storage stability was shown for **75 months in high water matrices** (clover), for **18 months in high protein matrices** (dry beans), for **48 months in high starch matrices** (sorghum grain), for **24 months in high oil matrices** (soybean seeds), for **24 months in high acid matrices** (orange) and for **71 months in dry matrices** (sorghum stover).

For **AMPA** maximum storage stability was shown for **31 months in high water matrices** (tomato), for **48 months in high starch matrices** (sorghum grain), for **24 months in high oil matrices** (soybean seeds), for **24 months in high acid matrices** (orange) and for **24 months in dry matrices** (soybean straw).

For ***N*-acetyl-glyphosate** maximum storage stability was shown for **12 months in high water matrices** (maize forage), **in high starch matrices** (maize grain) and **in high oil matrices** (soybean seed) and for **23 months in dry matrices** (maize stover).

For ***N*-acetyl-AMPA** maximum storage stability was shown for **23 months in high water matrices** (maize forage, maize green plant), **in high starch matrices** (maize grain) and **in dry matrices** (maize stover) and for **18 months in high oil matrices** (soybean seed).

In animal matrices, maximum storage stability for **glyphosate** and **AMPA** was shown for **26 months in tissues**, **16 months in milk** and **23 months in eggs**.

In **honey**, maximum storage stability for **glyphosate** and **AMPA** was shown for **6 months**.

Most studies demonstrate stability of the analytes for the maximum period tested. For plant matrices, a decline was found for AMPA in soybean forage, clover and sorghum stover (Mueth, M.G., 1991) and in wheat/rye grain and straw ([redacted] 1995). For animal matrices, a decline was found for glyphosate and AMPA in eggs.

The procedural recoveries for glyphosate and its metabolites were sometimes in the lower acceptable range (i.e. between 70-85 %). In such cases also recovered residues corrected by the freshly fortified samples and recovered residues in relation to the actual day 0 concentration were presented to ease the interpretation of the results.

Study not previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/001
Report author	[redacted]
Report year	2020
Report title	ILV of method ME-2220-01 and short term storage stability of glyphosate and its metabolite AMPA in honey
Report No	S19-04663
Document No	M-681330-01-1
Guidelines followed in study	OECD 506
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in honey was investigated. Samples were spiked separately with the test items at concentration levels of 0.250 mg/kg glyphosate and AMPA (10x LOQ). The samples were stored at ≤ -18°C in the dark until analysis for about 6 months. Glyphosate and AMPA in honey were stable for the maximum period tested: 6 months.

I. Materials and Methods

A. Materials			
1. Test material:			
	Identification:	Glyphosate	AMPA
	Description:	Solid/whitish	Solid/white
	Lot/Batch #:	107671	107466
	Purity:	99.9 %	98.5 %
	CAS # :	1071-83-6	1066-51-9
	Spiking levels:	0.250 mg/kg	0.250 mg/kg

2.	**Test Commodity:**	
	Commodity:	Honey
	Sample size:	2 g

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in honey was investigated. Triplicate samples were spiked with the test items at a concentration level of 0.250 mg/kg (separate samples were used for each test item). At day 0 five replicate samples were prepared. The spiked samples were stored at ≤ -18°C until analysis. At four storage intervals over a period of 6 months, the samples were tested for the stability of glyphosate and AMPA.
Each analytical set for storage stability analysis included the following samples: a non-treated control, four concurrent freshly fortified matrix samples (two with glyphosate, two with AMPA), and six aged (storage stability) samples, three fortified with glyphosate and three fortified with AMPA.

2. Description of analytical procedures

Analysis was done according to procedures described in Residue Analytical Method ME-2220-01 (refer to CA 4.1.2). In summary, honey samples were diluted with 0.1 % formic acid prior to addition of internal standard. An aliquot was centrifuged, filtered and analysed by high performance liquid chromatography and detected by tandem mass spectrometry with electrospray ionisation (HPLC-MS/MS).
The limit of quantification (LOQ) of this method was 0.025 mg kg for glyphosate and AMPA.
A variant of the analytical method with calibration using matrix-matched standard solutions was used for the investigation of the storage stability and successfully validated. For confirmation of the validity of the analytical method duplicate samples of honey spiked with 0.250 mg/kg glyphosate and AMPA at storage intervals of 0, 1, 3 and 6 months were analysed for the concentration of glyphosate and AMPA using the analytical method. Recovery values were in the acceptable range of 70-110 %. The relative standard deviations (RSDs) were below 20 %.

II. Results and Discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 is presented (italic).

Table 6.1-2: Storage stability of glyphosate and AMPA in honey

Commodity	Analyte	Storage period months (days)	Residue level in stored samples[1] (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples[1] (%) (mean)
Honey	Glyphosate	0	0.250, 0.253, 0.233, 0.233, 0.250 (0.244)	100, 94, 93, 93, 100 (96)	*100*	-
		1 (31)	0.261, 0.253, 0.259 (0.258)	104, 101, 104 (103)	*107*	109, 108 (109)
		3 (92)	0.247, 0.270, 0.241 (0.253)	99, 108, 96 (101)	*105*	102, 96 (99)
		6 (185)	0.259, 0.269, 0.258 (0.262)	104, 108, 103 (105)	*109*	104, 100 (102)
	AMPA	0	0.233, 0.237, 0.237, 0.248, 0.241 (0.239)	93, 95, 95, 99, 96 (96)	*100*	-
		1 (31)	0.247, 0.254, 0.253 (0.251)	99, 102, 101 (101)	*105*	100, 100 (100)
		3 (92)	0.232, 0.231, 0.229 (0.231)	93, 92, 92 (92)	*97*	97, 93 (95)
		6 (185)	0.265, 0.260, 0.254 (0.260)	106, 104, 102 (104)	*109*	96, 99 (98)

[1] Fortification level of 0.25 mg/kg

III. Conclusion

In this study, glyphosate and AMPA were proven to be stable in honey samples for at least 6 months when stored at ≤ -18°C.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessed the storage stability of glyphosate and AMPA in honey and was not previously evaluated at EU level. It was performed under GLP and is considered to be scientifically valid. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/003
Report author	[redacted]
Report year	2010
Report title	Storage stability of residues of Glyphosate and AMPA in various plant materials
Report No	FCS-0707
Document No	ASB2012-12488
Guidelines followed in study	EU Guidance Appendix H: Storage Stability of Residue Samples (7032/VI/95 Rev. 5, 22/Jul/1997) EPA OPPTS 860.1380 – Storage Stability Data (1996)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in barley (grain and straw), maize (grain) and sugar beet (root and leaves) stored at about ≤ -18°C was investigated. The samples were spiked separately with glyphosate and AMPA at a concentration level of 1 mg/kg (>10x LOQ). In all matrices investigated, glyphosate and AMPA residues were stable for the maximum period tested: 18 months.

I. Materials and methods

A. Materials		
1. Test material:		
Identification:	Glyphosate	AMPA
Description:	Not reported	Crystalline solid
Lot/Batch #:	3223X	70516
Purity:	99.2 %	98.5 %
CAS # :	1071-83-6	1066-51-9
Spiking levels:	1.0 mg/kg	1.0 mg/kg

2. Test Commodity:	
Crop:	Barley, maize, sugar beet
Type:	Barley, maize: Cereals Sugar beet: Root vegetable
Variety:	Not reported
Botanical name:	*Hordeum vulgare, Zea mays, Beta vulgaris*
Crop parts(s) or processed commodity:	Barley (grain and straw), maize (grain), sugar beet (root and leaves)
Sample size:	5-10 g

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in barley (grain and straw), maize (grain) and sugar beet (root and leaves) stored at about ≤ -18 °C was investigated.
Homogenised samples were spiked separately with the test items at a concentration level of 1.0 mg/kg for both glyphosate and AMPA. The samples were stored in coloured (brown) glass jars at -18 °C or lower until analysis. At four samplings over a period of 18 months the samples were tested for the stability of glyphosate and AMPA.
Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples (one with glyphosate, one with AMPA), and six aged (storage stability) samples, three fortified with glyphosate and three fortified with AMPA.

2. Description of analytical procedures

The samples were analysed with DFG method 405 (refer to CA 4.1.2). For the determination of glyphosate and the metabolite AMPA the samples were extracted with hydrochloric acid. After clean-up of the aqueous fraction by elution through Chelex 100 resin in the Fe(III) form glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron removed using an anion exchange resin. After concentration to dryness to remove the hydrochloric acid and dissolving in water, glyphosate and AMPA were quantified separately by means of HPLC equipped with a post derivatisation unit and a fluorescence detector.
Determination involves post-column hypochlorite oxidation for glyphosate and reaction of the amine product with o-phthaldialdehyde and mercaptoethanol to produce a fluorescent derivative.
The LOQ was 0.05 mg/kg for each analyte.
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from freshly spiked samples at a concentration of 1.0 mg/kg. All recovery values were in the acceptable range of 70-110 % and relative standard deviations (RSDs) <20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-3: Storage stability of glyphosate and AMPA in various plant matrices

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples[1] (%)	*Recovery of stored samples corrected for procedural recoveries[1] (%)*
Barley grain	Glyphosate	0	0.773, 0.738, 0.709 (0.740)	77, 74, 71 (74)	*100*	-	-
		6	0.795, 0.759, 0.658 (0.737)	80, 76, 66 (74)	*100*	73	*101*
		12	0.731, 0.637, 0.753 (0.707)	73, 64, 75 (71)	*96*	70	*101*
		18	0.686, 0.734, 0.679 (0.700)	69, 73, 68 (70)	*95*	71	*99*
	AMPA	0	0.952, 1.024, 0.948 (0.975)	95, 102, 95 (98)	*100*	-	-
		6	0.812, 0.815, 0.862 (0.830)	81, 82, 86 (83)	*85*	75	*110*
		12	0.721, 0.679, 0.771 (0.724)	72, 68, 77 (72)	*74*	82	*88*
		18	0.736, 0.661, 0.637 (0.678)	74, 66, 64 (68)	*70*	71	*96*
Barley straw	Glyphosate	0	0.747, 0.723, 0.749 (0.740)	75, 72 75 (74)	*100*	-	-
		6	0.669, 0.644, 0.665 (0.659)	67, 64, 67 (66)	*89*	72	*92*
		12	0.671, 0.700, 0.683 (0.685)	67, 70, 68 (69)	*93*	78	*87*
		18	0.768, 0.632, 0.750 (0.717)	77, 63, 75 (72)	*97*	84	*86*
	AMPA	0	0.790, 0.722, 0.751 (0.754)	79, 72, 75 (75)	*100*	-	-
		6 [2]	0.512, 0.487, 0.499 (0.499)	51, 49, 50 (50)	*66*	71	*75*
		12	0.334, 0.403, 0.362 (0.366)	33, 40, 36 (37)	*49*	85	*43*
		18	0.769, 0.736, 0.797 (0.767)	77, 74, 80 (77)	*102*	77	*100*
Maize grain	Glyphosate	0	0.808, 0.774, 0.821 (0.801)	81, 77, 82 (80)	*100*	-	-
		6	0.643, 0.662, 0.675 (0.660)	64, 66, 68 (66)	*82*	76	*87*
		12	0.802, 0.787, 0.781 (0.790)	80, 79, 79 (79)	*99*	79	*100*
		18	0.718, 0.742, 0.734 (0.731)	72, 74, 73 (73)	*91*	76	*96*
	AMPA	0	0.822, 0.954, 1.035 (0.937)	82, 95, 104 (94)	*100*	-	-
		6	0.826, 0.898, 0.731 (0.818)	83, 90, 73 (82)	*87*	77	*106*
		12	0.720, 0.836, 0.801 (0.786)	72, 84, 70 (79)	*84*	85	*93*
		18	0.896, 0.834, 0.837 (0.856)	90, 83, 84 (86)	*91*	80	*108*

Table 6.1-3: Storage stability of glyphosate and AMPA in various plant matrices

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples[1] (%)	*Recovery of stored samples corrected for procedural recoveries[1] (%)*
Sugar beet root	Glyphosate	0	0.823, 0.847, 0.859 (0.843)	82, 85, 86 (84)	*100*	-	-
		6	0.910, 0.916, 0.837 (0.888)	91, 92, 84 (89)	*105*	94	*95*
		12	0.763, 0.777, 0.672 (0.737)	76, 78, 67 (74)	*87*	79	*95*
		18	0.795, 0.862, 0.769 (0.809)	80, 86, 77 (81)	*96*	71	*114*
	AMPA	0	0.940, 0.868, 0.902 (0.903)	94, 87, 90 (90)	*100*	-	-
		6	0.880, 0.791, 0.959 (0.877)	88, 79, 96 (88)	*97*	80	*110*
		12	0.711, 0.668, 0.717 (0.699)	71, 67, 72 (70)	*77*	79	*89*
		18	0.674, 0.670, 0.658 (0.667)	67, 67, 66 (67)	*74*	74	*91*
Sugar beet leaves	Glyphosate	0	0.810, 0.908, 0.808 (0.842)	81, 91, 81 (84)	*100*	-	-
		6	0.748, 0.699, 0.706 (0.718)	75, 70, 71 (72)	*85*	80	*90*
		12	0.663, 0.635, 0.703 (0.667)	66, 64, 70 (67)	*79*	70	*96*
		18	0.637, 0.743, 0.657 (0.679)	64, 74, 66 (68)	*81*	80	*85*
	AMPA	0	0.839, 0.885, 0.891 (0.872)	84, 89, 89 (87)	*100*	-	-
		6	0.759, 0.665, 0.665 (0.696)	76, 67, 67 (70)	*80*	81	*86*
		12	0.539, 0.591, 0.559 (0.563)	54, 59, 56 (56)	*65*	81	*69*
		18	0.735, 0.814, 0.674 (0.741)	74, 81, 67 (74)	*85*	73	*101*

[1] Fortification level of 1.0 mg/kg for glyphosate and AMPA
[2] Low recoveries for the stored samples due to problems within the extraction of these samples.

III. Conclusion

The results of this study are inconsistent. For glyphosate in barley grain and sugar beet roots as well as for AMPA in maize grain no significant degradation was observed within 18 months. In barley straw (glyphosate and AMPA), maize grain (glyphosate) and sugar beet leaves (AMPA) intermediate samples showed a significant decline, while final samples collected after 18 months were stable (>70 % remaining). In view of the generally low procedural recoveries it can be concluded that these sample are still within the normal variation of residue, especially since day 0 values also gave recoveries between 70-90 %.

Barley grain (AMPA), sugar beet roots (AMPA) and sugar beet leaves (glyphosate) showed a decline at the end of the storage interval investigated. However, under consideration of the procedural recoveries, the remaining levels found were above 70 % of the fortification level.

In summary both glyphosate and AMPA showed a strong variation in the results, generally tending towards low recovery values between 70-90 %. Under consideration of the procedural recoveries and the concentrations measured in day 0 samples an overall stability of both analytes in the matrices investigated seems plausible for a storage interval of 18 months.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in high starch content matrices (barley and maize grain and sugar beet root), high water content matrices (sugar beet leaves) and straw was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/002
Report author	[redacted]
Report year	2012
Report title	Storage stability of residues of Glyphosate and AMPA in citrus fruit
Report No	REG-09-234
Document No	MSL0023608
Guidelines followed in study	EU Guidance Appendix H: Storage Stability of Residue Samples (7032/VI/95 Rev. 5, 22/Jul/1997)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary
The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in citrus fruit (oranges) stored at about ≤ -18 °C was investigated. The samples were spiked separately with glyphosate and AMPA at a concentration level of 0.5 mg/kg (10x LOQ). Glyphosate and AMPA residues were stable in orange fruit for the maximum period tested: 24 months.

I. Materials and methods

A. Materials

1. Test material:

Identification:	Glyphosate	AMPA
Description:	Not reported	Not reported
Lot/Batch #:	GLP-0810-19515-A	GLP-0811-19540-A
Purity:	Not reported	Not reported
CAS # :	1071-83-6	1066-51-9
Spiking levels:	0.5 mg/kg	0.5 mg/kg

2. Test Commodity:

Crop:	Orange, whole fruit
Type:	Orange: Citrus fruit
Variety:	Valencia
Botanical name:	*Citrus Sinensis*
Crop parts(s) or processed Commodity:	Whole fruit
Sample size:	10 g

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in orange (whole fruit) stored at ≤ -18°C was investigated. Duplicate samples (homogenised) were separately spiked with the test items at a concentration of 0.5 mg/kg glyphosate and AMPA. At day 0 five replicate samples were prepared. The samples (except for the day 0 samples) were stored in polypropylene bottles at -18 °C or lower until analysis.
At the target storage intervals of 0, 1, 3, 6, 9, 12, 18 and 24 months the samples were tested for the stability of glyphosate and AMPA.
Each analytical set for storage stability determination included the following samples: two non-treated control, four concurrent freshly fortified matrix samples (two with glyphosate, two with AMPA), and four aged (storage stability) samples, two fortified with glyphosate and two fortified with AMPA.

2. Description of analytical procedures

All samples were analysed using validated analytical method ES-ME-1294-01/AG-ME-1294-01 (refer to CA 4.1.2). Glyphosate and AMPA were isolated from crop matrices by high speed blender extraction using 0.1 % formic acid in water and methylene chloride. Following centrifugation, an aliquot of the aqueous phase extract was mixed with isotopically labelled glyphosate and AMPA internal standards then passed through solid phase extraction media for final clean-up. The samples were analysed by LC-MS/MS using a cation exchange column and quantitated using one specific precursor/product ion transition for each analyte.
The LOQ was 0.05 mg/kg for each analyte
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from freshly spiked samples of orange whole fruit. The recoveries were in the acceptable range of 70-110 % and the relative standard deviations (RSDs) were below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results used for the stability calculation were not corrected for recoveries.

Table 6.1-4: Storage stability of glyphosate and AMPA in orange fruits

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	Procedural recovery of freshly fortified samples[1] (%) (mean)
Orange whole fruit	Glyphosate	0	0.450, 0.446, 0.443, 0.435, 0.432 (0.441)	90, 89, 89, 87, 86 (88)	-
		1 (30)	0.477, 0.456 (0.467)	95, 91 (93)	91, 92 (91)
		3 (97)	0.458, 0.454 (0.456)	92, 91 (91)	92, 89 (90)
		6 (196)	0.463, 0.458 (0.461)	93, 92 (92)	89, 87 (88)
		9 (273)	0.434, 0.438 (0.436)	87, 88 (87)	86, 87 (86)
		12 (372)	0.471, 0.461 (0.466)	94, 92 (93)	85, 91 (88)
		18 (546)	0.445, 0.448 (0.447)	89, 90 (89)	89, 89 (89)
		24 (727)	0.442, 0.443 (0.443)	88, 89 (89)	87, 84 (86)
	AMPA	0	0.440, 0.428, 0.426, 0.444, 0.435 (0.435)	88, 86, 85, 89, 87 (87)	-
		1 (30)	0.452, 0.455 (0.454)	90, 91 (91)	92, 92 (92)
		3 (97)	0.453, 0.439 (0.446)	91, 88 (89)	92, 87 (89)
		6 (196)	0.448, 0.446 (0.447)	90, 89 (89)	88, 88 (88)
		9 (273)	0.436, 0.435 (0.436)	87, 87 (87)	88, 86 (87)
		12 (372)	0.460, 0.454 (0.457)	92, 91 (92)	86, 86 (86)
		18 (546)	0.439, 0.436 (0.438)	88, 87 (88)	86, 85 (85)
		24 (727)	0.422, 0.420 (0.421)	84, 84 (84)	92, 92 (92)

[1] Fortification level of 0.5 mg/kg for glyphosate and AMPA

III. Conclusion

In this study, glyphosate and AMPA were proven to be stable in oranges (high acid commodity) for at least 24 months when stored at ≤ -18°C.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the storage stability of glyphosate and metabolite AMPA in high acid content matrices was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/004
Report author	[redacted]
Report year	2007
Report title	Stability of glyphosate and metabolites in corn green plant, forage, grain, and stover containing the GAT and ZM-HRA genes during frozen storage
Report No	60874
Document No	ASB2008-2656
Guidelines followed in study	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Appendix H: Storage Stability of Residue Samples (7032/VI/95 Rev. 5, 22/Jul/1997)
Deviations from current test guideline	Yes (OECD 506): • A mixed spiking solution was used for glyphosate, *N*-acetyl-glyphosate and AMPA
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA (aminomethylphosphonic acid) and *N*-acetyl-AMPA in green plant, forage, grain and stover from maize containing the GAT and ZM-HRA genes was investigated at about ≤ -20 °C. The samples were spiked with glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-glyphosate at a concentration level of 0.5 mg/kg (10x LOQ). The residues of glyphosate, *N*-acetyl-glyphosate and AMPA were stable when stored at approximately -20 °C for the maximum period tested: at least 12 months in corn green plant, forage, and grain and stable for 23 months in stover. Residues of *N*-acetyl-AMPA were also stable for the maximum period tested: at least 23 months in corn green plant, forage, grain, and stover when stored at approximately -20 °C.

I. Materials and methods

A. Materials

1. Test material:

Identification:	Glyphosate	*N*-acetyl-glyphosate	AMPA	*N*-acetyl-AMPA
Description:	Not reported	Not reported	Not reported	Not reported
Lot/Batch #:	014	000	10003440	001

Purity:	97 %	84.3 % as sodium salt 67.4 % as free acid	99.5 %	76 %
CAS # :	1071-83-6	129660-96-4	1066-51-9	57637-97-5
Spiking levels:	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg

2. Test Commodity:

Crop:	Maize
Type:	Cereals
Variety:	GAT and ZM-HRA modified maize
Botanical name:	*Zea mays*
Crop parts(s) or processed commodity:	Green plant, forage, grain, stover
Sample size:	5 g (maize green plant, forage and grain), 10 g (maize stover)

B. Study design

1. Test procedure

The storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in green plant, forage, grain and stover from maize containing the GAT and ZM-HRA genes was investigated at about ≤ -20°C.

Homogenised samples were spiked with the test items at a concentration level of 0.5 mg/kg for glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA. Samples were spiked with glyphosate, *N*-acetyl-glyphosate and AMPA together. Separate stability samples were prepared at a later time to test the frozen storage stability of *N*-acetyl AMPA. The samples were stored in polypropylene bottles at approximately -20°C until analysis. Maize green plant, forage and grain samples were tested for the stability of glyphosate, *N*-acetyl-glyphosate and AMPA at six storage intervals over a period of 12 months and *N*-acetyl-AMPA at six storage intervals over a period of 23 months. Maize stover samples were tested for the stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA at seven storage intervals over a period of 23 months.

Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples (fortified with glyphosate, *N*-acetyl-glyphosate, AMPA and

N-acetyl-AMPA), and two aged (storage stability) samples fortified with glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA.

2. Description of analytical procedures

Samples were analysed using procedures based on the method DuPont-15444, "Analytical Method for the Determination of Glyphosate and Respective Metabolite Residues in Various Crop Matrices Using LC/MS/MS" with modifications. For the determination of glyphosate and the metabolites *N*-acetyl-glyphosate and AMPA duplicate samples were extracted using 0.1 % formic acid/methanol (96/4 v/v), cleaned by SPE and analysed using LC/MS/MS.

The analytical method was fully validated during the study DuPont-15444 ([redacted] 2007; refer to CA 4.1.2).

The LOQ was 0.05 mg/kg for each analyte.

In order to check the validity of the method, procedural recoveries were determined from samples freshly fortified with glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA at 0.5 mg/kg.

The procedural recoveries of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA were between 70 % and 110 % with a few exceptions for glyphosate in maize green plant, forage and stover and *N*-acetyl-glyphosate and *N*-acetyl-AMPA in maize stover. The relative standard deviations (RSDs) per analyte and commodity were below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-5: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
Maize green plant	Glyphosate	0	0.434, 0.421 (0.428)	87, 85 (86)	*100*	-	
		1	0.434, 0.440 (0.437)	87, 88 (88)	*102*	105, 92 (99)	*89, 90 (90)*
		3	0.487, 0.475 (0.481)	98, 95 (97)	*112*	98, 98 (98)	*99, 97 (98)*
		6	0.448, 0.435 (0.442)	90, 87 (89)	*103*	90, 87 (89)	*102, 99 (101)*
		9	0.427, 0.416 (0.422)	85, 83 (84)	*99*	85, 90 (88)	*97, 95 (96)*
		12	0.547, 0.559 (0.553)	109, 112 (111)	*129*	115, 110 (113)	*97, 99 (98)*
	N-acetyl-glyphosate	0	0.399, 0.426 (0.413)	80, 86 (83)	*100*	-	
		1	0.407, 0.424 (0.416)	82, 85 (84)	*101*	97, 85 (91)	*90, 94 (92)*
		3	0.454, 0.450 (0.452)	91, 90 (91)	*109*	94, 97 (96)	*95, 95 (95)*
		6	0.483, 0.474 (0.479)	97, 95 (96)	*116*	88, 92 (90)	*107, 106 (107)*
		9	0.419, 0.470 (0.445)	84, 94 (89)	*108*	100, 80 (90)	*93, 105 (99)*
		12	0.533, 0.522 (0.528)	107, 104 (106)	*128*	95, 95 (95)	*112, 110 (111)*
	AMPA	0	0.441, 0.417 (0.429)	88, 84 (86)	*100*	-	
		1	0.353, 0.377 (0.365)	71, 76 (74)	*85*	92, 87 (90)	*79, 85 (82)*
		3	0.374, 0.407 (0.391)	75, 82 (79)	*91*	92, 95 (94)	*80, 87 (84)*
		6	0.373, 0.360 (0.367)	75, 72 (74)	*86*	90, 89 (90)	*84, 81 (83)*
		9	0.320, 0.322 (0.321)	64, 64 (64)	*75*	80, 74 (77)	*83, 83 (83)*
		12	0.369, 0.349 (0.359)	74, 70 (72)	*84*	85, 95 (90)	*82, 77 (80)*

Table 6.1-5: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
	N-acetyl-AMPA	0	0.478, 0.489 (0.484)	96, 98 (97)	*100*	-	
		1	0.409, 0.413 (0.411)	82, 83 (83)	*85*	84, 85 (85)	*97, 98 (98)*
		3	0.456, 0.489 (0.473)	92, 98 (95)	*98*	88, 88 (88)	*104, 111 (108)*
		6	0.409, 0.361 (0.385)	82, 72 (77)	*80*	75, 86 (81)	*103, 90 (97)*
		9	N/A	N/A	N/A	N/A	N/A
		12	0.507, 0.530 (0.519)	102, 106 (104)	*107*	101, 100 (101)	*101, 106 (104)*
		23	0.430, 0.421 (0.426)	86, 85 (86)	*88*	77, 81 (79)	*110, 107 (109)*
Maize forage	Glyphosate	0	0.466, 0.481 (0.474)	94, 96 (95)	*100*	-	-
		1	0.441, 0.429 (0.435)	88, 86 (87)	*92*	86, 77 (82)	*109, 106 (108)*
		3	0.490, 0.459 (0.475)	98, 92 (95)	*100*	103, 98 (101)	*98, 92 (95)*
		6	0.472, 0.458 (0.465)	94, 91 (93)	*98*	88, 90 (89)	*105, 102 (104)*
		9	0.452, 0.455 (0.454)	90, 91 (91)	*96*	80, 85 (83)	*109, 109 (109)*
		12	0.580, 0.566 (0.573)	116, 114 (115)	*121*	110, 116 (113)	*102, 101 (102)*
	N-acetyl-glyphosate	0	0.467, 0.471 (0.469)	94, 94 (94)	*100*	-	
		1	0.351, 0.353 (0.352)	70, 71 (71)	*75*	85, 70 (78)	*90, 91 (91)*
		3	0.473, 0.439 (0.456)	95, 88 (92)	*97*	94, 90 (92)	*103, 96 (100)*
		6	0.430, 0.437 (0.434)	85, 87 (86)	*93*	87, 88 (88)	*98, 99 (99)*
		9	0.452, 0.492 (0.472)	90, 98 (94)	*101*	83, 77 (80)	*112, 122 (117)*
		12	0.457, 0.489 (0.473)	91, 98 (95)	*101*	94, 97 (96)	*95, 103 (99)*
	AMPA	0	0.452, 0.459 (0.456)	91, 92 (92)	*100*	-	
		1	0.364, 0.363 (0.364)	73, 73 (73)	*80*	74, 76 (75)	*97, 97 (97)*
		3	0.416, 0.432 (0.424)	83, 87 (85)	*93*	94, 90 (92)	*91, 95 (93)*
		6	0.374, 0.431 (0.403)	74, 86 (80)	*88*	88, 89 (89)	*84, 96 (90)*
		9	0.385, 0.364 (0.375)	77, 73 (75)	*82*	89, 90 (90)	*86, 81 (84)*

Table 6.1-5: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
		12	0.379, 0.374 (0.377)	76, 75 (76)	*83*	90, 103 (97)	*78, 78 (78)*
	N-acetyl-AMPA	0	0.476, 0.485 (0.481)	96, 97 (97)	*100*	-	
		1	0.457, 0.453 (0.455)	92, 91 (92)	*95*	87, 87 (87)	*105, 104 (105)*
		3	0.434, 0.439 (0.437)	87, 88 (88)	*91*	88, 85 (87)	*101, 102 (102)*
		6	0.385, 0.414 (0.400)	77, 83 (80)	*83*	79, 69 (74)	*105, 113 (109)*
		9	N/A	N/A	N/A	N/A	N/A
		12	0.471, 0.584 (0.528)	95, 118 (107)	*110*	96, 106 (101)	*94, 116 (105)*
		23	0.402, 0.411 (0.407)	81, 83 (82)	*85*	75, 77 (76)	*106, 109 (108)*
Maize grain	Glyphosate	0	0.447, 0.421 (0.434)	89, 85 (87)	*100*	-	
		1	0.467, 0.470 (0.469)	93, 94 (94)	*108*	93, 92 (93)	*101, 101 (101)*
		3	0.407, 0.444 (0.426)	82, 89 (86)	*98*	88, 101 (95)	*87, 94 (91)*
		6	0.477, 0.453 (0.465)	95, 90 (93)	*107*	84, 95 (90)	*106, 100 (103)*
		9	0.412, 0.422 (0.417)	83, 85 (84)	*96*	79, 85 (82)	*101, 104 (103)*
		12	0.469, 0.509 (0.489)	94, 102 (98)	*113*	95, 105 (100)	*93, 102 (98)*
	N-acetyl-glyphosate	0	0.464, 0.420 (0.442)	93, 84 (89)	*100*	-	
		1	0.470, 0.489 (0.480)	94, 98 (96)	*109*	88, 92 (90)	*104, 108 (106)*
		3	0.439, 0.509 (0.474)	88, 102 (95)	*107*	90, 106 (98)	*90, 104 (97)*
		6	0.425, 0.417 (0.421)	85, 83 (84)	*95*	85, 81 (83)	*102, 99 (101)*
		9	0.378, 0.411 (0.395)	76, 83 (80)	*89*	79, 75 (77)	*99, 107 (103)*
		12	0.394, 0.397 (0.396)	79, 80 (80)	*90*	75, 78 (77)	*103, 104 (104)*
	AMPA	0	0.476, 0.413 (0.445)	95, 83 (89)	*100*	-	
		1	0.364, 0.361 (0.363)	72, 72 (72)	*82*	82, 74 (78)	*93, 92 (93)*
		3	0.435, 0.458 (0.447)	87, 91 (89)	*100*	88, 90 (89)	*99, 103 (101)*
		6	0.484, 0.481 (0.483)	97, 96 (97)	*109*	97, 96 (97)	*100, 99 (100)*

Table 6.1-5: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
		9	0.425, 0.434 (0.430)	86, 88 (87)	*97*	80, 90 (85)	*101, 103 (102)*
		12	0.463, 0.472 (0.468)	92, 95 (94)	*105*	94, 95 (95)	*98, 101 (100)*
	N-acetyl-AMPA	0	0.459, 0.448 (0.454)	92, 90 (91)	*100*	-	
		1	0.427, 0.420 (0.424)	85, 84 (85)	*93*	78, 85 (82)	*104, 103 (104)*
		3	0.382, 0.383 (0.383)	77, 77 (77)	*84*	76, 78 (77)	*100, 100 (100)*
		6	0.410, 0.368 (0.389)	82, 74 (78)	*86*	79, 77 (78)	*106, 95 (101)*
		9	N/A	N/A	N/A	N/A	N/A
		12	0.441, 0.419 (0.430)	88, 84 (86)	*95*	81, 90 (86)	*103, 98 (101)*
		23	0.435, 0.413 (0.424)	87, 83 (85)	*93*	81, 76 (79)	*111, 106 (109)*
Maize stover	Glyphosate	0	0.455, 0.538 (0.497)	91, 108 (100)	*100*	-	
		1	0.528, 0.533 (0.531)	106, 107 (107)	*107*	105, 101 (103)	*103, 104 (104)*
		3	0.504, 0.561 (0.533)	101, 112 (107)	*107*	98, 108 (103)	*98, 109 (104)*
		6	0.543, 0.516 (0.530)	109, 103 (106)	*107*	98, 106 (102)	*107, 101 (104)*
		9	0.492, 0.514 (0.503)	99, 103 (101)	*101*	96, 100 (98)	*101, 105 (103)*
		12	0.527, 0.538 (0.533)	105, 108 (107)	*107*	110, 112 (111)	*95, 97 (96)*
		23	0.488, 0.516 (0.502)	98, 103 (101)	*101*	99, 109 (104)	*94, 99 (97)*
	N-acetyl-glyphosate	0	0.450, 0.484 (0.467)	90, 97 (94)	*100*	-	
		1	0.528, 0.564 (0.546)	106, 113 (110)	*117*	92, 99 (96)	*111, 119 (115)*
		3	0.453, 0.464 (0.459)	91, 93 (92)	*98*	94, 91 (93)	*98, 100 (99)*
		6	0.433, 0.425 (0.429)	87, 85 (86)	*92*	80, 90 (85)	*102, 100 (101)*
		9	0.484, 0.467 (0.476)	97, 94 (96)	*102*	93, 94 (94)	*103, 100 (102)*
		12	0.419[3], 0.393[3] (0.406)	84[3], 79[3] (82)	*87*	81, 92 (87)	*97, 91 (94)*
		23	0.315[3], 0.306[3] (0.311)	63[3], 61[3] (62)	*67*	64, 64 (64)	*98, 95 (97)*

Table 6.1-5: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
	AMPA	0	0.420, 0.445 (0.433)	84, 89 (87)	*100*	-	
		1	0.427, 0.437 (0.432)	86, 88 (87)	*100*	87, 91 (89)	*96, 99 (98)*
		3	0.415, 0.440 (0.428)	83, 88 (86)	*99*	91, 95 (93)	*89, 95 (92)*
		6	0.337, 0.371 (0.354)	68, 74 (71)	*82*	80, 86 (83)	*82, 90 (86)*
		9	0.362, 0.371 (0.367)	73, 75 (74)	*85*	87, 84 (86)	*85, 88 (87)*
		12	0.403, 0.377 (0.390)	81, 75 (78)	*90*	96, 102 (99)	*82, 76 (79)*
		23	0.335, 0.342 (0.339)	67, 68 (68)	*78*	93, 101 (97)	*69, 70 (70)*
	N-acetyl-AMPA	0	0.444, 0.426 (0.435)	89, 85 (87)	*100*	-	
		1	0.481, 0.451 (0.466)	97, 91 (94)	*107*	83, 81 (82)	*118, 111 (115*
		3	0.419, 0.451 (0.435)	84, 90 (87)	*100*	86, 91 (89)	*95, 102 (99)*
		6	0.379, 0.410 (0.395)	76, 82 (79)	*91*	78, 75 (77)	*100, 108 (104)*
		9	0.638, 0.575 (0.607)	128, 115 (122)	*140*	115, 119 (117)	*109, 99 (104)*
		12	0.532, 0.567 (0.550)	106, 113 (110)	*126*	101, 109 (105)	*101, 108 (105)*
		23	0.409, 0.428 (0.419)	82, 86 (84)	*96*	77, 77 (77)	*106, 111 (109)*

[1] Fortification level of 0.5 mg/kg for glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-glyphosate

[2] Corrected % Recovery = Stored Sample % Recovery / Average % Recovery of Fresh Recovery Samples × 100; All corrected recoveries are based on unrounded values stated in the study report. Hand calculations may vary from reported values because of rounding.

[3] Due to matrix interference for the *N*-acetyl-glyphosate 212>88 mass transition, the results from the 212>170 mass transition for the 12 and 23 months storage intervals (mg/kg, % Recovery) were used:
12 months: Stored Fort. A: 0.41 mg/kg, 82 %; Stored Fort. B: 0.40 mg/kg, 80 %; Fresh Fort. A: 0.39 mg/kg, 78 %; Fresh Fort. B: 0.41 mg/kg, 83 %; Corrected Rec. A: 102 %; Normalised Rec. B: 98 %
23 months: Stored Fort. A: 0.36 mg/kg, 71 %; Stored Fort. B: 0.34 mg/kg, 68b%; Fresh Fort. A: 0.34 mg/kg, 69 %; Fresh Fort. B: 0.36 mg/kg, 72 %; Corrected Rec. A: 102 %; Normalised Rec. B: 97 %

III. Conclusion

In this study glyphosate, *N*-acetyl-glyphosate and AMPA proved to be stable in GAT and ZM-HRA maize green plant, forage and grain for at least 12 months when stored at ≤ -20°C. For *N*-acetyl-AMPA samples were stored for 23 months and the analyses showed no significant degradation. In maize stover all analytes were stored for 23 months without significant degradation.

In maize green plants only 64 % of the applied concentration of AMPA was recovered after 9 months of storage. However, the procedural recovery was also rather low for these samples (77 %). In addition, in maize forage, which is a closely related matrix to green maize plants, no significant degradation was observed. Therefore, it can be concluded that AMPA is also stable for at least 12 months in green maize plants.
In maize stover only 62 % of the applied concentration of *N*-acetyl-glyphosate and 68 % of the applied AMPA were recovered after 23 months of storage. However, under consideration of the procedural recoveries no significant decline was observed for *N*-acetyl-glyphosate. For AMPA residues after 23 months were still 78 % of the day 0 concentration.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and its metabolites *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in high starch content matrix (maize grain), high water content matrices (maize green plant and forage) and dry matrix (maize stover) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with one deviation. A mixed spiking solution was used for glyphosate, *N*-acetyl-glyphosate and AMPA. However, the storage stability data for each analyte shows that there is no significant change in the concentration of any of the analytes. Hence, transformation from one compound to another is very unlikely.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/005
Report author	[redacted]
Report year	2007
Report title	Stability of Glyphosate, *N*-Acetylglyphosate, Aminomethyl phosphonic acid and *N*-Acetyl AMPA in GAT soybean forage, seed, and hay stored frozen
Report No	49990
Document No	ASB2008-2654
Guidelines followed in study	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Appendix H: Storage Stability of Residue Samples (7032/VI/95 Rev. 5, 22/Jul/1997)
Deviations from current test guideline	Yes (OECD 506): • A mixed spiking solution was used for glyphosate, *N*-acetyl-glyphosate and AMPA
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA (aminomethylphosphonic acid) and *N*-acetyl-AMPA in GAT soybean forage, seed and hay stored at about ≤ -20 °C was investigated. The samples were spiked with glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-glyphosate at a concentration level of 0.5 mg/kg (10x LOQ). The residues of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA were stable in soybean forage, seed and hay when stored at approximately -20 °C for the maximum period stored: 12 months for glyphosate, *N*-acetyl-glyphosate and AMPA and 18 months for *N*-acetyl-AMPA.

I. Materials and methods

A. Materials

1. Test material:

Identification:	Glyphosate	*N*-acetyl-glyphosate	AMPA	*N*-acetyl-AMPA
Description:	Not reported	Not reported	Not reported	Not reported
Lot/Batch #:	014	000	10003440	001
Purity:	97 %	84.3 % as sodium salt 67.4 % as free acid	99.5 %	76 %
CAS # :	1071-83-6	129660-96-4	1066-51-9	57637-97-5
Spiking levels:	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	GAT modified soybean
Botanical name:	*Glycine max*
Crop parts(s) or processed commodity:	Forage, seeds, hay
Sample size:	5 g (forage and seed), 10 g (hay)

B. Study design

1. Test procedure

The storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in GAT soybean forage, seed and hay stored at about ≤ -20°C was investigated.
Homogenised samples were spiked with the test items at a concentration level of 0.5 mg/kg for glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA. Samples were spiked with glyphosate, *N*-acetyl-glyphosate and AMPA together. Separate stability samples were prepared for *N*-acetyl AMPA. The samples were stored in plastic bottles at approximately -20°C until analysis. Soybean forage, seed and hay samples were tested for the stability of glyphosate, *N*-acetyl-glyphosate and AMPA at six storage intervals over a period of 12 months and *N*-acetyl-AMPA at six storage intervals over a period of 18 months.

Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples (fortified with glyphosate, *N*-acetyl-glyphosate, AMPA and
N-acetyl-AMPA), and two aged (storage stability) samples fortified with glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA.

2. Description of analytical procedures

Samples were analysed using procedures based on enforcement method DuPont-15444, "Analytical Method for the Determination of Glyphosate and Respective Metabolite Residues in Various Crop Matrices Using LC/MS/MS" with modifications. For the determination of glyphosate and the metabolites *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA duplicate samples were extracted using 0.1 % formic acid/methanol (96/4 v/v), cleaned by SPE and analysed using LC/MS/MS.
The analytical method was fully validated during the study DuPont-15444 ([redacted], 2007; refer to CA 4.1.2). The LOQ was 0.05 mg/kg for each analyte.
In order to confirm the accuracy of the residues determination, procedural recoveries were determined from samples fortified with glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA at 0.5 mg/kg.
The procedural recoveries of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA were between 70 % and 110 % except for AMPA in soybean forage. The relative standard deviations (RSDs) per analyte and commodity were below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-6: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various soybean commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)	*Recovery of stored samples corrected for procedural recoveries[2] (%)*
Soybean forage	Glyphosate	0[3]	0.485, 0.490 (0.488)	97, 98 (98)	*100*	-	-
		1[4]	0.427, 0.464 (0.446)	86, 93 (90)	*91*	92, 95 (94)	*92, 99 (96)*
		3	0.382, 0.390 (0.386)	77, 78 (78)	*79*	65, 77 (71)	*108, 110 (109)*
		6	0.434, 0.444 (0.439)	87, 89 (88)	*90*	87, 92 (90)	*97, 99 (98)*
		9	0.394, 0.431 (0.413)	79, 87 (83)	*85*	85, 88 (87)	*92, 101 (97)*
		12	0.450, 0.450 (0.450)	90, 90 (90)	*92*	85, 85, (85)	*106, 106 (106)*
	N-acetyl-glyphosate	0[3]	0.532, 0.531 (0.532)	106, 106 (106)	*100*	-	-
		1[4]	0.485, 0.464 (0.475)	98, 93 (96)	*89*	90, 94 (92)	*107, 101 (104)*
		3	0.530, 0.517 (0.524)	107, 103 (105)	*98*	106, 109 (108)	*100, 97 (99)*
		6	0.474, 0.530	95, 106	*94*	98, 99 (99)	*96, 108 (102)*

Table 6.1-6: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various soybean commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)	*Recovery of stored samples corrected for procedural recoveries[2] (%)*
			(0.502)	(101)			
		9	0.457, 0.464 (0.461)	92, 93 (93)	*87*	84, 95 (90)	*103, 104 (104)*
		12	0.533, 0.525 (0.529)	107, 105 (106)	*99*	105, 106 (106)	*101, 100 (101)*
	AMPA	0	0.447, 0.450 (0.449)	89, 90 (90)	*100*	-	-
		1[4]	0.432, 0.414 (0.423)	87, 83 (85)	*94*	79, 85 (82)	*106, 101 (104)*
		3	0.408, 0.401 (0.405)	82, 80 (81)	*90*	93, 87 (90)	*91, 89 (90)*
		6	0.511, 0.470 (0.491)	102, 94 (98)	*109*	112, 114 (113)	*90, 83 (87)*
		9	0.393, 0.378 (0.386)	79, 76 (78)	*86*	85, 78 (82)	*97, 93 (95)*
		12	0.372, 0.367 (0.370)	75, 74 (75)	*82*	79, 83 (81)	*92, 91 (92)*
	N-acetyl-AMPA	0	0.406, 0.411 (0.409)	81, 82 (82)	*100*	-	-
		1[4]	0.407, 0.378 (0.393)	82, 76 (79)	*96*	87, 87 (87)	*94, 87 (91)*
		3	0.364, 0.365 (0.365)	73, 73 (73)	*89*	74, 82 (78)	*93, 94 (94)*
		6	0.404, 0.423 (0.414)	81, 85 (83)	*101*	87, 80 (84)	*97, 102 (100)*
		9	N/A	N/A	*N/A*	N/A	*N/A*
		12[5]	0.488, 0.474 (0.481)	98, 95 (97)	*118*	92, 89 (91)	*109, 105 (107)*
		18	0.454, 0.456 (0.455)	91, 91 (91)	*111*	90, 82 (86)	*106, 106 (106)*
Soybean seeds	Glyphosate	0	0.384, 0.373 (0.379)	77, 75 (76)	*100*	-	-
		1	0.394, 0.414 (0.404)	79, 83 (81)	*107*	80, 79 (80)	*99, 105 (102)*
		3	0.374, 0.379 (0.377)	75, 76 (76)	*99*	76, 80 (78)	*96, 97 (97)*
		6	0.405, 0.394 (0.400)	81, 79 (80)	*106*	79, 85 (82)	*99, 96 (98)*
		9[7]	0.353, 0.371 (0.362)	71, 75 (73)	*96*	72, 72 (72)	*98, 103 (101)*
		12[8]	0.376, 0.377 (0.377)	75, 76 (76)	*99*	75, 77 (76)	*99, 99 (99)*
	N-acetyl-glyphosate	0	0.470, 0.441 (0.456)	94, 88 (91)	*100*	-	-
		1[3]	0.430, 0.398 (0.414)	86, 80 (83)	*91*	81, 89 (85)	*101, 95 (98)*
		3	0.390, 0.385 (0.388)	78, 78 (78)	*85*	79, 78 (79)	*99, 98 (99)*

Table 6.1-6: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various soybean commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)	*Recovery of stored samples corrected for procedural recoveries[2] (%)*
		6	0.399, 0.394 (0.397)	80, 79 (80)	*87*	76, 82 (79)	*101, 100 (101)*
		9[7]	0.375, 0.370 (0.373)	75, 74 (75)	*82*	77, 74 (76)	*100, 99 (100)*
		12[8]	0.424, 0.420 (0.422)	85, 84 (85)	*93*	83, 83 (83)	*102, 102 (102)*
	AMPA	0	0.364, 0.404 (0.384)	73, 81 (77)	*100*		-
		1[6]	0.386, 0.360 (0.373)	77, 72 (75)	*97*	71, 81 (76)	*102, 96 (99)*
		3	0.420, 0.390 (0.405)	84, 78 (81)	*105*	89, 80 (85)	*100, 93 (97)*
		6	0.513, 0.531 (0.522)	103, 107 (105)	*136*	106, 110 (108)	*95, 98 (97)*
		9	0.365, 0.387 (0.376)	74, 78 (76)	*98*	82, 79 (81)	*92, 97 (95)*
		12[5]	0.382, 0.391 (0.387)	76, 78 (77)	*101*	78, 76 (77)	*99, 102 (101)*
	N-acetyl-AMPA	0	0.402, 0.397 (0.400)	81, 79 (80)	*100*	-	-
		1[6]	0.344, 0.382 (0.363)	69, 77 (73)	*91*	70, 71 (71)	*99, 110 (105)*
		3	0.351, 0.395 (0.373)	70, 79 (75)	*93*	75, 73 (74)	*94, 106 (100)*
		6	0.394, 0.431 (0.413)	79, 86 (83)	*103*	80, 83 (82)	*97, 106 (102)*
		9	N/A	N/A	*N/A*	N/A	*N/A*
		12[5]	0.532, 0.564 (0.548)	106, 113 (110)	*137*	105, 108 (107)	*100, 106 (103)*
		18	0.388, 0.386 (0.387)	78, 77 (78)	*97*	74, 72 (73)	*106, 106 (106)*
Soybean hay	Glyphosate	0[3]	0.358, 0.464 (0.411)	72, 93 (83)	*100*	-	-
		1	0.371, 0.365 (0.368)	74, 73 (74)	*90*	65, 74 (70)	*107, 106 (107)*
		3	0.379, 0.388 (0.384)	76, 78 (77)	*93*	71, 73 (72)	*106, 108 (107)*
		6	0.381, 0.370 (0.376)	77, 74 (76)	*91*	69, 75 (72)	*107, 103 (105)*
		9	0.382, 0.401 (0.392)	76, 80 (78)	*95*	76, 82 (79)	*96, 101 (99)*
		12[5]	0.345, 0.336 (0.341)	69, 67 (68)	*83*	73, 73 (73)	*95, 92 (94)*
	N-acetyl-glyphosate	0[3]	0.513, 0.522 (0.518)	103, 105 (104)	*100*	-	-
		1	0.409, 0.420 (0.415)	82, 84 (83)	*80*	83, 79 (81)	*101, 104 (103)*
		3	0.576, 0.509	116, 102	*105*	80, 98 (89)	*130, 115*

Table 6.1-6: Storage stability of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in various soybean commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)	*Recovery of stored samples corrected for procedural recoveries[2] (%)*
			(0.543)	(109)			*(123)*
		6	0.416, 0.398 (0.407)	84, 80 (82)	*79*	73, 80 (77)	*109, 104 (107)*
		9	0.358, 0.434 (0.396)	72, 87 (80)	*76*	86, 76 (81)	*88, 107 (98)*
		12[5]	0.471, 0.437 (0.454)	94, 87 (91)	*88*	98, 96 (97)	*97, 90 (94)*
	AMPA	0	0.367, 0.442 (0.405)	73, 89 (81)	*100*	-	-
		1[4]	0.528, 0.484 (0.506)	106, 97 (102)	*125*	97, 99 (98)	*108, 99 (104)*
		3	0.454, 0.421 (0.438)	91, 84 (88)	*108*	75, 86 (81)	*113, 105 (109)*
		6	0.428, 0.370 (0.399)	86, 74 (80)	*99*	75, 89 (82)	*105, 91 (98)*
		9	0.368, 0.389 (0.379)	74, 78 (76)	*94*	77, 80 (79)	*93, 99 (96)*
		12[5]	0.331, 0.326 (0.329)	66, 65 (66)	*81*	79, 71 (75)	*88, 86 (87)*
	N-acetyl-AMPA	0	0.410, 0.414 (0.412)	82, 83 (83)	*100*	-	-
		1[4]	0.313, 0.406 (0.360)	63, 81 (72)	*87*	81, 74 (78	*81, 105 (93)*
		3	0.364, 0.329 (0.347)	73, 66 (70)	*84*	75, 71 (73)	*99, 90 (95)*
		6	0.350, 0.336 (0.343)	70, 67 (69)	*83*	71, 70 (71)	*100, 96 (98)*
		9	N/A	N/A	*N/A*	N/A	*N/A*
		12[5]	0.523, 0.504 (0.514)	105, 101 (103)	*125*	101, 96 (99)	*106, 103 (105)*
		18	0.337, 0.350 (0.344)	68, 70 (69)	*83*	71, 69 (70)	*97, 100 (99)*

[1] Fortification level of 0.5 mg/kg for glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA
[2] Corrected % Recovery = Stored Sample % Recovery / Average % Recovery of Fresh Recovery Samples * 100
[3] Glyphosate, *N*-acetyl-glyphosate, and AMPA samples were stored for 7 days; *N*-acetyl-AMPA samples were stored for 3 weeks
[4] AMPA samples were stored for 1 month + 10 days and *N*-acetyl-AMPA samples were stored for 1 month + 23 days
[5] *N*-acetyl-AMPA samples were stored for 12 months + 2 weeks.
[6] AMPA samples were stored for 1 month + 10 days and *N*-acetyl-AMPA samples were stored for 1 month +2 weeks
[7] Glyphosate and *N*-acetyl-glyphosate samples were stored for 9 months + 9 days
[8] Glyphosate and *N*-acetyl-glyphosate samples were stored for 12 months + 9 days. *N*-acetyl-AMPA samples were stored for 12 months + 2 weeks

III. Conclusion

In this study residues of glyphosate, *N*-acetyl-glyphosate and AMPA were proven to be stable in GAT soybean forage, seeds and hay for at least 12 months when stored at ≤ -20 °C. In soybean hay, at the 12 months storage interval, only 68 % of applied concentration of glyphosate and 66 % of the applied

concentration of AMPA were recovered. However, the procedural recoveries were 73 % and 75 %, respectively, suggesting a higher true residue concentration > 70 %.
Residues of *N*-acetyl-AMPA were proven to be stable in soybean forage, seeds and hay for 18 months. In soybean hay, only 69 % of applied concentration of *N*-acetyl-AMPA was recovered at 6 months and 18 months storage interval, however, the procedural recoveries were 71 % and 70 %, respectively, suggesting a higher true residue concentration > 70 %.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and its metabolites *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA in high oil content matrix (soybean seed), high water content matrix (soybean forage) and dry matrix (soybean hay) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with one deviation. A mixed spiking solution was used for glyphosate, *N*-acetyl-glyphosate and AMPA. However, the storage stability data for each analyte shows that there is no significant change in the concentration of any of the analytes. Hence, transformation from one compound to another is very unlikely.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/006
Report author	[redacted]
Report year	2007
Report title	Stability of glyphosate, *N*-Acetylglyphosate and Aminomethyl phosphonic acid in GAT corn forage, grain, and stover, stored frozen
Report No	49991
Document No	ASB2008-2655
Guidelines followed in study	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Appendix H: Storage Stability of Residue Samples (7032/VI/95 Rev. 5, 22/Jul/1997)
Deviations from current test guideline	Yes (OECD 506): • A mixed spiking solution was used
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (I, docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate, AMPA (aminomethylphosphonic acid) and *N*-acetyl-glyphosate in maize forage, grain and stover from GAT maize (stored at about ≤ -20°C) was investigated. The samples

were spiked with glyphosate, AMPA and *N*-acetyl-glyphosate together at a concentration level of 0.5 mg/kg (10x LOQ). In all matrices investigated, glyphosate, AMPA and *N*-acetyl-glyphosate residues were stable for the maximum period tested: 12 months.

I. Materials and methods

A. Materials			
1. Test material:			
Identification:	Glyphosate	*N*-acetyl-glyphosate	AMPA
Description:	Not reported	Not reported	Not reported
Lot/Batch #:	014	000	10003440
Purity:	97 %	84.3 % as sodium salt 67.4 % as free acid	99.53 %
CAS # :	1071-83-6	129660-96-4	1066-51-9
Spiking levels:	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg
2. Test Commodity:			
Crop:	Maize		
Type:	Cereals		
Variety:	GAT modified maize		
Botanical name:	*Zea mays*		
Crop parts(s) or processed commodity:	Grain, forage, stover		
Sample size:	5 g (maize forage and grain), 10 g (maize stover)		

B. Study design

1. Test procedure

The storage stability of glyphosate, *N*-acetyl-glyphosate and AMPA in forage, grain and stover stored from GAT maize at about ≤ -20 °C was investigated.
Homogenised samples were spiked with the test items together at a concentration level of 0.5 mg/kg (10x LOQ) for glyphosate, *N*-acetyl-glyphosate and AMPA. The samples were stored in plastic bottles at approximately -20°C until analysis. At five storage intervals over a period of 12 months the samples were tested for the stability of glyphosate, *N*-acetyl-glyphosate and AMPA.
Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples (fortified with glyphosate, AMPA and *N*-acetyl-glyphosate), and two aged (storage stability) samples fortified with glyphosate, AMPA and *N*-acetyl-glyphosate.

2. Description of analytical procedures

Samples were analysed using procedures based on enforcement method DuPont-15444, "Analytical Method for the Determination of Glyphosate and Respective Metabolite Residues in Various Crop Matrices Using LC/MS/MS" with modifications. For the determination of glyphosate and the metabolites *N*-acetyl-glyphosate and AMPA duplicate samples were extracted using 0.1 % formic acid/methanol (96/4 v/v), cleaned by SPE and analysed using LC/MS/MS.
The analytical method was fully validated during the study DuPont-15444 ([redacted] 2007; refer to CA 4.1.2). The LOQ was 0.05 mg/kg for each analyte.

In order to check the validity of the method, procedural recoveries were determined from samples freshly fortified with glyphosate, *N*-acetyl-glyphosate and AMPA at 0.5 mg/kg.
All procedural recoveries of glyphosate, *N*-acetyl-glyphosate and AMPA were between 70 % and 110 % and relative standard deviations (RSDs) per analyte and commodity were below 20 %, thus confirming the accuracy of the residue determination.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-7: Storage stability of glyphosate, *N*-acetyl-glyphosate and AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
Maize forage	Glyphosate	0	0.520, 0.497 (0.509)	105, 99 (102)	*100*	-	-
		1	0.502, 0.508 (0.505)	102, 101 (102)	*99*	105, 106 (106)	*96, 97 (97)*
		3	0.479, 0.455 (0.467)	96, 91 (94)	*92*	89, 85 (87)	*110, 104 (107)*
		6	0.403, 0.409 (0.406)	81, 82 (82)	*80*	84, 81 (83)	*98, 100 (99)*
		12	0.489, 0.477 (0.483)	98, 96 (97)	*95*	101, 96 (99)	*100, 98 (99)*
	N-acetyl-glyphosate	0	0.455, 0.458 (0.457)	92, 92 (92)	*100*	-	-
		1	0.358, 0.353 (0.356)	72, 71 (72)	*78*	74, 70 (72)	*99, 98 (99)*
		3	0.410, 0.404 (0.407)	82, 81 (82)	*89*	83, 75 (79)	*105, 103 (104)*
		6	0.372, 0.380 (0.376)	75, 76 (76)	*82*	83, 78 (81)	*93, 95 (94)*
		12	0.435, 0.437 (0.436)	88, 88 (88)	*95*	99, 98 (99)	*89, 89 (89)*
	AMPA	0	0.468 0.494 (0.481)	94, 99 (97)	*100*	-	-
		1	0.446, 0.452 (0.449)	90, 91 (91)	*93*	95, 96 (96)	*94, 95 (95)*
		3	0.503, 0.464 (0.484)	101, 93 (97)	*101*	100, 93 (97)	*105, 97 (101)*
		6	0.370, 0.371 (0.371)	74, 74 (74)	*77*	79, 84 (82)	*91, 91 (91)*
		12	0.384, 0.353 (0.369)	77, 71 (74)	*77*	99, 91 (95)	*81, 75 (78)*

Table 6.1-7: Storage stability of glyphosate, *N*-acetyl-glyphosate and AMPA in various maize commodities

Commodity	**Analyte**	**Storage period (months)**	**Residue level in stored samples (mg/kg) (mean)**	**Recovery of stored samples (% of nominal spiking level[1]) (mean)**	***% of initial value at day 0***	**Procedural recovery of freshly fortified samples (%) (mean)**	***Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)***
Maize grain	Glyphosate	0	0.521, 0.502 (0.512)	105, 100 (103)	*100*	-	-
		1	0.542, 0.571 (0.557)	108, 114 (111)	*109*	105, 106 (106)	*103, 109 (106)*
		3	0.409, 0.440 (0.425)	82, 88 (86)	*83*	93, 82 (88)	*93, 101 (97)*
		6	0.402, 0.418 (0.410)	80, 84 (82)	*80*	80, 80 (80)	*100, 105 (103)*
		12	0.442, 0.525 (0.484)	89, 105 (97)	*95*	89, 93 (91)	*98, 115 (107)*
	N-acetyl-glyphosate	0	0.486, 0.491 (0.489)	98, 98 (98)	*100*	-	-
		1	0.372, 0.469 (0.421)	74, 94 (84)	*86*	84, 77 (81)	*93, 117 (105)*
		3	0.398, 0.407 (0.403)	80, 82 (81)	*82*	81, 78 (80)	*100, 103 (102)*
		6	0.366, 0.368 (0.367)	73, 74 (74)	*75*	79, 78 (79)	*93, 94 (94)*
		12	0.398, 0.407 (0.403)	80, 81 (81)	*82*	74, 84 (79)	*101 103 (102)*
	AMPA	0	0.497, 0.483 (0.490)	100, 97 (99)	*100*	-	-
		1	0.490, 0.474 (0.482)	98, 95 (97)	*98*	108, 102 (105)	*94, 90 (92)*
		3	0.500, 0.509 (0.505)	100, 102 (101)	*103*	91, 92 (92)	*110, 112 (111)*
		6	0.420, 0.409 (0.415)	84, 82 (83)	*85*	87, 86 (87)	*97, 95 (96)*
		12	0.400, 0.469 (0.435)	81, 94 (88)	*89*	84, 85 (85)	*95, 111 (103)*
Maize stover	Glyphosate	0	0.526, 0.529 (0.528)	105, 106 (106)	*100*	-	-
		1	0.499, 0.501 (0.500)	100, 100 (100)	*95*	96, 96 (96)	*104, 104 (104)*
		3	0.437, 0.389 (0.413)	88, 78 (83)	*78*	92, 87 (90)	*98, 87 (93)*
		6	0.442, 0.427 (0.435)	89, 86 (88)	*82*	100, 100 (100)	*88, 86 (87)*
		12	0.446, 0.444 (0.445)	89, 89 (89)	*84*	92, 93 (93)	*97, 96 (97)*

Table 6.1-7: Storage stability of glyphosate, *N*-acetyl-glyphosate and AMPA in various maize commodities

Commodity	Analyte	Storage period (months)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[2] (%) (mean)*
	N-acetyl-glyphosate	0	0.488, 0.450 (0.469)	98, 90 (94)	*100*	-	-
		1	0.426, 0.433 (0.430)	85, 87 (86)	*92*	85, 89 (87)	*97, 99 (98)*
		3	0.405, 0.380 (0.393)	81, 76 (79)	*84*	73, 81 (77)	*105, 98 (102)*
		6	0.462, 0.476 (0.469)	93, 95 (94)	*100*	102, 100 (101)	*92, 95 (94)*
		12	0.519, 0.505 (0.512)	104, 101 (103)	*109*	104, 98 (101)	*102, 100 (101)*
	AMPA	0	0.472, 0.472 (0.472)	94, 95 (95)	*100*	-	-
		1	0.451, 0.475 (0.463)	90, 95 (93)	*98*	95, 90 (93)	*98, 103 (101)*
		3	0.415, 0.429 (0.422)	83, 86 (85)	*89*	90, 101 (96)	*87, 90 (89)*
		6	0.352, 0.387 (0.370)	71, 78 (75)	*78*	92, 92 (92)	*77, 85 (81)*
		12	0.323, 0.320 (0.322)	65, 64 (65)	*68*	87, 85 (86)	*75, 74 (75)*

[1] Fortification level of 0.5 mg/kg for glyphosate, AMPA and *N*-acetyl-glyphosate

[2] Recoveries of stored fortifications were corrected based on the average of the two fresh fortifications for the analyte at each storage interval. (Corrected % Recovery = Stored Sample % Recovery / Average % Recovery of Fresh Recovery Samples * 100). All corrected recoveries are based on unrounded values stated in the study report. Hand calculations may vary from reported values because of rounding.

III. Conclusion

This study demonstrates the storage stability of glyphosate, *N*-acetyl-glyphosate and AMPA in GAT maize matrices (grain, forage and stover) for a period of at least 12 months when stored at ≤ -20°C.
For AMPA in maize stover the final sample collected after 12 months showed residues <70 % of the nominal level. However, under consideration of the procedural recoveries, the recovery rate from stored samples was > 70 %. Moreover, results of a separate storage stability study (see CA 6.1/004) confirm stability of AMPA for at least 12 months in maize stover.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolites *N*-acetyl-glyphosate and AMPA in high starch content matrix (maize grain), high water content matrix (maize forage) and dry matrix (maize stover) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with one deviation. A mixed spiking solution was used for glyphosate, *N*-acetyl-glyphosate and AMPA. However, the storage stability data for each analyte shows that there is no significant change in the concentration of any of the analytes. Hence, transformation from one compound to another is very unlikely.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/007
Report author	[redacted]
Report year	1997
Report title	Determination of the Storage Stability of Glyphosate in Beans, Oilseed Rape and Linseed
Report No	IF-94/13882-00
Document No	394 GLY
Guidelines followed in study	EPA OPPTS 860.1380 – Storage Stability Data (1996) EU Guidance Appendix H: Storage Stability of Residue Samples (7032/VI/95 Rev. 5, 22/Jul/1997)
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary
The storage stability of glyphosate in beans, oilseed rape and linseed stored at about ≤-18 °C was investigated. The samples were spiked with glyphosate at a concentration level of at least 10x the LOQ: 2.6 mg/kg, 0.6 and 5.6 mg/kg, respectively. In all matrices investigated (representatives of high oil content and high protein matrices), glyphosate residues were stable for the maximum period tested: 18 months.

I. Materials and methods

A.	**Materials**			
1.	**Test material:**			
	Identification:	Glyphosate		
	Description:	Not reported		
	Lot/Batch #:	185-ff-131		
	Purity:	99.5 %		
	CAS # :	1071-83-6		
	Spiking levels:	2.6 mg/kg (beans), 0.6 mg/kg (oilseed rape), 5.7 mg/kg (linseed)		
2.	**Test Commodity:**			
	Crop:	Beans	Oilseed rape	Linseed
	Type:	Pulses	Oilseeds	Oilseeds
	Variety:	Not reported		
	Botanical name:	*Phaseolus vulgaris*	*Brassica napus*	*Linum usitatissimum*
	Crop parts(s) or processed			
	Commodity:	Not reported		
	Sample size:	10 g each		

B. Study design

1. Test procedure

The storage stability of glyphosate in beans, oilseed rape and linseed was investigated. Bean samples were homogenised, oilseed rape and linseed samples were used unprocessed. Duplicate samples were spiked with the test item at a concentration level of 2.6 mg/kg, 0.6 mg/kg and 5.6 mg/kg, respectively. The spiked samples were stored in plastic bottles at about ≤ -18°C until analysis. At five samplings over a period of 551 days (18 months) the samples were tested for the stability of glyphosate.
Each analytical set for storage stability analysis included the following samples: a non-treated control, a concurrent freshly fortified matrix sample, and two aged (storage stability) samples fortified with glyphosate.

2. Description of analytical procedures

The analytical method based on DFG 405 (refer to CA 4.1.2) has been already used and described in projects IF-93/13833-01 (beans), IF-93/13831-01 (oilseed rape) and IF-93/13836-01 (linseed). For the determination of glyphosate samples were extracted with aqueous hydrochloric acid. After clean-up by elution through Chelex-100-ligand exchange and anion exchange resin, the eluate was evaporated to dryness to remove the hydrochloric acid. The samples were analysed by HPLC equipped with post-column derivatisation and a fluorescence detector. Determination involves post-column hypochlorite oxidation and reaction with o-phthalaldehyde and mercaptoethanol to produce a fluorescent derivative.
The limit of quantification (LOQ) in the study was reported as 0.05 mg/kg for beans and 0.06 mg/kg for oilseed rape and linseed.
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from samples of beans, oilseed rape and linseed freshly spiked with glyphosate at a concentration of 2.6 mg/kg, 0.6 mg/kg and 5.7 mg/kg, respectively. Mean recovery values were in the acceptable range of 70-110 %. The relative standard deviations (RSDs) were below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results used for the stability calculation were not corrected for recoveries.

Table 6.1-8: Storage stability of glyphosate in plant matrices

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	Procedural recovery of freshly fortified samples[1] (%)
Bean, dry seed	Glyphosate	0	2.30, 2.34 (2.32)	89, 90 (90)	81
		6 (174)	2.45, 2.33 (2.39)	94, 90 (92)	89
		12 (371)	2.84, 2.76 (2.8)	109, 106 (108)	79
		15 (456)	2.70, 2.75 (2.73)	104, 106 (105)	84
		18 (551)	2.56, 2,51 (2.54)	99, 96 (98)	97
Rapeseeds	Glyphosate	0	0.584, 0.470 (0.527)	96, 77 (87)	78
		6 (174)	0.529, 0.531 (0.53)	87, 88 (88)	85
		12 (371)	0.564, 0.589 (0.577)	93, 97 (95)	68
		15 (456)	0.633, 0.698 (0.666)	104, 115 (111)	83
		18 (551)	0.59, 0.58 (0.585)	97, 95 (96)	102
Linseeds	Glyphosate	0	5.34, 5.18 (5.26)	94, 91 (93)	86
		6 (182)	5.17, 4.82 (0.50)	91, 85 (88)	96
		12 (371)	4.98, 6.03 (0.55)	88, 106 (97)	74
		15 (456)	6.22, 5.82 (0.602)	109, 103 (106)	87
		18 (551)	5.05, 5.06 (5.06)	89, 89 (89)	87

[1] Fortification level of 2.6 mg/kg for bean, 0.6 mg/kg for rapeseeds and 5.7 mg/kg for linseeds

III. Conclusion

In this study, glyphosate was proven to be stable in dry beans, rapeseeds and linseeds for at least 18 months when stored at ≤ -18 °C.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate in high protein content matrices (bean, dry seeds), high oil content matrices (rape seeds and linseeds) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/008
Report author	[redacted]
Report year	1996
Report title	Determination of glyphosate in soybean raw agricultural commodities (RAC) - stability report
Report No	91210
Document No	455 GLY (June 1993)
Guidelines followed in study	US EPA Pesticides Assessment Guideline (171-4)
Deviations from current test guideline	Yes (OECD 506): • Storage at -10°C instead of -18°C or lower • First sampling of soybean seeds at day 5 instead of day 0
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in soybean seed and straw was investigated. Homogenised samples were spiked separately with the test items at a concentration level of 1.0 mg/kg (10x LOQ) each and stored at < -10°C. Glyphosate and AMPA were stable for the maximum period tested: in soybean seeds (representative of high oil content oilseed crops) for at least 6 months and in soybean straw for at least 13 months when stored ≤-10 °C.

I. Materials and methods

A.	**Materials**		
1.	**Test material:**		
	Identification:	Glyphosate	AMPA
	Description:	Not reported	Not reported
	Lot/Batch #:	185-FF-131	45-95B
	Purity:	99.5 %	98.0 %
	CAS # :	1071-83-6	1066-51-9
	Spiking levels:	0.10 – 1.0 mg/kg	0.10 – 1.0 mg/kg
2.	**Test Commodity:**		
	Crop:	Soybean	
	Type:	Oilseeds	

Variety:	Not reported
Botanical name:	*Glycine max*
Crop parts(s) or processed commodity:	Seeds and straw
Sample size:	30 g (seeds), 15 g(straw)

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in soybean seed and straw was investigated.
Duplicate samples (homogenised) were spiked separately with the test items at a concentration level of 1.0 mg/kg, each. The spiked samples were stored in amber jars at about -10°C until analysis. At six samplings over a period of 398 days (13 months) for soybean straw and at four samplings over a period of 183 days (6 months) for soybean seeds the samples were tested for the stability of glyphosate.

Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples, and four aged (storage stability) samples, two fortified with glyphosate and two fortified with AMPA.

2. Description of analytical procedures

For the determination of glyphosate and the metabolite AMPA the Huntingdon Life Science method BD-045-91 based on DFG 405 (refer to CA 4.1.2) was used.
Samples were extracted with a chloroform hydrochloric acid mixture. After clean-up of the aqueous fraction by elution through Chelex 100 resin in the Fe(III) form glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron removed using an anion exchange resin. After concentration to dryness to remove the hydrochloric acid, samples were analysed by HPLC equipped with an o-phthalaldehyde (OPA) post-column reactor and a fluorescence detector. Determination involves post-column hypochlorite oxidation and reaction of the amine product with o-phthalaldehyde and mercaptoethanol to produce a fluorescent derivative.
The LOQ was 0.1 mg/kg each for glyphosate and AMPA.
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from freshly spiked samples of soybean seeds and straw. The samples were fortified with glyphosate and AMPA at concentrations of 0.1 mg/kg and 1.0 mg/kg each. The mean recoveries per analyte and commodity were in the acceptable range of 70-110 %. The relative standard deviations (RSDs) were below 20 % for AMPA. For glyphosate the RSDs were above 20 %.

II. Results and discussion

The results are presented in the table below. At the storage period of 45 days the results of the two glyphosate samples deviated by more than 20 %. However, since the difference of the results of all other storage periods was not greater than 20 %, this is negligible and has no influence on the validity of the study. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).
Glyphosate and AMPA in soybean seeds were stable for about 6 months (183 days) and in soybean straw for about 13 months (398 days).

Table 6.1-9: Storage stability of glyphosate and AMPA in soybean seeds and straw

Commodity	Analyte	Storage period (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0[2]*	Procedural recovery of freshly fortified samples[3] (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[3] (%) (mean)*
Soybean seeds	Glyphosate	5	0.762, 0.739 (0.751)	76, 74 (75)	*100*	64, 65 (65)	*115*
		14	0.740, 0.701 (0.721)	74, 70 (72)	*96*	73, 70 (72)	*100*
		45	0.526, 0.751 (0.639)	53, 75 (64)	*85*	68, 50 (59)	*108*
		183	0.857, 0.797 (0.827)	86, 80 (83)	*111*	108, 64 (86)	*97*
	AMPA	5	0.789, 0.779 (0.784)	79, 78 (78)	*100*	68, 69 (69)	*113*
		14	0.739, 0.805 (0.772)	74, 81 (77)	*98*	84, 87 (86)	*91*
		45	0.673, 0.769 (0.721)	67, 77 (72)	*92*	79, 52 (66)	*109*
		183	0.729, 0.704 (0.717)	73, 70 (72)	*91*	61, 72 (66)	*109*
Soybean straw	Glyphosate	0	0.846, 0.705 (0.776)	85, 71 (78)	*100*	50, 74 (62)	*126*
		45	0.759, 0.608 (0.684)	76, 61 (68)	*87*	55, 80 (67)	*101*
		44	0.846, 0.803 (0.825)	85, 80 (83)	*106*	114, 68 (91)	*91*
		102	0.709, 0.633 (0.671)	71, 63 (67)	*86*	78, 75 (77)	*87*
		300	0.666, 0.765 (0.712)	67, 77 (71)	*92*	99, 91 (95)	*75*
		398	0.718, 0.791 (0.755)	72, 79 (76)	*97*	126, 71 (99)	*78*

Table 6.1-9: Storage stability of glyphosate and AMPA in soybean seeds and straw

Commodity	Analyte	Storage period (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0[2]*	Procedural recovery of freshly fortified samples[3] (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[3] (%) (mean)*
	AMPA	0	0.802, 0.733 (0.768)	80, 73 (77)	*100*	66, 74 (70)	*110*
		15	0.625, 0.704 (0.665)	63, 70 (67)	*87*	66, 76 (71)	*94*
		44	0.687, 0.681 (0.684)	69, 68 (68)	*88*	68, 65 (67)	*101*
		102	0.515, 0.552 (0.534)	52, 55 (53)	*69*	62, 70 (66)	*80*
		300	0.406, 0.564 (0.485)	41, 56 (49)	*64*	82, 88 (85)	*54*
		398	0.605, 0.516 (0.561)	61, 52 (56)	*73*	62, 65 (63)	*89*

[1] Nominal spiking level: 1 mg/kg
[2] For soybean seeds day 5 is the reference as first analysis was done at day 5.
[3] Fortification level of 0.1 mg/kg (first value) and 1.0 mg/kg (second value)

III. Conclusion

In this study the procedural recoveries were generally very low, often not achieving a recovery rate of 70 % of the freshly fortified concentrations. Since this is an overall pattern within the study, it can be concluded that the analytical method used had a low precision, resulting in a large variation of the results. For glyphosate intermediate samples for seeds and straw showed uncorrected recovery values below the significance trigger of 70 % remaining. However, samples collected after longer storage intervals were above this trigger, suggesting the overall stability of glyphosate in both matrices for up to 6 months in soybean seeds and up to 13 months in soybean straw. Uncorrected residues recovered for AMPA from soybean seeds were between 72-78 % after up to 6 months. In soybean straw all samples except at fortification gave recovered residues below 70 % of the nominal concentration. Taking into account the low procedural recoveries, most samples lay between 70-100 % remaining, however one single corrected value after 10 months storage was 54 %. However, samples collected after longer storage intervals were above the trigger of 70 %. In summary, a slight degradation was observed for AMPA in soybean straw but the poor procedural recoveries do not allow the estimation of reliable storage intervals based on this study.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in high oil content and dry matrix was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with minor deviations. One deviation is that the samples were stored at -10°C instead of -18°C or lower, but since stability of glyphosate and AMPA is shown at -10°C it can be concluded that stability is also ensured at -18°C. Another deviation is that the first sampling of soybean seeds was at day 5, but has no influence on the validity of the study since stability was shown for all storage periods up to 6 months.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/009
Report author	[redacted]
Report year	1996
Report title	Determination of glyphosate in pasture grasses stability report
Report No	91212
Document No	456 GLY
Guidelines followed in study	US EPA Pesticides Assessment Guideline (171 4)
Deviations from current test guideline	Yes (OECD 506): • Storage at -10°C instead of -18°C or lower • First sampling of pasture grass at day 6 instead of day 0
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

[2] RMS to check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinica

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in pasture grasses was investigated. Homogenised samples were spiked separately with the test items at a concentration level of 1.0 mg/kg (10x LOQ) each and stored at <-10 °C for about one year. Glyphosate and AMPA were stable in pasture grasses (representative of high water content fodder crops) for the maximum period tested: 12 months when stored ≤-10 °C.

I. Materials and methods

A. Materials

1. Test material:

Identification:	Glyphosate	AMPA
Description:	Not reported	Not reported
Lot/Batch #:	185-FF-131	45-95B
Purity:	99.5 %	98.0 %
CAS #:	1071-83-6	1066-51-9
Spiking levels:	0.10 – 1.0 mg/kg	0.10 – 1.0 mg/kg

2. Test Commodity:

Crop:	Pasture grasses
Type:	Not applicable
Variety:	Not reported
Botanical name:	Not applicable
Crop parts(s) or processed Commodity:	Grasses
Sample size:	15 g

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in pasture grasses was investigated.
Duplicate samples (homogenised) were spiked separately with the test items at a concentration level of 1.0 mg/kg each. The spiked samples were stored in amber jars at <-10°C until analysis. At seven samplings over a period of 362 days the samples were tested for the stability of glyphosate and AMPA, respectively. Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples, and four aged (storage stability) samples, two fortified with glyphosate and two fortified with AMPA The concurrent matrix spike samples were fortified with a combined glyphosate/AMPA solution on the day of analysis.

2. Description of analytical procedures

For the determination of glyphosate and the metabolite AMPA the Huntingdon Life Science method BD-045-91 based on DFG 405 (refer to CA 4.1.2) was used.
Samples were extracted with a chloroform hydrochloric acid mixture. After clean-up of the aqueous fraction by elution through Chelex 100 resin in the Fe(III) form glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron removed using an anion exchange resin. After concentration to dryness to remove the hydrochloric acid, samples were analysed by HPLC equipped with an o-phthalaldehyde (OPA) post-column reactor and a fluorescence detector. Determination involves post-column hypochlorite oxidation and reaction of the amine product with o-phthalaldehyde and mercaptoethanol to produce a fluorescent derivative.
The LOQ was 0.1 mg/kg for each analyte.

The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from freshly spiked samples of pasture grass. The samples were fortified with glyphosate and AMPA at concentrations of 0.1 mg/kg and 1.0 mg/kg each. The mean recoveries per analyte and commodity were in the acceptable range of 70-110 %. The relative standard deviation (RSD) was below 20 % for AMPA. For glyphosate the RSD was above 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic). Glyphosate and AMPA in pasture grasses were stable for about 12 months.

Table 6.1-10: Storage stability of glyphosate and AMPA in pasture grasses

Commodity	Analyte	Storage period (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0[2]*	Procedural recovery of freshly fortified samples[3] (%) (mean)	*Recovery of stored samples corrected for procedural recoveries[3] (%) (mean)*
Pasture grasses	Glyphosate	6	0.781, 0.929 (0.855)	78, 93 (86)	*100*	110, 86 (98)	*88*
		10	1.02, 1.09 (1.06)	102, 109 (106)	*124*	122, 104 (113)	*94*
		19	0.922, 0.911 (0.917)	92, 91 (92)	*107*	140, 91 (115)	*80*
		51	0.831, 0.697 (0.764)	83, 70 (76)	*88*	244, 98 (98[4])	*78*
		95	0.755, 0.637 (0.696)	76, 64 (70)	*81*	74, 83 (79)	*87*
		187	0.706, 0.772 (0.739)	71, 77 (74)	*86*	98, 79 (89)	*83*
		362	0.849, 0.758 (0.804)	85, 76 (80)	*94*	102, 77 (90)	*89*
	AMPA	6	0.626, 0.553 (0.590)	63, 55 (59)	*100*	67, 81 (74)	*80*
		10	0.706, 0.731 (0.719)	71, 73 (72)	*122*	77, 91 (84)	*86*
		19	0.688, 0.686 (0.687)	69, 69 (69)	*116*	75, 80 (77)	*90*
		51	0.690, 0.638 (0.664)	69, 64 (66)	*113*	70, 74 (72)	*92*
		95	0.540, 0.634 (0.587)	54, 63 (59)	*99*	73, 80 (77)	*77*
		187	0.554, 0.654 (0.604)	55, 65 (60)	*102*	80, 74 (77)	*78*
		362	0.756, 0.729 (0.743)	76, 73 (74)	*126*	84, 78 (81)	*91*

[1] Nominal spiking level: 1 mg/kg
[2] Day 6 is the reference as first analysis was done at day 6.
[3] Fortification level of 0.1 mg/kg (first value) and 1.0 mg/kg (second value)
[4] based on 1.0 mg/kg fortification level only

III. Conclusion

In this study the results of the procedural recoveries based on freshly fortified samples ranged from 55-244 % for glyphosate and 67-91 % for AMPA, suggesting a relative low precision of the analytical method.
For glyphosate all uncorrected recoveries, except one sample after 3 months, were above the trigger value for a significant degradation of 70 % remaining. For AMPA samples collected after 6, 19, 51, 95 and 187 days gave unsatisfactory recoveries of 59-69 % remaining. However, corresponding procedural recoveries were also relatively low, suggesting corrected recoveries well above 70 % remaining. The samples collected after 10 and 362 days gave 72 % and 74 % remaining.
In summary, it can be concluded that both glyphosate and AMPA are stable during the 12 months storage interval investigated. However, the high amplitude in the procedural recoveries and the low initial residues directly after fortification lead to a strong variation in the results, suggesting to consider the study as additional information.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in high water content matrix was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with two minor deviations. One deviation is that the samples were stored at -10 °C instead of -18 °C or lower, but since stability of glyphosate and AMPA is shown at -10 °C it can be concluded that stability is also ensured at -18 °C. Another deviation is that the first sampling of grass was at day 6, but has no influence on the validity of the study since stability was shown for all storage periods up to 12 months.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/010
Report author	[redacted]
Report year	1996
Report title	Storage stability of residues of *N*-(phosphonomethyl) glycine and trimethylsulphonium cation in banana
Report No	RJ 2161B
Document No	33010290 (94JH232)
Guidelines followed in study	Not reported
Deviations from current test guideline	Yes, (OECD 506): • No details on sample preparation and storage condition is given
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate (*N*-(phosphonomethyl) glycine) and TMS (glyphosate trimesium (trimethylsolfonium cation)) in banana whole fruits (peel plus flesh) was investigated. Samples were spiked with the test items at concentration levels of 0.50 mg/kg glyphosate and TMS (10 × LOQ). The samples were stored at about ≤-18 °C until analysis for about 12 months. Glyphosate and TMS in banana samples (representatives of high water content matrices) were stable for the maximum period tested: 12 months.
TMS is not a relevant analyte in this dossier; therefore, data with respect to this analyte is not presented in the following summary.

I. Materials and methods

A. Materials			
1. Test material:			
Identification:	Glyphosate		
Description:	Not reported		
Lot/Batch #:	Not reported		
Purity:	99.2 %		
CAS # :	1071-83-6		
Spiking levels:	0.50 mg/kg		
2. Test Commodity:			
Crop:	Banana		
Type:	Miscellaneous fruits with inedible peel, large		
Variety:	Not reported		
Botanical name:	Not reported		
Crop parts(s) or processed			
Commodity:	Whole fruit (skin plus flesh)		
Sample size:	Not reported		

B. Study design

1. Test procedure

The storage stability of glyphosate in banana whole fruits (peel plus flesh) was investigated. Triplicate samples were spiked with the test item at a concentration level of 0.50 mg/kg. The spiked samples were stored at about ≤-18 °C until analysis. At three storage intervals over a period of 12 months, the samples were tested for the stability of glyphosate.
Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples, and three aged (storage stability) samples fortified with glyphosate.

2. Description of analytical procedures

Analysis was done according to procedures described in Residue Analytical Method 245/02 (refer to CA 4.1.2). In summary, glyphosate was extracted from the samples by maceration with water. The extracts were then cleaned-up by partitioning with chloroform followed by cation exchange

chromatography. An aliquot of the glyphosate-containing fraction was then derivatised with heptafluorobutanol and trifluoroacetic anhydride. The glyphosate derivative was analysed by gas chromatography with mass selective detection (GC-MSD). Residues were quantified by external standardisation. The limit of determination of this method was 0.05 mg/kg.
In order to confirm the accuracy of the residue determination, procedural recoveries were determined from banana whole fruit samples freshly spiked with 0.5 mg/kg glyphosate.
The procedural recovery values were in the acceptable range of 70-110 %. The relative standard deviation (RSD) was below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 is presented (italic).

Table 6.1-11: Storage stability of glyphosate in banana whole fruits

Commodity	Analyte	Storage period (months)	Residue level in stored samples[1] (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[2]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples[2] (%) (mean)
Banana whole fruit (peel plus flesh)	Glyphosate	0	0.36, 0.33, 0.42 (0.37)	72, 66, 84 (74)	*100*	77, 82 (80)
		6	0.47, 0.40, 0.35 (0.41)	94, 80, 70 (81)	*111*	88, 91 (90)
		12	0.37, 0.38, 0.35 (0.37)	74, 76, 70 (73)	*100*	71, 73 (72)

[1] Residues have been rounded to two significant figures
[2] Fortification level of 0.50 mg/kg

III. Conclusion

In this study, glyphosate was proven to be stable in banana whole fruit (peel plus flesh) samples for at least 12 months when stored at ≤ -18 °C.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessed the storage stability of glyphosate in high water content matrices (banana) was not previously evaluated at EU level a. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/011
Report author	[redacted]
Report year	1995
Report title	Storage Stability of Glyphosate and AMPA in Wheat Grain and Straw and in Rye Grain and Straw
Report No	303614
Document No	325 GLY
Guidelines followed in study	Biologische Bundesanstalt (BBA) Richtlinie Teil VI, Reihe 2: Rückstandsanalytik (1986), BBA-Merkblatt Nr.58, Rückstandsuntersuchungen – Richtlinie zur Durchführung der Analysen (1983) Industrieverband Agrar (IVA) Guidelines Rückstandsversuche
Deviations from current test guideline	Yes, (OECD 506): • Samples were not prepared as duplicates
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in wheat grain and straw and in rye grain and straw was investigated. Samples were spiked separately with the test items at a concentration level of at least 10x the LOQ, 1.0 mg/kg glyphosate and 0.5 mg/kg AMPA. The samples were stored at about -20°C until analysis for about 3.5 years.
Glyphosate is stable in wheat and rye matrices (grain and straw) (representative of high starch content cereal crops) for the maximum period tested: at least 3.5 years (45 months) when stored under deep freeze conditions. AMPA in cereal grain is stable for at least 288 days (10 months) and in straw for at least 190 days (6 months) under freezer conditions.

I. Materials and methods

A. Materials		
1. Test material:		
Identification:	Glyphosate	AMPA
Description:	White solid	Crystalline
Lot/Batch #:	185-ff-131	108F3811
Purity:	99.5 %	98.6 %
CAS # :	1071-83-6	1066-51-9
Spiking levels:	1.0 mg/kg	0.5 mg/kg
2. Test Commodity:		
Crop:	Wheat, rye	
Type:	Cereals	
Variety:	Not reported	
Botanical name:	*Triticum aestivum, Secale cereale*	
Crop parts(s) or processed		
Commodity:	Grain and straw	
Sample size:	15 g	

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in wheat grain and straw and in rye grain and straw was investigated.
Samples were spiked separately with the test items at a concentration level of 1.0 mg/kg glyphosate and 0.5 mg/kg AMPA. The samples were stored at about -20 °C in plastic bottles until analysis. At six samplings over a period of 1349 days the samples were tested for the stability of glyphosate and AMPA.
Each analytical set for storage stability analysis included the following samples: a non-treated control, two concurrent freshly fortified matrix samples (one with glyphosate, one with AMPA), and two aged (storage stability) samples, one fortified with glyphosate and one fortified with AMPA.

2. Description of analytical procedures

All samples were analysed using an adaptation of the analytical method DFG 405 (refer to CA 4.1.2).
For the determination of glyphosate and the metabolite AMPA the samples were extracted with hydrochloric acid. After clean-up of the aqueous fraction by elution through Chelex 100 resin in the Fe(III) form glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron removed using an anion exchange resin. After concentration to dryness to remove the hydrochloric acid, glyphosate and AMPA were quantified separately by means of HPLC equipped with a post derivatisation unit and a fluorescence detector.
Determination involves post-column hypochlorite oxidation for glyphosate and reaction of the amine product with o-phthalaldehyde and mercaptoethanol to produce a fluorescent derivative.
The limit of determination was 0.03 mg/kg for each analyte.
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from samples of wheat and rye grain and straw freshly spiked with glyphosate and AMPA at concentrations of 1.0 mg/kg and 0.5 mg/kg, respectively.

The mean recoveries were in acceptable range of 70-110 %.except for wheat grain (68 %). The relative standard deviations (RSDs) were below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-12: Storage stability of glyphosate and AMPA in grain and straw of wheat and rye

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)	*Recovery of stored samples corrected for procedural recoveries (%)*
Wheat grain	Glyphosate	0	0.761	76	*100*	-	-
		6 (190)	0.799	80	*105*	NP	-
		10 (288)	0.823	82	*108*	74	*111*
		21 (643)	0.648	65	*85*	57	*114*
		45 (1349)	0.688	69	*90*	72	*96*
	AMPA	0	0.393	79	*100*	-	-
		6 (190)	0.413	83	*105*	NP	-
		10 (288)	0.405	81	*103*	80	*101*
		21 (643)	0.276	55	*70*	64	*86*
		45 (1349)	0.230	46	*59*	76	*61*
Wheat straw	Glyphosate	0	0.873	87	*100*	-	-
		6 (190)	0.860	86	*99*	86	*100*
		10 (288)	0.803	80	*92*	82	*98*
		21 (643)	0.733	73	*84*	75	*97*
		45 (1349)	1.083	108	*124*	108	*100*
	AMPA	0	0.361	72	*100*	-	-
		6 (190)	0.413	83	*114*	86	*97*
		10 (288)	0.316	63	*88*	76	*83*
		21 (643)	0.245	49	*68*	68	*72*
		45 (1349)	0.286	57	*79*	89	*64*
Rye grain	Glyphosate	0	0.712	71	*100*	-	-
		6 (190)	0.884	88	*124*	106	*83*
		10 (288)	0.876	88	*123*	84	*105*
		21 (643)	0.752	75	*106*	73	*103*
		45 (1349)	0.683	68	*96*	90	*76*
	AMPA	0	0.399	80	*100*	-	-
		6 (190)	0.395	79	*99*	89	*89*
		10 (288)	0.395	79	*99*	79	*100*
		21 (643)	0.328	66	*82*	66	*100*
		45 (1349)	0.266	53	*67*	91	*58*
Rye straw	Glyphosate	0	0.850	85	*100*	-	-
		6 (190)	0.956	96	*112*	NP	-
		10 (288)	0.777	78	*91*	82	*95*
		21 (643)	0.599	60	*70*	82	*73*
		45 (1349)	0.945	95	*111*	114	*83*
	AMPA	0	0.429	86	*100*	-	-
		6 (190)	0.395	79	*92*	NP	-
		10 (288)	0.295	59	*69*	71	*83*

Table 6.1-12: Storage stability of glyphosate and AMPA in grain and straw of wheat and rye

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg)	Recovery of stored samples (% of nominal spiking level[1])	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)	*Recovery of stored samples corrected for procedural recoveries (%)*
		21 (643)	0.226	45	*53*	75	*60*
		45 (1349)	0.195	39	*45*	92	*42*

[1] Fortification level of 1.0 mg/kg for glyphosate and 0.5 mg/kg for AMPA
[NP] Not performed

III. Conclusion

The results of this study indicate that glyphosate is stable in wheat and rye (grain and straw) for a period of at least 45 months (3.5 years) when stored at ≤-20 °C. Although samples near the maximum storage interval tested gave residues slightly below the trigger value of 70 % remaining, the procedural recoveries and the relatively low recovery at day 0 suggest stability of the residue.
For AMPA a significant decline was observed in all grain samples stored longer than 10 months and in all straw samples stored longer than 6 months.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in high starch content matrices (grain) and straw was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with the deviation that samples were not prepared as duplicates. Nevertheless, stability for glyphosate and decline for AMPA at longer storage times can still clearly be seen.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/012
Report author	[redacted]
Report year	1991
Report title	Storage stability of glyphosate residues in crop commodities
Report No	MSL10843
Document No	M-644183-01-1
Guidelines followed in study	US EPA Pesticides Assessment Guideline (171-4)
Deviations from current test guideline	Yes, (OECD 506): • Incurred residue samples were not prepared as duplicates • The fortification level for the procedural recoveries of the incurred residues is not reported • Limited data on residue and recovery levels of spiked samples available (analysed in duplicates/triplicates for 0 and 1 month but only mean value given in report)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1,2]	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in various crop matrices (maize grain, soybean forage, sorghum stover, clover and tomatoes) stored at about ≤-18 °C was investigated. The samples were spiked separately with glyphosate and AMPA at a concentration level of 0.5 mg/kg (10 × LOQ). Glyphosate residues were stable for at least 31 months in all matrices tested and AMPA residues significantly declined after longer storage intervals for some matrices.

I. Materials and methods

A. Materials		
1. Test material:		
Identification:	Glyphosate	AMPA
Description:	Not reported	Not reported
Lot/Batch #:	3214314-6	3058808
Purity:	99.9 %	97.0 %
CAS # :	1071-93-6	1066-51-9
Spiking levels:	0.5 mg/kg	0.4845 mg/kg

2. Test Commodity:		
Crop:	Exogenous spiking: Maize, soybean, sorghum, clover, tomatoes Incurred residues: Maize, soybean, sorghum, clover, tomatoes, alfalfa, potatoes	
Type:	Maize, sorghum: Cereals Tomatoes: Fruiting vegetables Soybean; Oilseeds Potatoes: Root and tuber vegetables Clover, alfalfa: Forages	
Variety:	Not reported	
Botanical name:	*Zea mays, Glycine max, Sorghum bicolor, Trifolium, Solanum lycopersicum, Medicago sativa, Solanum tuberosum*	
Crop parts(s) or processed		
Commodity:	Maize (grain), sorghum (stover), tomato (fruits), soybean (forage), clover, alfalfa (seeds), potato (tuber)	
Sample size:	30 g (maize, tomato, potato), 15 g (soybean, sorghum, clover, alfalfa)	

B. Study design

1. Test procedure

The storage stability of incurred residues of glyphosate and AMPA in maize (grain), sorghum (stover/straw), tomato (fruits), soybean (forage), clover, alfalfa (seeds) and potato (tubers) stored at ≤-18 °C was investigated.

Determination of the storage stability of the incurred residues of glyphosate and AMPA started between 1 month and 32 months of frozen storage. Then 3-4 storage intervals were analysed. Longest storage of incurred residues in total was 25-75 months.

In addition, the storage stability of glyphosate and AMPA of exogenously spiked maize (grain), sorghum (stover/straw), tomato (fruits), soybean (forage) and clover samples stored at ≤-18 °C was investigated. These spiked samples were also used to cover the period between harvest and the first analysis of the incurred residues (between 1 month and 32 months of frozen storage).

Duplicate samples (homogenised) were spiked separately with the test items at a concentration level of 0.5 mg/kg glyphosate and AMPA. The samples were stored at ≤ -18°C until analysis.

At the nine target storage intervals of 0, 1, 3, 6, 9, 12, 18, 24 and 31 months the samples were tested for the stability of glyphosate and AMPA.

Each analytical set for storage stability analysis included the following samples: two non-treated control, four concurrent freshly fortified matrix samples (two with glyphosate, two with AMPA), and four aged (storage stability) samples, two fortified with glyphosate and two fortified with AMPA (for the 0 and 1 month analyses, samples were analysed in duplicate).

2. Description of analytical procedures

All samples were analysed using the analytical method based on the well-established method DFG 405 (refer to CA 4.1.2). For the determination of glyphosate and the metabolite AMPA the samples were extracted with hydrochloric acid. After clean-up of the aqueous fraction by elution through a Chelex 100 resin in the Fe(III) form, glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron removed using an anion exchange resin. After concentration to dryness to remove the hydrochloric acid, glyphosate and AMPA were quantified separately by means of HPLC equipped with a post derivatisation unit and a fluorescence detector.
Determination involves post-column hypochlorite oxidation for glyphosate and reaction of the amine product with o-phthalaldehyde and mercaptoethanol to produce a fluorescent derivative.
The LOQ was 0.05 mg/kg for each analyte.
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from freshly spiked samples of maize (grain), soybean (forage), clover, tomatoes, sorghum (stover/straw), alfalfa seed and potato. The recovery values were between 70 % and 110 % except for some recoveries of glyphosate in maize grain samples and AMPA in maize grain, tomato and sorghum stover/straw. The overall mean recovery value for each analyte and crop was in the acceptable range of 70 - 110 % and RSDs were below 20 %.

II. Results and discussion

The results from the stored samples containing incurred residues and from the stored samples with spiked residues are presented in the tables below. Endogenous AMPA residues were very low in the five matrices primarily chosen for storage stability testing, therefore two additional crops, alfalfa seed and potatoes, were added to the study in order to generate additional endogenous residue data.

Table 6.1-13: Storage stability of glyphosate and AMPA from samples with incurred/endogenous residues

Commodity	Analyte	Storage period months (years)	Residue level in stored samples (mg/kg)	% of initial value at day 0 (first analysis of incurred residues)	Procedural recovery of freshly fortified samples (%)
Maize grain	Glyphosate	13 (1)	1.47	100	83.1
		23 (2)	1.38	94	82.0
		37 (3)	1.17	80	68.6
	AMPA	13 (1)	n.d.	100	88.2
		23 (2)	0.19	>100	81.5
		37 (3)	0.12	>100	71.9
Soya bean forage	Glyphosate	32 (2.5)	0.75	100	112.1
		57 (4.75)	0.50	67	75.5
		71 (6)	0.53	71	74.8
	AMPA	32 (2.5)	0.02	100	102.2
		57 (4.75)	n.d.	-	70.2
		71 (6)	n.d.	-	72.9
Sorghum stover	Glyphosate	7 (0.5)	1.46	100	89.1
		45 (3.75)	2.22	152	92.4
		59 (5)	2.08	142	73.7
		71 (6)	1.61	110	80.8
	AMPA	7 (0.5)	0.03	100	78.6
		45 (3.75)	0.11	367	80.6
		59 (5)	0.13	433	69.2
		71 (6)	0.07	233	80.9
Clover	Glyphosate	17 (1)	3.51	100	96.1
		61 (5)	2.99	85	80.7
		75 (6)	2.74	78	73.2

Table 6.1-13: Storage stability of glyphosate and AMPA from samples with incurred/endogenous residues

Commodity	Analyte	Storage period months (years)	Residue level in stored samples (mg/kg)	% of initial value at day 0 (first analysis of incurred residues)	Procedural recovery of freshly fortified samples (%)
	AMPA	17 (1)	0.01	100	87.5
		61 (5)	n.d.	-	82.7
		75 (6)	n.d.	-	73.0
Tomatoes	Glyphosate	4	0.09	100	83.3
		48 (4)	0.08	89	75.4
		62 (5)	0.04	44	77.6
	AMPA	4	n.d.	100	82.1
		48 (4)	n.d.	-	67.4
		62 (5)	n.d.	-	69.1
Alfalfa seed	Glyphosate	2	15.5	100	87.0
		13 (1)	12.7	82	74.0
		25 (2)	13.9	90	99.3
	AMPA	2	0.20	100	77.2
		13 (1)	0.19	95	72.8
		25 (2)	0.23	115	91.0
Potato	Glyphosate	1	n.d.	100	79.5
		2	0.01	-	102.9
		11 (1)	n.d.	-	85.4
		27 (2)	n.d.	-	92.3
	AMPA	1	0.19	100	75.1
		2	0.23	121	97.0
		11 (1)	0.23	121	80.6
		27 (2)	0.16	84	89.7

n.d. - not detected

The analytical results for the exogenously spiked samples are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-14: Storage stability of glyphosate and AMPA in various fortified crop samples

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries (%) (mean)*
Maize grain	Glyphosate	0	0.410	82.0	*100*	82.0	*100*
		1 (34)	0.396	79.2	*97*	78.4	*101*
		3 (95)	0.397	79.4	*97*	78.0	*102*
		6 (181)	0.312	62.4	*76*	60.7	*103*
		9 (271)	0.292	58.4	*71*	63.3	*92.2*
		12 (376)	0.337	67.4	*82*	68.6	*98.2*
		18 (528)	0.309	61.8	*75*	69.4[2]	*89.0*
		24 (742)	0.354	70.8	*86*	72.0	*98.4*
		31 (944)	0.286	57.2	*70*	75.3	*76.0*
	AMPA	0	0.395	81.5	*100*	81.5	*100*
		1 (34)	0.351	72.4	*89*	73.8	*98.2*
		3 (95)	0.302	62.3	*76*	63.1	*98.9*
		6 (181)	0.319	65.8	*81*	65.0	*101*
		9 (271)	0.313	64.6	*79*	69.3	*93.3*
		12 (376)	0.297	61.3	*75*	71.9	*85.2*
		18 (528)	0.336	69.3	*85*	72.8	*95.4*
		24 (742)	0.358	73.9	*91*	81.5	*90.6*
		31 (944)	0.270	55.7	*68*	70.7	*78.8*
Soya bean forage	Glyphosate	0	0.375	75.5	*100*	75.5	*99.8*
		1 (27)	0.428	85.6	*114*	83.8	*102*
		3 (92)	0.372	74.4	*99*	77.2	*96.4*
		6 (182)	0.382	76.4	*101*	80.1	*95.4*
		9 (274)	0.379	75.8	*101*	77.6	*97.6*
		12 (379)	0.406	81.2	*108*	74.8	*109*
		18 (531)	0.422	84.8	*112*	80.2	*106*
		24 (743)	0.409	81.8	*108*	81.7	*100*
		31 (958)	0.290	58.0	*77*	80.3	*72.2*
	AMPA	0	0.340	70.2	*100*	70.2	*99.9*
		1 (27)	0.387	79.8	*114*	80.4	*99.3*
		3 (92)	0.367	75.7	*108*	78.8	*96.2*
		6 (182)	0.351	72.4	*103*	81.0	*89.4*
		9 (274)	0.296	61.1	*87*	77.1	*79.3*
		12 (379)	0.329	67.9	*97*	72.9	*93.1*
		18 (531)	0.348	71.8	*102*	79.7	*90.2*
		24 (743)	0.360	74.3	*106*	91.0	*81.7*
		31 (958)	0.228	47.1	*67*	78.6	*59.9*
Clover	Glyphosate	0	0.403	80.7	*100*	80.7	*99.8*
		1 (28)	0.430	86.0	*107*	85.3	*101*
		3 (96)	0.398	79.6	*99*	81.0	*98.2*
		6 (183)	0.383	76.6	*95*	77.5	*98.8*
		9 (273)	0.352	70.4	*87*	77.9	*90.4*
		12 (376)	0.385	77.0	*96*	73.2	*105*
		18 (530)	0.361	72.2	*90*	77.9	*92.6*
		24 (741)	0.391	78.2	*97*	83.7	*93.4*
		31 (944)	0.519	103.8	*129*	98.2	*106*

Table 6.1-14: Storage stability of glyphosate and AMPA in various fortified crop samples

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries (%) (mean)*
	AMPA	0	0.401	82.7	*100*	82.7	*100*
		1 (28)	0.347	71.6	*87*	85.6	*83.6*
		3 (96)	0.293	60.5	*73*	70.0	*86.5*
		6 (183)	0.334	68.9	*83*	85.7	*80.5*
		9 (273)	0.242	50.2	*60*	77.1	*64.8*
		12 (376)	0.242	50.2	*60*	73.1	*68.5*
		18 (530)	0.242	50.2	*60*	78.9	*63.6*
		24 (741)	0.230	47.5	*57*	84.7	*56.1*
		31 (944)	0.227	46.9	*57*	88.6	*52.8*
Tomatoes	Glyphosate	0	0.377	75.4	*100*	75.4	*100*
		1 (34)	0.405	81.0	*107*	78.4	*103*
		3 (91)	0.375	75.0	*99*	78.3	*95.8*
		6 (188)	0.422	84.6	*112*	81.0	*104*
		9 (272)	0.370	74.0	*98*	79.0	*93.6*
		12 (379)	0.393	78.6	*104*	77.6	*101*
		18 (528)	0.418	83.6	*111*	85.4	*97.8*
		24 (735)	0.423	84.6	*112*	81.8	*104*
		31 (938)	0.427	85.4	*113*	81.5	*105*
	AMPA	0	0.327	67.4	*100*	67.4	*100*
		1 (34)	0.340	70.2	*104*	66.7	*105*
		3 (91)	0.340	70.2	*104*	72.7	*96.6*
		6 (188)	0.407	84.0	*124*	72.6	*116*
		9 (272)	0.328	67.7	*100*	74.6	*90.8*
		12 (379)	0.341	70.4	*104*	69.1	*102*
		18 (528)	0.381	78.6	*117*	83.4	*94.1*
		24 (735)	0.361	74.5	*110*	81.7	*91.2*
		31 (938)	0.356	73.5	*109*	74.1	*99.1*
Sorghum stover/straw	Glyphosate	0	0.461	92.4	*100*	92.4	*100*
		1 (29)	0.392	78.4	*85*	78.4	*100*
		3 (92)	0.422	84.6	*92*	84.9[3]	*99.6*
		6 (182)	0.381	76.2	*83*	78.1	*97.6*
		9 (274)	0.398	79.6	*86*	74.5	*107*
		12 (377)	0.389	77.8	*84*	73.7	*106*
		18 (531)	0.410	82.0	*89*	84.2	*97.4*
		24 (743)	0.409	81.8	*89*	80.8	*101.2*
		31 (958)	0.323	64.6	*70*	73.5[4]	*87.8*
	AMPA	0	0.391	80.6	*100*	80.6	*100*
		1 (29)	0.319	65.8	*82*	73.3	*89.8*
		3 (92)	0.333	68.7	*85*	78.5[3]	*87.5*
		6 (182)	0.296	61.1	*76*	80.9	*75.5*
		9 (274)	0.263	54.3	*67*	75.9	*71.6*
		12 (377)	0.221	45.6	*57*	69.2	*65.8*
		18 (531)	0.237	48.9	*61*	81.1	*60.3*
		24 (743)	0.278	57.4	*71*	80.9	*71.0*
		31 (958)	0.124	25.6	*32*	69.1[4]	*36.9*

Table 6.1-14: Storage stability of glyphosate and AMPA in various fortified crop samples

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%) (mean)	*Recovery of stored samples corrected for procedural recoveries (%) (mean)*

[1] Fortification level of 0.5 mg/kg for glyphosate and 0.4845 for AMPA,
[2] One fortified sample of maize grain from the 528 day analysis contained very low levels of glyphosate and AMPA. This sample was not used in these calculations.
[3] One fortified sample of sorghum straw from the 92 day analysis contained very high levels of glyphosate and AMPA. This sample was not used in these calculations.
[4] Based on background levels from only one control sample. The second control sample was lost during sample workup.

III. Conclusion

Endogenous AMPA residues were very low in the five matrices primarily chosen for storage stability testing, therefore two additional crops, alfalfa seed and potatoes, were added to the study in order to generate additional endogenous residue data.
Endogenous residues of both glyphosate and AMPA were very low in some commodities. Commodities with residues <0.5 mg/kg (10x LOQ) are not considered to provide reliable results and are therefore considered as additional/supportive data. This is the case for all endogenous residues of AMPA.
Endogenous residues of glyphosate were shown to be stable in maize grain, soybean forage, sorghum stover, clover and alfalfa after ca. 2-6 years in frozen storage. In soybean forage glyphosate was found slightly <70 % after 57 months however procedural recoveries were only ca. 75 % as well.

Based on fortified samples glyphosate was stable (>70 % remaining) for at least 31 months in tomatoes and clover. In soybean forage and sorghum stover the last sample collected after 31 months was slightly below 70 % remaining, but under consideration of the procedural recoveries within an acceptable range. For maize grain intermediate samples collected after 6, 9, 12, 18 and 31 months gave recoveries below 70 % of the fortified level. However, under consideration of the procedural recoveries no significant decline was observed. In addition, the day zero sample already gave a low recovery of 82 %, suggesting a lesser decline of the stored samples compared to the nominal concentration.
For AMPA samples collected after longer storage intervals gave a significant decline for some matrices. The following intervals were proven to be stable within this study: maize grain (at least 31 months), soybean forage (24 months), clover (6 months), tomatoes (at least 31 months) and sorghum stover (9 months).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in high starch content matrices (maize grain, potato, alfalfa seed), high water content matrices (tomatoes, clover, soybean forage) and dry matrices (sorghum straw/stover) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with minor deviations. The incurred residue samples were not prepared as duplicates, and the fortification level for the procedural recoveries of the incurred residues is not reported. The spiked samples were prepared and analysed in duplicates (in triplicates for 0 and 1 month) but only the mean value is reported. Nevertheless, stability for glyphosate and decline for AMPA at longer storage times can still clearly be seen.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/013
Report author	[redacted]
Report year	1989
Report title	Storage stability validation for ICIA0224 in raw agricultural commodities
Report No	WRC 89-22
Document No	VV-320945
Guidelines followed in study	US EPA Pesticides Assessment Guideline (171 4)
Deviations from current test guideline	Yes (OECD 506): • No freshly spiked procedural recoveries
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate, AMPA (aminomethylphosphonic acid) and TMS (glyphosate-trimesium (trimethylsolfonium-cation)) in sorghum grain, soybean seeds and straw and in wheat grain was investigated. Samples were spiked with the test items at concentration levels of 1.0 mg/kg or 5.0 mg/kg glyphosate and AMPA and 0.46 mg/kg or 2.3 mg/kg TMS. The samples were stored at about -20 °C until analysis for about 4 years.
Glyphosate, AMPA and TMS are stable for the maximum period tested: in soybean seeds (representative of high oil content oilseed crops) and straw and in wheat grain (representative of high starch content cereal crops) for at least 24 months and in sorghum grain (representative of high starch content cereal crops) for at least 48 months when stored under deep freeze conditions.
TMS is not a relevant analyte in this dossier, therefore data with respect to this analyte is not presented in the following summary.

I. Materials and methods

A. Materials				
1. Test material:				
	Identification:	Glyphosate	AMPA	
	Description:	Not reported	Not reported	
	Lot/Batch #:	Not reported	Not reported	
	Purity:	Not reported	Not reported	
	CAS # :	1071-83-6	1066-51-9	

Spiking levels:	1.0-5.0 mg/kg	1.0-5.0 mg/kg	
2. Test Commodity:			
Crop:	Sorghum, soybean, wheat		
Type:	Sorghum, wheat: Cereals Soybean: Oilseeds		
Variety:	Sorghum: Not reported Soybean: Coker 136 Wheat: Spring		
Botanical name:	*Sorghum bicolor, Glycine max, Triticum aestivum*		
Crop parts(s) or processed			
Commodity:	Sorghum, wheat: Grain Soybean: Seeds and straw		
Sample size:	25 g		

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in sorghum (grain), soybean (seeds and straw) and wheat (grain) stored at -20 °C was investigated.
Duplicate or triplicate samples (homogenised) were spiked with the test items at concentration levels of 1.0-5.0 mg/kg glyphosate and AMPA. The samples were stored in glass bottles in the dark at -20 °C until analysis. At four samplings over a period of 24 months for soybean (seeds and straw) and wheat (grain) and four sampling over a period of 48 months for sorghum (grain) the samples were tested for the stability of glyphosate and AMPA.
Each analytical set for storage stability analysis included the following samples: one non-treated control and two or three aged (storage stability) samples for glyphosate and AMPA. No samples for measuring the procedural recovery were freshly spiked.

2. Description of analytical procedures

Glyphosate and AMPA were analysed with method RRC 85-34 (refer to CA 4.1.2). Samples were extracted with water. The extract was cleaned up using a cation exchange column, the analytes were converted to a fluorescing derivative with a 9-fluorenytmethyl chloroformate and the derivative was determined by HPLC using an anion exchange column with fluorescence detection
Within this study, for confirmation of the accuracy of the analytical method, samples of sorghum grain, soybean seed and straw and wheat grain prepared at 0 day of storage were analysed for the concentration of the glyphosate and AMPA. The nominal spiking levels were 1.0 mg/kg for wheat grain, soybean seeds, soybean straw, and 5.0 mg/kg for sorghum grain. All recoveries of glyphosate and AMPA were between 70 % and 110 % and relative standard deviations (RSDs) below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 is presented (italic).

Table 6.1-15: Storage stability of glyphosate and AMPA in various crops

Commodity	Analyte	Storage period months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1,2]) (mean)	*% of initial value at day 0*
Sorghum grain	Glyphosate	0	4.79	93	*100*
		6 (191)	N/A	N/A	*N/A*
		12 (285)	4.60, 4.40 (4.50)	92, 88 (90)	*94*
		24 (633)	4.67,4.72 (4.70)	93, 94 (94)	*98*
		48 (1462)	4.90, 5.10, 4.90 (5.00)	98, 101, 98 (99)	*104*
	AMPA	0	4.20	83	*100*
		6 (191)	N/A	N/A	*N/A*
		12 (285)	4.05, 4.35 (4.20)	81, 87 (84)	*100*
		24 (633)	3.65, 3.23 (3.44)	73, 65 (69)	*82*
		48 (1462)	4.25, 4.00, 4.1 (4.12)	85, 80, 82 (82)	*98*
Soybean seeds	Glyphosate	0	0.88, 1.05, 1.06 (1.00)	88, 105, 106 (100)	*100*
		6 (191)	1.18, 0.98, 1.09 (1.08)	118, 98, 109 (108)	*108*
		12 (394)	0.76, 0.73, 0.75 (0.75)	76, 73, 75 (75)	*75*
		24 (786)	1.04, 1.04, 1.04 (1.04)	104, 104, 104 (104)	*104*
		48 (1462)	N/A	N/A	*N/A*
	AMPA	0	0.92, 0.9, 0.87 (0.90)	92, 90, 87 (90)	*100*
		6 (191)	0.73, 0.83, 1.03 (0.86)	73, 83, 103 (86)	*96*
		12 (394)	1.11, 1.07, 1.01 (1.06)	111, 107, 101 (106)	*118*
		24 (786)	0.83, 0.77, 0.83 (0.81)	83, 77, 83 (81)	*90*
		48 (1462)	N/A	N/A	*N/A*
Soybean straw	Glyphosate	0	1.05, 0.99, 0.96 (1.00)	105, 99, 96 (100)	*100*
		6 (191)	0.94, 1.08, 1.03 (1.02)	94, 108, 103 (102)	*102*
		12 (394)	0.80, 0.80, 0.91 (0.84)	80, 80, 91 (84)	*84*
		24 (786)	1.04, 1.10, 1.04 (1.06)	104, 110, 104 (106)	*106*
		48 (1462)	N/A	N/A	*N/A*
	AMPA	0	0.77, 0.70, 0.74 (0.74)	77, 70, 74 (74)	*100*
		6 (191)	0.72, 0.73, 0.66 (0.70)	72, 73, 66 (70)	*95*
		12 (394)	0.9, 0.94, 0.96 (0.93)	90, 94, 96 (93)	*126*
		24 (786)	0.81, 0.83, 0.81 (0.82)	81, 83, 81 (82)	*111*
		48 (1462)	N/A	N/A	*N/A*
Wheat grain	Glyphosate	0	0.98, 0.95, 0.83 (0.92)	98, 95, 83 (92)	*100*
		6 (191)	0.86, 0.89 (0.88)	86, 89 (88)	*96*
		12 (394)	0.85, 0.84, 0.77 (0.82)	85, 84, 77 (82)	*89*
		24 (786)	0.77, 0.8, 0.71 (0.76)	77, 80, 71 (76)	*83*
		48 (1462)	N/A	N/A	*N/A*
	AMPA	0	1.00, 0.98, 0.95 (0.99)	100, 98, 95 (98)	*100*
		6 (191)	0.96, 0.78 (0.87)	96, 78 (87)	*88*
		12 (394)	0.74, 0.69, 0.69 (0.71)	73, 68, 68 (70)	*72*
		24 (786)	0.83, 0.61, 0.66 (0.70)	83, 61, 66 (70)	*71*
		48 (1462)	N/A	N/A	*N/A*

[1] Nominal spiking levels for glyphosate and AMPA: 1.0 mg/kg for wheat grain, soybeans seed and straw, 5.0 mg/kg for sorghum grain
[2] Corrected for contamination in untreated control sample
N/A Not analysed

III. Conclusion

This study demonstrates that glyphosate and AMPA are stable in soybean seeds and straw and in wheat grain for at least 24 months and in sorghum grain for at least 48 months when stored at ≤-20 °C.
Although single values <70 % of the nominal concentration of AMPA were found, over the whole storage period, the residue levels remained above 70 % of the day zero values, hence suggesting stability of glyphosate and AMPA over the tested storage periods.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in high starch content matrices (wheat and sorghum grain), high oil content matrix (soybean seeds) and dry matrix (soybean straw) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with the deviation that no freshly spiked procedural recoveries were analysed. Nevertheless, stability for glyphosate and AMPA could be demonstrated.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/014
Report author	[redacted]
Report year	1988
Report title	Storage stability of Glyphosate and AMPA in swine tissues, dairy cow tissues and milk laying hen tissues and eggs
Report No	[redacted]-7515
Document No	M-645906-01-1
Guidelines followed in study	Not stated
Deviations from current test guideline	Yes, (OECD 506): • Spiking level of glyphosate not at least 10xLOQ for fat and muscle (pig, cow, chicken), cow milk and eggs (0.2 mg/kg); • Spiking level of AMPA not at least 10xLOQ for fat and muscle (pig, cow, chicken), cow milk and chicken eggs (0.05 mg/kg), for pig liver (0.1 mg/kg) and for chicken liver (0.25 mg/kg) • A mixed spiking solution of glyphosate and AMPA was used
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in animal matrices was investigated. Samples were spiked with the test items at concentration levels between 0.2 and 6.0 mg/kg for glyphosate and between 0.05 mg/kg and 1.5 mg/kg for AMPA. The samples were stored at <-20 °C. Glyphosate and AMPA were stable for the maximum period tested: 26 months in pig fat, muscle, liver and kidney, 16 months in cattle milk, 24 months in cattle fat, muscle, liver and kidney, 13 months in chicken kidney, 25 months in chicken fat, muscle and liver, except in chicken eggs where instability was observed at later intervals and the maximum period of stable storage was 14 months.

I. Materials and methods

A. Materials		
1. Test material:		
Identification:	Glyphosate	AMPA
Description:	Not reported	Not reported
Lot/Batch #:	Not reported	Not reported
Purity:	Not reported	Not reported
CAS # :	1071-83-6	1066-51-9
Spiking levels:	0.2 – 6.0 mg/kg	0.05 – 1.5 mg/kg
2. Test Commodity:		
Animal:	Pig, cow, chicken	
Commodities:	Fat, muscle, liver, kidney, milk (cow), eggs (chicken)	
Sample size:	10 g (fat, muscle, liver, kidney), 20 g (eggs), 60 g (milk)	

B. Study design

1. Test procedure

The storage stability of glyphosate and AMPA in animal matrices was investigated.
Duplicate samples (at day 0 three replicate samples) were spiked with the test items at a concentration level between 0.2 and 6.0 mg/kg for glyphosate and between 0.05 mg/kg and 1.5 mg/kg for AMPA. The spiked samples were stored in glass jars except for milk samples which were stored in polypropylene bottles at <-20 °C until analysis in the dark. At five samplings over a period of 715-852 days (24-28 months) for pig, cow and chicken tissues (except chicken kidney) and chicken eggs and at four samplings over a period of 390-473 days (13-16 months) for cow milk and chicken kidney the samples were tested for the stability of glyphosate and AMPA.

Each analytical set for storage stability analysis included the following samples: two non-treated control, two concurrent freshly fortified matrix samples (each for glyphosate and AMPA), and two aged (storage stability) samples, each for glyphosate and AMPA.

2. Description of analytical procedures

All samples were analysed using the analytical method based on DFG 405 (refer to CA 4.1.2). Samples were extracted with water and chloroform. After concentration of the extract, a clean-up was performed with a Chelex column and ion-exchange chromatography. After concentration, glyphosate and AMPA were analysed by HPLC using post-column derivatisation (o-phthalaldehyde) with fluorescence detection. The LOQ for tissues was 0.05 mg/kg each of glyphosate and AMPA and for milk and eggs was 0.025 mg/kg each of glyphosate and AMPA.
The accuracy of the residue determination at the different storage intervals was confirmed by procedural recoveries from freshly spiked samples. The samples were fortified with glyphosate and AMPA at the same concentrations as the stored samples. The mean recoveries per analyte and commodity were in the acceptable range of 70-110 %. The relative standard deviations (RSDs) per analyte and commodity were below 20 %.

II. Results and discussion

The results are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are presented (italic).

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
Pig fat	Glyphosate	0	0.164, 0.145, 0.154 (0.154)	82, 73, 77 (77)	*100*	-	-
		7 (210)	0.182, 0.190 (0.186)	91, 95 (93)	*121*	98, 98 (98)	*95*
		15 (437)	0.161, 0.159 (0.160)	81, 80 (81)	*104*	95, 97 (96)	*84*
		17 (524)	0.169, 0.165 (0.167)	85, 83 (84)	*108*	101, 102 (101)	*83*
		26 (794)	0.172, 0.178 (0.175)	86, 86 (86)	*114*	82, 88 (85)	*103*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
	AMPA	0	0.0416, 0.0376, 0.0392 (0.0395)	83, 75, 78 (79)	*100*	-	-
		7 (210)	0.0422, 0.0430 (0.0426)	84, 86 (85)	*108*	93, 93 (93)	*91*
		15 (437)	0.0376, 0.0355 (0.0366)	75, 67 (71)	*93*	92, 101 (96)	*74*
		17 (524)	0.0336, 0.0331 (0.0334)	67, 66 (67)	*85*	91, 91 (91)	*74*
		26 (794)	0.0320, 0.0318 (0.0319)	64, 64 (64)	*81*	79, 90 (84)	*76*
Pig muscle	Glyphosate	0	0.207, 0.187, 0.180 (0.191)	104, 94, 90 (96)	*100*	-	-
		7 (213)	0.167, 0.165 (0.166)	84, 83 (83)	*87*	89, 93 (91)	*91*
		13 (382)	0.201, 0.205 (0.203)	101, 103 (102)	*106*	105, 108 (106)	*96*
		16 (467)	0.180, 0.188 (0.184)	90, 94 (92)	*96*	100, 103 (101)	*91*
		26 (794)	0.172, 0.166 (0.169)	86, 83 (85)	*88*	110, 96 (103)	*83*
	AMPA	0	0.0501, 0.0505, 0.0469 (0.0492)	100, 101, 94 (98)	*100*	-	-
		7 (213)	0.0419, 0.0411 (0.0415)	84, 82 (83)	*84*	93, 94 (93)	*89*
		13 (382)	0.0403, 0.0412 (0.0408)	81, 82 (82)	*83*	96, 97 (96)	*85*
		16 (467)	0.0372, 0.0415 (0.0394)	74, 83 (79)	*80*	92, 96 (94)	*84*
		26 (794)	0.0351, 0.0354 (0.0353)	70, 71 (71)	*72*	103, 85 (94)	*75*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
Pig liver	Glyphosate	0	0.678, 0.639, 0.663 (0.660)	85, 80, 83 (83)	*100*		-
		14 (417)	0.609, 0.601 (0.605)	76, 75 (76)	*92*	82, 87 (84)	*90*
		16 (468)	0.650, 0.646 (0.648)	81, 81 (81)	*98*	92, 94 (93)	*87*
		17 (521)	0.564, 0.562 (0.563)	71, 70 (70)	*85*	76, 82 (79)	*89*
		26 (790)	0.559, 0.612 (0.586)	70, 77 (73)	*89*	88, 93 (90)	*81*
	AMPA	0	0.0927, 0.0854, 0.0883 (0.0888)	93, 85, 88 (89)	*100*	-	-
		14 (417)	0.0625, 0.0606 (0.0616)	63, 61 (62)	*69*	74, 81 (78)	*79*
		16 (468)	0.0758, 0.0788 (0.0773)	76, 79 (77)	*87*	93, 94 (93)	*83*
		17 (521)	0.0811, 0.0786 (0.0799)	81, 79 (80)	*90*	95, 96 (95)	*84*
		26 (790)	0.0760, 0.0808 (0.0784)	76, 81 (78)	*88*	100, 96 (98)	*80*
Pig kidney	Glyphosate	0	4.027, 4.093, 3.942 (4.021)	101, 102, 99 (101)	*100*	-	-
		8 (241)	3.848, 3.615 (3.732)	96, 90 (93)	*93*	99, 96 (97)	*96*
		13 (377)	3.448, 3.650 (3.549)	86, 91 (89)	*88*	90, 91 (90)	*99*
		16 (469)	3.556, 3.132 (3.344)	89, 78 (84)	*83*	95, 100 (97)	*86*
		26 (790)	3.158, 3.602 (3.380)	79, 90 (85)	*84*	81, 86 (83)	*102*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
	AMPA	0	0.505, 0.505, 0.504 (0.505)	101, 101, 101 (101)	*100*		-
		8 (241)	0.510, 0.456 (0.483)	102, 91 (97)	*96*	106, 101 (104)	*93*
		13 (377)	0.474, 0.498 (0.486)	95, 100 (97)	*96*	99, 95 (97)	*100*
		16 (469)	0.465, 0.403 (0.434)	93, 81 (87)	*86*	98, 105 (102)	*85*
		26 (790)	0.389, 0.486 (0.438)	78, 97 (88)	*87*	98, 81 (89)	*98*
Cow fat	Glyphosate	0	0.172, 0.165, 0.177 (0.171)	86, 83, 89 (86)	*100*	-	-
		6 (175)	0.175, 0.165 (0.170)	88, 83 (85)	*99*	96, 95 (95)	*89*
		12 (349)	0.168, 0.170, (0.169)	84, 85 (85)	*99*	98, 100 (99)	*86*
		15 (436)	0.163, 0.164 (0.164)	82, 82 (82)	*96*	99, 93 (96)	*85*
		24 (715)	0.168, 0.199 (0.184)	84, 100 (92)	*108*	100, 111 (105)	*88*
	AMPA	0	0.0423, 0.0386, 0.0418 (0.0409)	85, 77, 84 (82)	*100*	-	-
		6 (175)	0.0420, 0.0385 (0.0403)	84, 77 (81)	*99*	98, 94 (96)	*84*
		12 (349)	0.0386, 0.0387, (0.0387)	77, 77, (77)	*95*	90, 97 (94)	*83*
		15 (436)	0.0323, 0.0327 (0.0325)	65, 65 (65)	*79*	85, 80 (82)	*79*
		24 (715)	0.0345, 0.0406 (0.0376)	69, 81 (75)	*92*	91, 95 (93)	*81*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
Cow muscle	Glyphosate	0	0.176, 0.163, 0.178 (0.172)	88, 82, 89 (86)	*100*	-	-
		6 (177)	0.151, 0.145 (0.148)	76, 73 (74)	*86*	80, 87 (83)	*89*
		10 (300)	0.174, 0.178 (0.176)	87, 89 (88)	*102*	98, 91 (95)	*93*
		13 (373)	0.175, 0.180 (0.178)	88, 90 (89)	*103*	98, 98 (98)	*91*
		24 (721)	0.184, 0.207 (0.196)	92, 104 (98)	*114*	89, 104 (96)	*102*
	AMPA	0	0.0422, 0.0428, 0.0439 (0.0430)	84, 86, 88 (86)	*100*	-	-
		6 (177)	0.0416, 0.0414 (0.0415)	83, 83 (83)	*97*	95, 100 (98)	*85*
		10 (300)	0.0379, 0.0381 (0.0380)	76, 76 (76)	*88*	93, 84 (89)	*86*
		13 (373)	0.0334, 0.0357 (0.0346)	67, 71 (69)	*80*	98, 97 (97)	*71*
		24 (721)	0.0358, 0.0410 (0.0384)	72, 82 (77)	*89*	82, 86 (84)	*92*
Cow liver	Glyphosate	0	3.936, 3.755, 3.785 (3.825)	98, 94, 95 (96)	*100*	-	-
		10 (288)	3.447, 3.265 (3.356)	86, 82 (84)	*88*	91, 93 (92)	*91*
		13 (380)	3.510, 3.417 (3.464)	88, 85 (87)	*91*	96, 97 (96)	*90*
		15 (433)	3.248, 3.275 (3.262)	81, 82 (82)	*85*	88, 89 (89)	*92*
		24 (717)	3.638, 3.726 (3.682)	91, 93 (92)	*96*	89, 96 (93)	*99*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
	AMPA	0	0.466, 0.463, 0.636 (0.522)	93, 93, 127 (104)	*100*		-
		10 (288)	0.433, 0.414 (0.424)	87, 83 (85)	*81*	93, 94 (94)	*90*
		13 (380)	0.438, 0.427 (0.433)	88, 85 (87)	*83*	98, 99 (98)	*88*
		15 (433)	0.437, 0.427 (0.432)	87, 85 (86)	*83*	96, 103 (100)	*87*
		24 (717)	0.448, 0.429 (0.439)	90, 86 (88)	*84*	92, 98 (95)	*93*
Cow kidney	Glyphosate	0	5.666, 5.451, 5.566 (5.561)	94, 91, 93 (93)	*100*	-	-
		6 (181)	5.221, 5.187 (5.204)	87, 86 (87)	*94*	94, 91 (93)	*94*
		10 (296)	5.358, 5.345 (5.352)	89, 89 (89)	*96*	93, 88 (90)	*99*
		13 (377)	5.395, 5.377 (5.386)	90, 90 (90)	*97*	95, 95 (95)	*95*
		24 (717)	5.459, 5.426 (5.443)	91, 90 (91)	*98*	92, 85 (89)	*102*
	AMPA	0	1.389, 1.328, 1.355 (1.357)	93, 89, 90 (90)	*100*	-	-
		6 (181)	1.218, 1.228 (1.223)	81, 82 (82)	*90*	92, 87 (90)	*91*
		10 (296)	1.198, 1.200 (1.199)	80, 80 (80)	*88*	85, 84 (84)	*95*
		13 (377)	1.229, 1.211 (1.220)	82, 81 (81)	*90*	90, 92 (91)	*90*
		24 (717)	1.299, 1.313 (1.306)	87; 88 (87)	*96*	79, 76 (78)	*112*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
Cow milk	Glyphosate	0	0.170, 0.167 (0.169)	85, 84 (84)	*100*	-	-
		5 (137)	0.162, 0.159 (0.161)	81, 80 (80)	*95*	96, 95 (95)	*84*
		7 (200)	0.158, 0.158 (0.158)	79, 79 (79)	*93*	92, 93 (93)	*86*
		16 (473)	0.192, 0.181 (0.187)	96, 91 (93)	*111*	105, 116 (110)	*85*
	AMPA	0	0.0409, 0.0431 (0.0420)	82, 86 (84)	*100*	-	-
		5 (137)	0.0369, 0.0365 (0.0367)	74, 73 (73)	*87*	90, 89 (89)	*82*
		7 (200)	0.0398, 0.0387 (0.0393)	80, 77 (79)	*94*	100, 105 (103)	*76*
		16 (473)	0.0374, 0.0338 (0.0356)	75, 68 (71)	*85*	78, 87 (82)	*87*
Chicken fat	Glyphosate	0	0.159, 0.183, 0.188 (0.177)	80, 92, 94 (88)	*100*	-	-
		12 (368)	0.162, 0.164 (0.163)	81, 82 (82)	*92*	98, 95 (97)	*85*
		14 (420)	0.163, 0.162 (0.163)	82, 81 (81)	*92*	93, 98 (95)	*85*
		16 (474)	0.160, 0.159 (0.160)	80, 80 (80)	*90*	98, 95 (96)	*83*
		25 (753)	0.156, 0.142 (0.149)	78, 71 (75)	*84*	87, 83 (85)	*88*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
	AMPA	0	0.0406, 0.0464, 0.0472 (0.0447)	81, 93, 94 (89)	*100*		-
		12 (368)	0.0382, 0.0387 (0.0385)	76, 77 (77)	*86*	92, 86 (89)	*87*
		14 (420)	0.0350, 0.0363 (0.0357)	70, 73 (71)	*80*	82, 84 (83)	*86*
		16 (474)	0.0380, 0.0390 (0.0385)	76, 78 (77)	*86*	93, 93 (93)	*83*
		25 (753)	0.0330, 0.0290 (0.0310)	71, 64 (68)	*69*	80, 73 (76)	*82*
Chicken muscle	Glyphosate	0	0.176, 0.173, 0.174 (0.174)	88, 87, 87 (87)	*100*	-	-
		12 (361)	0.176, 0.179 (0.178)	88, 90 (89)	*102*	95, 91 (93)	*96*
		14 (426)	0.181, 0.175 (0.178)	91, 88 (89)	*102*	100, 105 (102)	*87*
		16 (483)	0.173, 0.173 (0.173)	87, 87, (87)	*99*	95, 96 (96)	*91*
		25 (753)	0.173, 0.149 (0.161)	87, 75 (81)	*93*	95, 81 (88)	*91*
	AMPA	0	0.0484, 0.0478, 0.0485 (0.0482)	97, 96, 97 (96)	*100*	-	-
		12 (361)	0.0444, 0.0445 (0.0445)	89, 89 (89)	*92*	96, 93 (95)	*94*
		14 (426)	0.0467, 0.0472 (0.0470)	93, 94 (94)	*98*	102, 107 (105)	*90*
		16 (483)	0.0445, 0.0469 (0.0457)	89, 94 (91)	*95*	104, 105 (105)	*87*
		25 (753)	0.0390, 0.0350 (0.0370)	78, 70 (74)	*77*	87, 65 (76)	*98*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
Chicken liver	Glyphosate	0	1.766, 1.618, 1.639 (1.674)	88, 81, 82 (84)	*100*		-
		13 (384)	1.630, 1.557 (1.594)	82, 78 (80)	*95*	115, 114 (114)	*70*
		14 (426)	1.641, 1.654 (1.648)	82, 83 (82)	*98*	92, 94 (93)	*89*
		16 (474)	1.621, 1.616 (1.619)	81, 81 (81)	*97*	103, 104 (103)	*78*
		25 (747)	1.904, 1.919 (1.912)	95, 96 (96)	*114*	113, 108 (111)	*86*
	AMPA	0	0.196, 0.178, 0.187 (0.187)	78, 71, 75 (75)	*100*	-	-
		13 (384)	0.195, 0.184 (0.190)	78, 74 (76)	*102*	116, 116 (116)	*65*
		14 (426)	0.205, 0.200 (0.203)	82, 80 (81)	*109*	95, 100 (97)	*83*
		16 (474)	0.213, 0.216 (0.215)	85, 86 (86)	*115*	109, 110 (109)	*79*
		25 (747)	0.214, 0.213 (0.214)	86, 85 (86)	*114*	99, 93 (95)	*90*
Chicken kidney	Glyphosate	0	3.697, 3.685, 3.648 (3.677)	92, 92, 91 (92)	*100*	-	-
		1 (19)	3.894, 3.875 (3.885)	97, 97 (97)	*106*	98, 98 (98)	*99*
		4 (132)	3.658, 3.514 (3.586)	91, 88 (90)	*98*	92, 93 (92)	*97*
		13 (390)	4.107, 4.343 (4.225)	103, 109 (106)	*115*	115, 95 (105)	*101*

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*
	AMPA	0	0.508, 0.515, 0.506 (0.510)	102, 103, 101 (102)	*100*		-
		1 (19)	0.486, 0.498 (0.492)	97, 100 (98)	*96*	96, 96 (96)	*103*
		4 (132)	0.476, 0.435 (0.456)	95, 87 (91)	*89*	100, 98 (99)	*92*
		13 (390)	0.447, 0.461 (0.454)	89, 92 (91)	*89*	99, 84 (91)	*100*
Chicken eggs	Glyphosate	0	0.182, 0.169, 0.179 (0.177)	91, 85, 90 (88)	*100*	-	-
		12 (368)	0.158, 0.157 (0.158)	79, 79 (79)	*89*	90, 91 (90)	*87*
		14 (431)	0.159, 0.160 (0.160)	80, 80 (80)	*90*	90, 93 (91)	*87*
		25 (755)	0.0729, 0.0819 (0.0774)	36, 41 (39)	*44*	91, 91 (91)	*43*
		28 (852)	0.0630, 0.0640 (0.0635)	32, 32 (32)	*36*	65, 67 (66)	*48*
	AMPA	0	0.0494, 0.0448, 0.0479 (0.0474)	99, 90, 96 (95)	*100*	-	-
		12 (368)	0.0401, 0.0395 (0.0398)	80, 79 (80)	*84*	89, 89 (89)	*90*
		14 (431)	0.0418, 0.0418 (0.0418)	84, 84 (84)	*88*	92, 96 (94)	*89*
		25 (755)	0.0165, 0.0180 (0.0173)	33, 36 (35)	*36*	79, 77 (78)	*45*
		28 (852)	0.0174, 0.0169 (0.0172)	35, 34 (34)	*36*	70, 72 (71)	*49*

[1] Nominal spiking levels for glyphosate: 0.2 mg/kg for fat and muscle (pig, cow, chicken), cow milk and chicken eggs; 0.8 mg/kg for pig liver; 2 mg/kg for chicken liver; 4 mg/kg for pig and chicken kidney; 4.9 mg/kg for cow liver; 6 mg/kg for cow kidney; Nominal spiking levels for AMPA: 0.05 mg/kg for fat and muscle (pig, cow, chicken), cow milk and chicken eggs; 0.1 mg/kg for

Table 6.1-16: Storage stability of glyphosate and AMPA in animal matrices

Commodity	Analyte	Storage period in months (days)	Residue level in stored samples (mg/kg) (mean)	Recovery of stored samples (% of nominal spiking level[1]) (mean)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)[1, 2] (mean)	*Recovery of stored samples corrected for procedural recoveries[1] (%) (mean)*

pig liver; 0.25 mg/kg for chicken liver; 0.5 mg/kg for pig and chicken kidney and cow liver; 1.5 mg/kg for cow kidney
[2] Hand calculations of the mean may vary from reported values because of rounding.

III. Conclusion

The storage stability for glyphosate and AMPA when stored at ≤ -20°C in animal matrices was investigated in swine, cattle and chicken samples.

For glyphosate no significant degradation during storage was observed for all matrices investigated except for chicken eggs. In eggs 14 months was the maximum storage period without a significant degradation of the residue. For all other matrices the maximum storage intervals were: 26 months for pig fat, muscle, liver and kidney; 16 months for cattle milk, 24 months for cattle fat, muscle, liver and kidney; 13 months for chicken kidney and 25 months for chicken fat, muscle and liver.

For AMPA the fortification levels and the corresponding recoveries after storage were generally lower compared to glyphosate. For pig fat and liver, for cattle fat and muscle and for chicken fat and liver single recoveries below 70 % were observed. However, either the low corresponding procedural recoveries or other samples stored for longer intervals suggest no true degradation of the residue. Under consideration of the overall samples for each commodity and the initial concentrations directly after fortification these single results do not indicate a significant degradation of the residue. For chicken eggs, corresponding to the results for glyphosate, the degradation of AMPA was significant during storage, indicating stable residues of AMPA after storage for only 14 months. Samples of chicken eggs stored for 25 and 28 months gave a significant decline of the AMPA residue. For all other animal commodities maximum storage intervals were: 26 months for pig fat, muscle, liver and kidney; 16 months for cattle milk, 24 months for cattle fat, muscle, liver and kidney; 13 months for chicken kidney and 25 months for chicken fat, muscle and liver.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in animal matrices (tissues, milk and eggs) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with two deviations. Some spiking levels of glyphosate and AMPA were not at least 10 × LOQ. Nevertheless, stability for glyphosate and AMPA in all tested matrices except eggs and also decline for glyphosate and AMPA in eggs at longer storage times can still clearly be seen. Secondly, a mixed spiking solution was used for glyphosate and AMPA. However, the storage stability data for each analyte shows that there is no significant change in the concentration of any of the analytes except in eggs where both glyphosate and AMPA show a decline. Hence, transformation from one compound to another is very unlikely.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.1/015
Report author	[redacted]
Report year	1987
Report title	Magnitude of SC-0224 residues in eggs and poultry
Report No	[redacted] 87-43
Document No	Not available
Guidelines followed in study	US EPA: Subdivision O, Pesticide Assessment Guidelines for Residue Chemistry
Deviations from current test guideline	Yes, (OECD 506): • No details on sample preparation and storage conditions are given • Freshly spiked procedural recoveries not available for each storage interval • Spiking level of AMPA not at least 10 × LOQ for liver • Limited data on residue and recovery levels of spiked samples at 0 storage interval for samples of egg (only one sample analysed for AMPA) • Incurred residue samples of liver and kidney were not prepared as duplicates at the day of first analysis
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

1. Information on the study

Data point:	CA 6.1/016
Report author	[redacted]
Report year	1987
Report title	Magnitude of SC-0224 residues in meat and milk
Report No	[redacted] 87-44
Document No	Not available
Guidelines followed in study	US EPA: Subdivision O, Pesticide Assessment Guidelines for Residue Chemistry
Deviations from current test guideline	Yes, (OECD 506): • No details on sample preparation and storage conditions are given • Freshly spiked procedural recoveries not available for each storage interval • Spiking level of AMPA not at least 10xLOQ for cow liver • Limited data on residue and recovery levels of spiked samples at 328 days storage interval for samples of milk (only one sample

	analysed) • Incurred residue samples of liver and kidney were not prepared as duplicates at the day of first analysis
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The frozen storage stability of glyphosate, AMPA (aminomethylphosphonic acid) and TMS (glyphosate trimesium (trimethylsolfonium cation)) in chicken eggs, cow milk and tissues (liver, fat, muscle, kidney) was investigated within the scope of the poultry and ruminant feeding studies. TMS is not a relevant analyte in this dossier; therefore, data with respect to this analyte is not presented in the following summary.

Samples were spiked with the test items at various concentration levels in range of 0.2-1.0 mg/kg for glyphosate and 0.1-1.0 mg/kg for AMPA. The samples were stored frozen until analysis for about 22 to 23 months. Glyphosate and AMPA in all animal matrices were stable for the maximum period tested: 23 months in eggs, 22 months in milk, 23 months in cow muscle and fat. AMPA was also shown to be stable in cow liver for the maximum period tested: 23 months.

The stability of glyphosate in cow liver and kidney and the stability of AMPA in cow kidney was tested with incurred residues samples over a period of approximately 44 months. The results indicate that there is no decline, however due to the high level of variation between the different storage intervals the results are not considered reliable.

I. Materials and methods

A. Materials				
1. Test material:				
Identification:	Glyphosate		AMPA	
Description:	Not reported		Not reported	
Lot/Batch #:	Not reported		Not reported	
Purity:	Not reported		Not reported	
CAS #:	1071-83-6		1066-51-9	
Spiking levels:	0.2-1.0 mg/kg		0.1-1.0 mg/kg	
2. Test Commodity:				
Animal:	Cow, chicken			
Commodities:	Cow fat , muscle, liver, kidney and milk, chicken eggs			
Sample size:	Not reported			

B. Study design

1. Test procedure

The frozen storage stability of glyphosate and AMPA in animal matrices was investigated.

Samples (single, duplicate or triplicate) were spiked with the test items at concentrations between 0.2 mg/kg and 1.0 mg/kg for glyphosate and between 0.1 mg/kg and 1.0 mg/kg for AMPA. At various storage intervals over a period of 23 months for chicken eggs, 22 months for milk, and 23 months for cow muscle and fat samples were tested for the stability of glyphosate and AMPA. Additionally, at 3 intervals over a period of 23 months cow liver samples were tested for the stability of AMPA.

The stability of glyphosate in cow liver and kidney and the stability of AMPA in cow kidney was tested with incurred residues samples over a period of approximately 44 months.

2. Description of analytical procedures

Samples were analysed using procedures based on SCC methods RRC87-41 and RRC87-42 (refer to CA 4.1.2).
Glyphosate and AMPA were extracted from animal matrices using deionised water and clean-up by a cation exchange column. Milk was diluted with glacial acetic acid and for liver a 2-part clean-up system with methanol/water (1/10, v/v) was used. After separate collection of glyphosate and AMPA by use of an ion exchange column the analytes were converted to fluorescent derivatives with 9-fluorenylmethyl chloroformate (FMCL or FMOC) by adding borate buffer and FMOC-Cl derivatisation solution. Quantitation was achieved by HPLC-fluorescence analysis.

The limit of quantitation (LOQ) of glyphosate and AMPA in animal tissue (kidney, liver, fat and muscle from cow and chicken) is 0.05 mg/kg (except cow liver, with LOQ of 0.2 mg/kg), while the LOQ of glyphosate and AMPA in milk and eggs is 0.02 mg/kg.

The accuracy of the residue determination was confirmed by procedural recoveries from freshly spiked samples fortified with glyphosate and AMPA at 0.5-1.0 mg/kg.

The procedural recoveries of glyphosate and AMPA were between 70 % and 110 %.

II. Results and discussion

The results from the stored samples with spiked residues and from the stored samples containing incurred residues are presented in the table below. The analytical results are presented as % recovery of the nominal spiking level. Additionally, % recovery of initial value at day 0 are presented (italic).

Table 6.1-17: Storage stability of glyphosate and AMPA in various animal matrices

Commodity	Analyte	Storage period days (months)	Spiking level (mg/kg)	Residue level in stored samples[1] (mg/kg)	Recovery of stored samples (% of nominal spiking level)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)
Egg	Glyphosate	0	0.2	0.19, 0.18 (0.19)	95, 89 (92)	*100*	-
		186 (6)		0.21, 0.21, 0.20 (0.21)	107, 107, 98 (104)	*111*	-
		0	0.5	0.50, 0.49	100, 98	*100*	-

Table 6.1-17: Storage stability of glyphosate and AMPA in various animal matrices

Commodity	Analyte	Storage period days (months)	Spiking level (mg/kg)	Residue level in stored samples[1] (mg/kg)	Recovery of stored samples (% of nominal spiking level)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)
				(0.50)	(99)		
		186 (6)		0.48, 0.56, 0.51 (0.52)	95, 111, 102 (103)	*104*	-
		683 (23)		0.64, 0.46, 0.42 (0.51)	128, 92, 84 (101)	*102*	109
	AMPA	0	0.5	0.52	105	-	-
		186 (6)	0.1	0.07, 0.06, 0.07 (0.07)	72, 64, 68 (68)	-	-
		186 (6)	0.2	0.19, 0.22, 0.21 (0.21)	96, 109, 107 (104)	-	-
		683 (23)		0.24, 0.13, 0.17 (0.18)	120, 65, 85 (90)	-	106[2]
Milk	Glyphosate	0	0.2	0.22, 0.24 (0.23)	110, 122 (116)	*100*	-
		186 (6)		0.25, 0.21, 0.26 (0.24)	123, 103, 128 (118)	*104*	-
		328 (11)		0.22	110	*96*	
		0	0.5	0.55, 0.56 (0.56)	110, 112 (111)	*100*	-
		186 (6)		0.51, 0.55, 0.53 (0.53)	101, 109, 105 (105)	*95*	-
		328 (11)		0.52	104	*93*	
		671 (22)		0.41, 0.39, 0.40 (0.40)	82, 78, 80 (80)	*71*	92
	AMPA	0	0.2	0.22	109	*100*	-
		186 (6)		0.26, 0.28, 0.23 (0.26)	130, 140, 115 (128)	*118*	-
		328 (11)		0.20	101	*91*	-
		0	0.5	0.64	128	*100*	-
		186 (6)		0.31, 0.34, 0.31 (0.32)	62, 68, 62 (64)	*50*	-
		328 (11)		0.54	107	*84*	-
		671 (22)		0.52, 0.52, 0.46 (0.50)	104, 104, 92 (100)	*78*	104

Table 6.1-17: Storage stability of glyphosate and AMPA in various animal matrices

Commodity	Analyte	Storage period days (months)	Spiking level (mg/kg)	Residue level in stored samples[1] (mg/kg)	Recovery of stored samples (% of nominal spiking level)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)
Cow muscle	Glyphosate	0	1.0	0.97, 0.96, 1.00 (0.98)	97, 96, 100 (98)	*100*	-
		182 (6)		0.81, 0.86, 0.81 (0.83)	81, 86, 81 (83)	*85*	-
		687 (23)		1.01, 1.05, 0.99 (1.02)	101, 105, 99 (102)	*104*	102
	AMPA	0	1.0	0.86, 0.83, 0.86 (0.85)	86, 83, 86 (85)	*100*	-
		182 (6)		1.03, 0.95, 1.11 (1.03)	103, 95, 111 (103)	*121*	-
		687 (23)		0.87, 0.88, 0.83 (0.86)	87, 88, 83 (86)	*101*	93
Cow fat	Glyphosate	0	1.0	0.87, 0.93, 0.90 (0.90)	87, 93, 90 (90)	*100*	-
		212 (7)		0.86, 0.97, 0.78 (0.87)	86, 97, 78 (87)	*97*	-
		687 (23)		0.74, 0.99, 0.93 (0.89)	74, 99, 93 (89)	*99*	105
	AMPA	0	1.0	0.68, 0.93, 0.82 (0.81)	68, 93, 82 (81)	*100*	-
		212 (7)		1.16, 1.10, 1.10 (1.12)	116, 110, 110 (112)	*138*	-
		687 (23)		1.00, 0.86, 0.86 (0.91)	100, 86, 86 (91)	*112*	90
Cow liver	Glyphosate	0[3]	-	0.51	-	*100*	-
		604 (20)[3]	-	1.4, 1.2, 1.3 (1.3)	275, 235, 255 (255)	*255*	77[4]
		1311 (44)[3]		1.3, 1.2, 1.5 (1.3)	255, 235, 294 (261)	*255*	80[4]
	AMPA	0	1.0	0.66, 0.62, 0.50 (0.59)	66, 62, 50 (59)	*100*	-
		182 (6)		1.2, 0.93 (1.1)	119, 93 (106)	*186*	-
		691 (23)		0.71, 0.76, 0.70 (0.72)	71, 76, 70 (72)	*122*	*75*
Cow kidney	Glyphosate	0[3]	-	7.6	-	*100*	-
		604 (20)[3]	-	17.2, 16.5, 17.0 (16.9)	226, 217, 224 (222)	*222*	83[4]
		1304 (43)[3]		17.1, 17.4, 17.2 (17.2)	225, 229, 226 (227)	*226*	94[4]
	AMPA	0[3]	-	1.7	-	*100*	-
		604 (20)[3]	-	2.6, 1.9, 2.5 (2.3)	154, 109, 149 (137)	*135*	87[4]
		1311 (44)[3]		2.9, 2.8, 2.8 (2.8)	170, 167, 166 (168)	*165*	97[4]

Table 6.1-17: Storage stability of glyphosate and AMPA in various animal matrices

Commodity	Analyte	Storage period days (months)	Spiking level (mg/kg)	Residue level in stored samples[1] (mg/kg)	Recovery of stored samples (% of nominal spiking level)	*% of initial value at day 0*	Procedural recovery of freshly fortified samples (%)

[1] Concentration corrected for amount in unfortified control samples.
[2] Fortification level of 0.5 mg/kg
[3] Results for treated sample (incurred residue); Day 0 is the initial value used as the basis for calculating recoveries for stored samples
[4] Fortification level of 1.0 mg/kg

III. Conclusion

The storage stability for glyphosate and AMPA in animal matrices was investigated in cow and chicken samples.
Glyphosate and AMPA were proved to be stable in eggs, milk and cow muscle and fat for the maximum period tested: 23 months in eggs, 22 months in milk and 23 months in cow muscle and fat.

For AMPA in eggs the samples collected after 6 months with a fortification level of 0.1 mg/kg showed residues <70 % of the nominal level. The 6 months samples of the higher fortification level (0.2 mg/kg) however showed residues >70 %. The results of the 0.2 mg/kg fortification level are considered more reliable since the residues are high enough (10x LOQ) to adequately determine the stability with less variability of the recoveries. In addition, the final samples of the 0.2 mg/kg fortification level taken after 23 months confirm the stability with a mean recovery of 90 % of the nominal level.

For AMPA in milk the samples collected after 6 months with a fortification level of 0.5 mg/kg showed residues <70 % of the nominal level. However, the samples of the longer storage intervals of 11 and 22 months showed recoveries of 92-107 %, indicating that there is no actual decline of AMPA for the tested period (22 months). In addition, recoveries of the 0.2 mg/kg fortification are >70 % for all storage intervals (0, 6 and 11 months) confirming stability of AMPA in milk.

For AMPA in cow liver at day 0 residues were <70 % of the nominal spiking level 1.0 mg/kg. Mean recoveries at the storage intervals of 6 and 23 months were 106 % and 72 % indicating that the performance of the initial sample weights or analysis at day 0 was not so good.

For cow kidney incurred residues of glyphosate and AMPA in treated samples were used for the evaluation of storage stability. For cow liver incurred residues of glyphosate in treated samples were used for the evaluation of storage stability. However, due to the high level of variation between the different storage intervals the results are not considered reliable. Nevertheless, the results indicate that there is no decline, thus confirming the results of the storage stability study on animal matrices presented in CA 6.1/014.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the storage stability of glyphosate and metabolite AMPA in animal matrices (tissues, milk and eggs) was previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 506 with some deviations. No details on sample preparation and storage conditions is given. Freshly spiked procedural recoveries are not available for each storage interval, but the available procedural recoveries of glyphosate and AMPA were between 70 % and 110 % indicating good performance of the analytical method. The spiking level of AMPA was not at least 10xLOQ for liver, but a fortification level of 1.0 mg/kg is high enough to determine a possible decline. Limited data on residue and recovery levels of spiked samples at day 0 of egg (only one sample analysed for AMPA) and day 328 of milk (only one sample analysed for each glyphosate and AMPA) is available. And incurred residue samples of liver and kidney were not prepared as duplicates at the day of first analysis.
Nevertheless, stability for glyphosate and AMPA in all tested matrices can clearly be seen.

Assessment and conclusion by RMS:

CA 6.2 Metabolism, Distribution and Expression of Residues

The metabolism of glyphosate has been investigated in all crop categories relevant to metabolism according to OECD 501. Different studies are available for non-tolerant plants/conventional plants and tolerant/genetically modified plants:

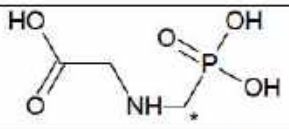
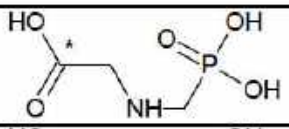
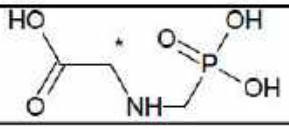
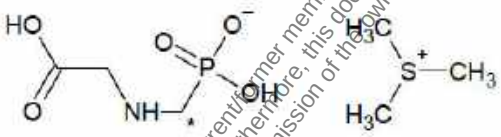
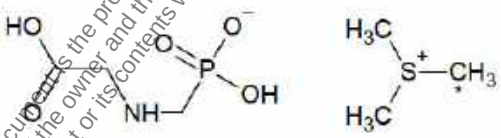
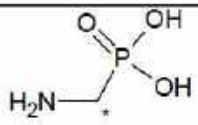
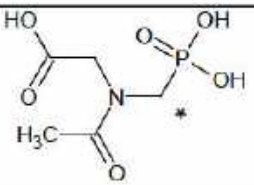
- non-tolerant plants: fruits (citrus (calamondin citrus), tree nuts (walnut, almond and pecan), apple, grape); root crops (potato, sugar beets), cereals/grass crops (wheat, barley, oats, rice, sorghum, maize, pasture), pulses and oilseeds (soybean, cotton), miscellaneous (coffee and sugarcane).
- genetically modified plants:
  CP4 EPSPS modification: root crops (sugar beets), pulses and oilseeds (soybean, cotton)
  CP4 EPSPS and GOX modification: cereals/grass crops (maize/corn), pulses and oilseeds (rape/canola)
  GAT modification: cereals/grass crops (maize/corn), pulses and oilseeds (rape/canola, soybean)

The metabolic fate of glyphosate has also been investigated in livestock (lactating goat and laying hen).

Within the different plant metabolism studies glyphosate, the trimesium salt of glyphosate or its metabolite AMPA (aminomethylphosphonic acid) were used. Three different glyphosate labels are possible, the first one (which is used on the majority of metabolism studies) is *N*-(phosphono-^{14}C-methyl)glycine (^{14}C-methane-glyphosate) where the methylene carbon is labelled. Two labels in the glycine moiety are possible, the one labelled on the carbon of the carboxyl group, named *N*-(phosphonomethyl)-^{14}C-carboxy-glycine and the other one labelled on the other carbon of the glycine group, named *N*-(phosphonomethyl)-^{14}C-methyl-glycine.

Within several animal studies glyphosate, the trimesium salt of glyphosate and/or its metabolite AMPA (aminomethylphosphonic acid) were used. Additionally *N*-acetyl glyphosate with a radioactive ^{14}C-label at the methylene carbon was utilised during the conduct of the livestock studies (laying hen and lactating goat).

The different labels used are shown in the table below. As the naming of labels differs in the different metabolism studies a common naming is used in the summary of this section. That one indicated in **bold** is the one used in the summaries within this dossier

Label	Structural formula (* indicates the label position)	Code Number (Synonyms) That indicated in bold was used in the summary dossier
Glyphosate CP 67573 *N*-(phosphono-methyl)glycine		
^{14}C-methane-label		• ***N*-(phosphono-^{14}C-methyl)glycine** • ^{14}C-methane-glyphosate
^{14}C-carboxy-glycine-label		• ***N*-(phosphonomethyl)-^{14}C-carboxy-glycine** • Glycine-1-^{14}C-glyphosate
^{14}C-methyl-glycine-label		• ***N*-(phosphonomethyl)-^{14}C-methyl-glycine** • Glycine-2-^{14}C-glyphosate
Glyphosate-trimesium ICIA0224 Trimesium salt of glyphosate *N*-(phosphono-methyl)glycine trimesium salt		
PMG-label		• ***N*-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labelled glyphosate-trimesium)** • PMG • [^{14}C]-phosphonomethylene glyphosate trimesium • *N*-phosphono-methylglycine anion • [^{14}C-PMG]glyphosate-trimesium • ^{14}C-PMG-labeled glyphosate-trimesium
TMS-label		• ***N*-(phosphonomethyl)glycine ^{14}C-trimesium salt (^{14}C-TMS labelled glyphosate-trimesium)** • trimethylsulfonium cation • ^{14}C-TMS labelled glyphosate-trimesium
AMPA (Aminomethylphosphonic acid) Monosodium salt of AMPA		
^{14}C-AMPA		• **Amino-^{14}C-methylphosphonic acid** • ^{14}C-AMPA • ^{14}C-aminomethyl-phosphonic acid
***N*-acetyl glyphosate** *N*-acetyl-*N*-(phosphonomethyl)glycine IN-MCK20		
^{14}C-*N*-acetyl glyphosate		• ***N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine** • [^{14}C]-*N*-acetyl glyphosate

Several metabolites were identified in the studies conducted with the different radiolabels of the active substance. The chemical structures and report names used in the summaries are provided in the List of Metabolites in Document N3.

In the following for the classification of a metabolite into major and minor the following definitions were used:

- Major metabolite in food ≥10 % TRR and ≥0.01 mg/kg or if TRR <10 % amount >0.05 mg /kg
- Major metabolite in processing study: TRR ≥10 %
- Major metabolite in feed and non food/non feed related commodities: ≥10 % TRR and ≥0.01 mg/kg

CA 6.2.1 Metabolism, distribution and expression of residues in plants

Glyphosate is an herbicide which is active against all plants by the inhibition of the shikimate cycle required for the formation of essential amino acids. In principle, it is systemic in plants. However, due to its high potency as herbicide, the translocation within crops is very limited before withering. The uptake via soil in confined rotational crop is very limited (see CA 6.6.1) and is mainly via uptake of glyphosate by treated leaves.

As a result, uptake of glyphosate is not expected or expected at only low rate into primary crops. The different metabolism studies dealt with the fact to find application scenarios which result in sufficient residues to investigate the nature of glyphosate-derived residues.

Therefore, a lot of plant metabolism studies represent artificial scenarios which are not related to current GAPs (as there are pre-sowing, pre-planting, pre-emergent uses):
foliar applications, stem or trunk applications, growing in treated hydroponic solution.

The different study designs reflect the need to identify at least the major chemical components of the residue although knowing that too high rates will induce severe phytotoxicity which may stress or even kill the crops.

Within the following table available plant metabolism studies are summarised.

Table 6.2.1-1: Overview on available plant metabolism studies

Crop Category	Crop
Non-tolerant plants	
Fruit	Citrus (calamondin citrus (mandarin), lemon) Tree nuts (walnut, almond and pecan) Apple Grape
Root crops	Potato Sugar beets Beet[1] Carrot[1]
Leafy crops[1]	Cabbage[1]
Cereal/grass crops	Wheat (primary crop also in rotational crop studies) [1] Barley Oats Rice Sorghum Maize Pasture
Pulses and oilseeds	Soybean (primary crop also in rotational crop studies) [1] Cotton Pea[1] String bean[1]
Miscellaneous	Coffee Sugarcane
Genetically modified plants	
CP4 EPSPS, CP4 EPSPS and GOX modification	
Root Crops	Sugar beets
Cereal/grass crops	Wheat Maize/corn
Pulses and oilseeds	Rape/canola Soybean Cotton
GAT modification	
Cereal/grass crops	Maize/corn
Pulses and oilseeds	Rape/canola Soybean

[1] marked crops were investigated as primary crops within confined rotational crops studies (for further details please refer to CA 6.6.1.

In the following the different metabolism studies on primary crops are summarised as full OECD summaries and are assessed again by the applicant.

Non-tolerant plants

Fruit crops

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/001
Report author	[redacted]
Report year	1975
Report title	The metabolism of CP 67573 by citrus
Report No	328
Document No	Not available
Guidelines followed in study	None
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Radioactive residues in RAC are expressed in % of applied radioactivity rather than in terms of mg/kg (TRR values) Recalculation is only possible for the 1 to 4 months untreated leaf samples from the soil and foliar application experiments • No distribution of the residue between pulp and peel is reported • Radioactive residues found in untreated leaves in the time course experiment were characterised by ion exchange chromatography, but not identified by two independent methods. Radioactive residues found after hydroponic treatment were not characterised • No flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed • Radioactive counting data available only for the soil and foliar application experiments • Unextracted radioactive residues not precisely quantified • No release and characterisation and/or identification was attempted on the unextracted radioactive residues • No description of conditions and length of storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities[1, 2]	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The metabolism of glyphosate (N-(phosphonomethyl)glycine) in citrus plant was studied after soil, hydroponic and foliar application in a series of experiments.
In soil uptake experiments, N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) and amino-^{14}C-methyl-phosphonic acid (^{14}C-AMPA), respectively, each were applied to the soil of plant pots containing calamondin citrus plants at rates equivalent to 2.24 kg as/ha. In parallel, the same amount of ^{14}C-glyphosate was applied to selected leaves simulating foliar treatment. Samples of leaves were collected at 1, 2 and 3 months after treatment. Samples of soil, roots, stems, leaves, immature and mature fruits were collected at 4 months at termination.
Hydroponic uptake was investigated in a hydroponic solution at 10 mg test substance/kg with ^{14}C-glyphosate or ^{14}C-AMPA.
Foliar application experiments were performed by applying 4 mg ^{14}C-glyphosate as drops to the leaf surface. One group of citrus trees was treated with a mixture of ^{13}C-glyphosate and ^{14}C-glyphosate for spectroscopic structure elucidation of glyphosate metabolite. Sampling was performed between 1 and 8 weeks in these experiments.

After soil treatment of calamondin citrus plants with ^{14}C-glyphosate at a rate of 2.24 kg a.s./ha, less than 0.1 % of the applied activity was absorbed from treated soil or translocated into the leaves, stems, immature fruit and mature fruit. Comparable low rates of absorption were observed after soil application of ^{14}C-AMPA. Foliar treatment at the same rate led to higher translocation of the applied activity into untreated leaves of the same plants at 0.27 % - 1.01 % between 1 - 4 months.

During hydroponic treatment for 1 week at 10 mg/L ^{14}C-glyphosate or ^{14}C-AMPA, respectively, in the nutrient solution, the percentage of radioactivity recovered was 1.3 % or 1.8 % in the leaves, 0.3 % in the stems both for ^{14}C-glyphosate or ^{14}C-AMPA treatment, and 4.2 % and 5.5 % in the roots, for ^{14}C-glyphosate or ^{14}C-AMPA, respectively. $^{14}CO_2$ amounted to 2.1 % and 1.4 % of the applied activity with ^{14}C-glyphosate or ^{14}C-AMPA, respectively. Replacement of the ^{14}C-glyphosate and ^{14}C-AMPA treatment solutions with fresh nutrient solution resulted in a decrease of $^{14}CO_2$ for the second week to 0.3 % and 0.2 % of the applied radioactivity for ^{14}C-glyphosate or ^{14}C-AMPA treatments, respectively.

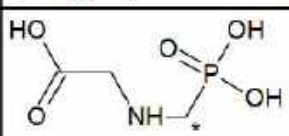
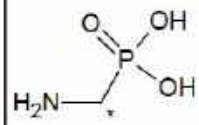
After foliar application of 4 mg ^{14}C-glyphosate, treated leaves contained 76.6 % of the applied radioactivity after one week. The radioactivity in treated leaves declined to 10.3 % after 6 weeks. Non-treated leaves of the same plant showed 0.8 - 2.6 % and stems 1.3 - 2.2 % of the applied radioactivity at 1 - 8 weeks after treatment. In fruit, <0.1 to 1.4 % were recovered at 1 - 6 weeks, while 9.8 % of the applied activity were found after 8 weeks. Thus, accidental treatment of the lower foliage in a citrus orchard could result in a detectable residue of glyphosate in mature fruit. A total of 79.7 % of the applied radioactivity was recovered after one week; the amount of recovered radioactivity declined during the course of the study, being 13.5 % of the applied radioactivity after 8 weeks.

^{13}C-NMR and GC-MS indicated the presence of glyphosate in purified calamondin citrus extracts. No indications of AMPA could be found.

High voltage electrophoresis showed similar electrophoretic mobility of the glyphosate metabolite fractions from commercial orange and calamondin citrus.

I. Materials and Methods

A. Materials

1. Test material	
Test Material:	a) N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate); batch nos. 191, 240 and 245 b) Amino-^{14}C-methyl-phosphonic acid (^{14}C-AMPA), batch no. 235 c) N-(phosphono-^{13}C-methyl)glycine
Chemical structure:	 a), c) * Position of label  b) * Position of label
Radiochemical purity:	a) batch no: 191: 88 % batch no: 240: 96.8 % batch no: 245: 97 % All three batches were purified to >99 % by ion exchange chromatography prior to use b) >99 % for batch 235
Specific activity:	a) 1.76 MBq/mg (8.03 mCi/mmol), batch no: 191 0.41 MBq/mg (1.87 mCi/mmol), batch no: 240 1.98 MBq/mg (9.07 mCi/mmol), batch no: 245 b) 2.96 MBq/mg (8.90 mCi/mmol), batch no: 235
CAS No:	Glyphosate: 1071-83-6 AMPA: 1066-51-9
Log $P_{o/w}$:	Glyphosate: -3.2 AMPA: -2.47

Test system:

Soil:	Norfolk sandy loam soil (2 % clay, 1 % organic matter with pH 5.7)
Crop:	Calamondin citrus Commercial orange variety
Botanical name:	*Citrus microcarpa, × Citrofortunella microcarpa or × Citrofortunella mitis*
Crop part(s):	Leaf, root, stem, immature and mature fruit

B. Study design

1. In-life phase

Uptake ("propensity") study
N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate, formulated with isopropylamine and G 3780A adjuvant) and Amino-^{14}C-methyl-phosphonic acid (^{14}C-AMPA), respectively, each were applied to the soil of plant pots containing calamondin citrus plants at rates equivalent to 2.24 kg as/ha. This rate corresponded to 5.6 mg and 5.93×10^8 dpm (9.88 MBq) applied radioactivity for ^{14}C-glyphosate or 5.6 mg and 1×10^9 dpm (16.67 MBq) applied radioactivity for ^{14}C-AMPA, respectively. Alternatively the same rate of ^{14}C-glyphosate was applied to selected leaves simulating foliar treatment, corresponding to 5.6 mg and 5.93×10^8 dpm. Plants were kept under greenhouse conditions for the duration of the study. One set of control plants was maintained in the same greenhouse cubicle as the ^{14}C-treated plants, while a second set of controls was maintained in an isolated greenhouse cubicle.

Transpiration study
Calamondin citrus plants were grown in a hydroponic solution at 10 mg test substance/L (^{14}C-glyphosate: 4.4×10^8 dpm (7.33 MBq), chambers no. 1 and no. 2 or ^{14}C-AMPA: 8.0×10^8 dpm (13.33 MBq), chambers no. 3 and no. 4). After one week the citrus plants in chambers no. 1 and 3 were harvested and the nutrient solutions in chambers no. 2 and 4 containing ^{14}C-glyphosate or ^{14}C-AMPA were replaced with fresh unlabelled nutrient solutions. The citrus plants in chambers no. 2 and no. 4 were harvested at the end of the 2nd week.

Foliar glyphosate ^{14}C time course study in calamondin
Six 16 month old calamondin citrus plants were treated by applying drops of formulated ^{14}C-glyphosate to the upper and lower surface of each of fifty lower leaves of each plant. The applied amount was equivalent to 4 mg ^{14}C-glyphosate with a total activity of 4.29×10^8 dpm (7.15 MBq).

Glyphosate metabolite production in calamondin
Thirty 16 month old calamondin citrus plants were treated by applying drops of a formulation containing both ^{14}C- and ^{13}C- isotopically labelled glyphosate to the upper and lower surface of each of fifty lower leaves of each plant. The applied amount was equivalent to 4 mg ^{14}C-glyphosate with a total activity of 2.2×10^7 dpm (0.37 MBq).

Comparative glyphosate metabolism in commercial orange
Commercial variety orange plants cultivated in plastic pots were treated by applying drops of a ^{14}C-glyphosate solution to the upper and lower surface of each of fifty lower leaves of each plant. The applied amount was equivalent to 4 mg ^{14}C-glyphosate with a total activity of 9.66×10^7 dpm (1.61 MBq).

2. Sampling

Uptake ("propensity") study
At 1, 2 and 3 months after treatment, random samples of 50 untreated leaves were collected from each calamondin plant. At 4 months after treatment the study was terminated and soil, roots, stems, leaves, immature and mature fruits were collected.

Transpiration study
Samples of leaves, stem, roots, as well as nutrient solutions, $^{14}CO_2$ traps, and volatile traps were investigated at termination of the experiments, after one or two weeks, respectively.

Foliar glyphosate ^{14}C time course study in calamondin
After 1, 2, 3, 4, 6 and 8 weeks, single plants were harvested and separated into treated leaves, untreated leaves, stems and fruits.

Glyphosate metabolite production in calamondin

Glyphosate metabolite production in calamondin was undertaken to provide sufficient material for spectroscopic structure elucidation of glyphosate metabolite. Plants were harvested 16 days after treatment and separated into treated leaves, untreated leaves, stems and fruits. After determination of the ^{14}C content by combustion, the untreated leaves from treated plants were separated into five different composites depending upon their ^{14}C concentration. All of the stems from treated plants were composited, as well as all of the fruit.

Comparative glyphosate metabolism in commercial orange

Plants were harvested 16 days after treatment and separated into treated leaves, untreated leaves and stems.

Plant samples were frozen immediately after harvesting and then lyophilised. The dried material was then ground to a fine powder that would pass a 60 mesh screen.

3. Analytical procedures

The total radioactive residues in harvested plant samples were determined by combustion and subsequent liquid scintillation counting (LSC). Liquid extracts were quantified by LSC.

Characterisation of the radioactivity in the samples was performed by ion exchange chromatography and GLC-FID/RAD.

Citrus leaf and fruit samples were pre-extracted with organic solvents before extraction with water. Leaf material was sequentially Soxhlet-extracted for 18 hours with each of hexane, diethyl ether, and acetone. Fruit material was extracted with acetone followed by two extractions with diethyl ether.

Soil or defatted plant material were extracted four times with water and the extracts combined and counted.

Plant extracts were purified and characterised by column chromatography using ion exchange resins (cation exchange: AG 50W-X4, AG 50W-X8; anion exchange AG 1-X8) as well as molecular sieve type supports (Bio-Gel P-2). Cation exchange resins were eluted with water while anion exchange columns were eluted with a 0 to 8 N formate gradient.

The quantification and identification of the residues in the extracts was performed following derivatisation to N-trifluoroacetyl-butyl-esters by GC/MS on glass columns packed with 1.5 % OV-17 on Chromosorb W-HP.

^{13}C NMR spectra of the residues in the purified extracts were obtained at 22.6 mHz and 35 °C by standard pulsed techniques.

High voltage electrophoresis was used for characterisation of the extract residues applying a chromatographic flat plate.

Standards of unlabelled N-(phosphonomethyl)glycine and aminomethylphosphonic acid were used in addition to the ^{13}C- and ^{14}C-labelled compounds as analytical reference substances.

Tri-n-butyl-N-trifluroacetyl glyphosate was prepared by derivatising glyphosate with trifluoroacetic anhydride/trifluoroacetic acid and diazobutane.

II. Results and Discussion

A. Total radioactive residues (TRRs)

In the table below the uptake of radioactivity observed in the uptake ("propensity") study, following soil or foliar treatment equivalent to 2.24 kg as/ha, is summarised. Radioactive residues in the analysed fractions were reported in the original study as percentage of applied radioactivity. Total radioactive residues, expressed as glyphosate equivalents, were calculated from the reported values upon dossier compilation; these values are shown in the table below in *italics.*

Less than 0.1 % of the 2.24 kg a.s./ha ^{14}C-glyphosate or ^{14}C-AMPA applied, respectively, was absorbed from treated soil and translocated into each leaves, stems, immature fruits or mature fruits at 1 to 4 months after application. Total radioactive residues (TRR) in untreated leaves ranged between 0.09 - 0.13 mg/kg after ^{14}C-glyphosate treatment and between 0.15 - 0.19 mg/kg after ^{14}C-AMPA treatment. After foliar treatment with ^{14}C-glyphosate equivalent to the same rate, the radioactivity found in the untreated leaves from treated plants ranged from 0.27 to 1.01 % of the applied radioactivity at 1 to 4 months after application. TRRs in untreated leaves ranged between 0.36 and 1.36 mg/kg. Four months after treatment, residues in treated leaves reached 11.92 % of the applied radioactivity, while 0.29 %, 0.41 % and 0.26 % were found in roots, stems and immature fruit, respectively. In mature fruit, 1.3 % of the applied radioactivity was recovered.

Table 6.2.1-2: Recovered radioactivity following foliar or soil treatment of citrus trees with ^{14}C-glyphosate or ^{14}C-AMPA at rates equivalent to 2.24 kg a.s./ha

Treatment	1 Month leaves[1]	2 Months leaves[1]	3 Months leaves[1]	4 Months Leaves[1]	Soil	Roots	Stems	Treated leaves	Immature fruit	Mature fruit	Total
^{14}C-glyphosate Initial: 5.93 x 10^8 dpm (5.6 mg)											(soil)
% AR	0.08	0.09	0.09	0.09	63.82	0.41	0.08	-	0.06	0.05	64.51
^{14}C dpm/g	9850	12408	12950	13580	n r.	n r.	n r.	n.r.	n r.	n r.	n r.
TRR (mg/kg)	*0.09*	*0.12*	*0.12*	*0.13*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
^{14}C-glyphosate (foliar) Initial: 5.93 x 10^8 dpm (5.6 mg)											
% AR	0.27	1.01	0.29	0.76	10.52	0.29	0.41	11.92	0.26	1.30	25.46
^{14}C dpm/g	38050	144790	46250	142950	n r.	n r.	n r.	n.r.	n r.	n r.	n r.
TRR (mg/kg)	*0.36*	*1.37*	*0.44*	*1.36*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
^{14}C-AMPA (soil) Initial: 1 x 10^9dpm (5.6 mg)											
% AR	0.08	0.06	0.06	0.07	64.67	0.34	0.09	-	0.04	0.04	65.25
^{14}C dpm/g	18345	18105	18602	21680	n r.	n r.	n r.	n.r.	n r.	n r.	n r.
TRR (mg/kg)	*0.16*	*0.15*	*0.16*	*0.19*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Exposed control plants											
^{14}C dpm/g	5430	6417	8831	7750	n r.	n.r.	n.r.	n r.	n.r.	n.r.	n r.
TRR (mg/kg)	*0.05*	*0.06*	*0.08*	*0.07*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
Isolated control plants											
^{14}C dpm/g	2260	2570	3600	675	n r.	n.r.	n.r.	n r.	n.r.	n.r.	n r.
TRR (mg/kg)	*0.02*	*0.02*	*0.03*	*0.01*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.

% AR Percentage of applied radioactivity (mean of 2 replications)
TRR Total radioactive residue, expressed as glyphosate equivalents (calculated upon dossier compilation; a conversion factor of 1.522 was applied to convert residues of AMPA into glyphosate equivalents)
n.r. = not reported
n.c. = not calculated

Table 6.2.1-2: Recovered radioactivity following foliar or soil treatment of citrus trees with ^{14}C-glyphosate or ^{14}C-AMPA at rates equivalent to 2.24 kg a.s./ha

Treatment	1 Month leaves[1]	2 Months leaves[1]	3 Months leaves[1]	4 Months							
				Leaves[1]	Soil	Roots	Stems	Treated leaves	Immature fruit	Mature fruit	Total

[1] = untreated leaves from treated plants
Values in *italics* were calculated from reported values upon dossier compilation

For the transpiration study recovered radioactivity is shown in the table below.
The radioactivity levels found in leaves and stems of citrus plant after hydroponic application of ^{14}C-glyphosate (chambers no. 1 and no. 2) were comparable after one or two weeks. In leaves, 1.3 % and 1.2 % of the applied activity were recovered after one or two weeks, respectively, while in stems 0.3 % and 0.4 %, respectively, were determined. In roots, 4.2 % of the applied radioactivity were found after one week, while after two weeks 2.6 % of the applied radioactivity was recovered.
The accumulated plant CO_2 evolution from chambers no. 1 and no. 2 at the end of one week was 2.1 % and 2.9 % of the applied activity, respectively. Replacement of the ^{14}C-labelled nutrient solution with fresh solution containing no ^{14}C-glyphosate after the first week in chamber no. 2 resulted in the accumulated plant $^{14}CO_2$ dropping to 0.3 % of the applied activity for the 2nd week. 2.3 % of the applied radioactivity found in the unlabelled nutrient solution at the end of the 2nd week correspond to the apparent decrease in radioactivity content of roots from 1st to 2nd week. <0.01 % $^{14}CO_2$ evolved from the nutrient solution in chambers no. 1 and no. 2. The ^{14}C accountability for chambers no. 1 and no. 2 was 95.2 % and 92.2 % of the applied radioactivity, respectively.

After hydroponic application of ^{14}C-AMPA (chambers no. 3 and no. 4), the ^{14}C content in leaves declined from 1.8 % of the applied radioactivity at 1 week to 0.8 % of the applied radioactivity by the second week. The ^{14}C content in stems remained at 0.3 % of the applied radioactivity for both the 1st and 2nd week. The decrease of radioactivity in roots between the 1st and 2nd week (from 5.5 % to 2.5 % of the applied activity) closely approximates the increase in radioactivity found in the 2nd week nutrient solution (3.3 % of the applied activity). 1.4 % and 0.8 % of the applied radioactivity evolved as $^{14}CO_2$ from citrus plants in chamber no. 3 and no. 4, respectively, by the end of the 1st week. The removal of ^{14}C-AMPA from the nutrient solution in chamber no. 4 after the 1st week resulted in the accumulated $^{14}CO_2$ evolution from citrus plants decreasing to only 0.2 % of the applied radioactivity by the end of the 2nd week. No $^{14}CO_2$ (<0.01 %) was detected from the nutrient solution containing ^{14}C-AMPA. The ^{14}C accountability for chambers no. 3 and no. 4 was 91.7 % and 93.2 % of the applied radioactivity, respectively.

Table 6.2.1-3: Uptake and distribution of radioactivity following hydroponic application of ^{14}C-glyphosate or ^{14}C-AMPA at 10 mg/L

Fraction	% AR			
	Chamber 1 (1 week) ^{14}C-glyphosate (10 mg/L) 4.4 x 10^8 dpm	Chamber 2 (2 weeks) ^{14}C-glyphosate (10 mg/L) 4.4 x 10^8 dpm	Chamber 3 (1 week) ^{14}C-AMPA (10 mg/L) 8.0 x 10^8 dpm	Chamber 4 (2 weeks) ^{14}C-AMPA (10 mg/L) 8.0 x 10^8 dpm
Leaves				
1 week	1.3	-	1.8	-
2 weeks	-	1.2	-	0.8
Stems				
1 week	0.3	-	0.3	-
2 weeks	-	0.4	-	0.3
Roots				
1 week	4.2	-	5.5	-
2 weeks	-	2.6	-	2.5
Plant $^{14}CO_2$				

Table 6.2.1-3: Uptake and distribution of radioactivity following hydroponic application of ^{14}C-glyphosate or ^{14}C-AMPA at 10 mg/L

Fraction	% AR			
	Chamber 1 (1 week) ^{14}C-glyphosate (10 mg/L) 4.4 x 10^8 dpm	Chamber 2 (2 weeks) ^{14}C-glyphosate (10 mg/L) 4.4 x 10^8 dpm	Chamber 3 (1 week) ^{14}C-AMPA (10 mg/L) 8.0 x 10^8 dpm	Chamber 4 (2 weeks) ^{14}C-AMPA (10 mg/L) 8.0 x 10^8 dpm
1 week	2.1	2.9	1.4	0.8
2 weeks	-	0.3	-	0.2
Nutrient $^{14}CO_2$				
1 week	<0.01	<0.01	<0.01	<0.01
2 weeks	-	<0.01	-	<0.01
Nutrient solution				
1 week	85.3	82.5	82.7	85.3
2 weeks	-	2.3[1]	-	3.3[1]
Total	95.2	92.2	91.7	93.2

[1] = after one week the nutrient solution was replaced by fresh, unlabelled nutrient solution

The results of the ^{14}C-glyphosate time course study are summarised in the table below. The amount of radioactivity translocated to the non-treated leaves of treated citrus plants varied from as low as 0.8 % of the applied radioactivity at 2 weeks up to 2.6 % of the applied radioactivity at 6 weeks. In stems, the translocated radioactivity ranged between 1.3 to 2.2 % of the applied radioactivity. The highest variability was found in the ^{14}C-content present in the fruit, ranging from <0.1 % of the applied radioactivity after one week after treatment to 9.8 % of the applied radioactivity after 8 weeks. The ^{14}C accountability ranged from 79.7 % of the applied radioactivity at one week to only 13.5 % of the applied radioactivity at 8 weeks and correlated with the number of treated leaves that had abscised by harvest time.

Table 6.2.1-4: Recovered radioactivity following foliar application of 4 mg ^{14}C-glyphosate per citrus plants (time course study)

Interval (weeks)	% AR 4 mg ^{14}C-glyphosate, 4.29 x 10^8dpm				
	Treated leaves[1]	Non-treated leaves	Stems	Fruit	Accountability
1	76.6	1.8	1.3	<0.1	79.7
2	23.0	0.8	1.6	1.0	26.4
3	26.8	1.8	1.9	0.3	30.8
4	24.5	1.6	2.2	-	28.3
6	10.3	2.6	1.7	1.4	16.0
8	5	2.2	1.5	9.8	13.5

1 = the number of treated leaves present at each harvest was as follows: 1 week=41, 2 weeks=20, 3 weeks=22, 4 weeks=19, 6 weeks=10, 8 weeks=0.

The distribution of radioactivity as the percentage of the applied ^{14}C- and ^{13}C- isotopically labelled glyphosate in citrus commodities 16 days after foliar treatment with ^{14}C- and ^{13}C- isotopically labelled glyphosate in the plant experiment for glyphosate metabolite production is summarised in the table below. In treated leaves, 72.3 - 74.4 % of the applied ^{14}C- and ^{13}C- isotopically labelled glyphosate were recovered; 38 % of the treated leaves had abscised before harvest. In non-treated leaves, stems and fruit the values ranged between 0.3 - 3.5 %, 1.0 - 2.7 % and 0.5 - 5.3 % of the applied ^{14}C- and ^{13}C- isotopically labelled glyphosate.

Table 6.2.1-5: Recovered radioactivity following foliar application of 4 mg $^{13}C/^{14}C$-glyphosate per citrus plants (metabolite production)

Commodity	% AR 4 mg $^{13}C/^{14}C$-glyphosate, 2.2 × 10^7dpm	
	Range (%)	Weighted average
Treated leaves	72.3 – 74.4	73.4
Non-treated leaves	0.3 – 3.5	1.4
Stems	1.0 – 2.7	1.9
Fruit	0.5 – 5.3	2.1

The distribution of radioactivity found in the various plant fractions of a commercial orange variety is listed in the table below. In treated leaves, 54.8 – 56.5 % of the applied radioactivity was recovered; only 3 % of the treated leaves had abscised before harvest. In untreated leaves and stems the values were 0.9 and 0.7 – 1.0 %, respectively.

Table 6.2.1-6: Recovered radioactivity following foliar application of 4 mg ^{14}C-glyphosate per citrus plants (commercial orange variety)

Commodity	% AR 4 mg ^{14}C-glyphosate, 9.66 × 10^7dpm	
	Range (%)	Average
Treated leaves	54.8 – 56.5	55.6
Non-treated leaves	0.9	0.9
Fruit	0.7 – 1.0	0.8

B. Extraction and characterisation of residues

Mature fruit from plants foliar treated with ^{14}C-glyphosate in the uptake ("propensity") study was pre-extracted with acetone and diethyl ether. The defatted material was subsequently extracted with water. The water extract was purified by sequential cation / anion / cation exchange chromatography. The elution patterns from the chromatographic columns were typical of glyphosate. High voltage electrophoresis (HVE) of the radioactive fraction demonstrated electrophoretic mobility of the radioactivity extracted from the mature fruit that was identical with that of ^{14}C-glyphosate standard spiked into the sample. No balance was reported for extraction and fractionation of the radioactive residues.

No extraction or characterisation was performed in the transpiration study.

Water extracts of the untreated leaves harvested at 1, 2 and 4 weeks in the foliar ^{14}C-glyphosate time course study contained 87, 95, and 98 % of the total radioactivity present in the respective samples. The water soluble components were characterised by cation exchange chromatography. The elution pattern revealed only one ^{14}C-labelled compound that had an elution volume similar to glyphosate in the presence of plant material.
However, the elution volume of glyphosate standard was less than that of the sample in the presence of plant material. There was no evidence of ^{14}C-AMPA in the extracts. No balance was reported for extraction and fractionation of the radioactive residues.

The treated citrus leaves from the glyphosate metabolite production study containing 73 % of the applied ^{14}C- and ^{13}C- isotopically labelled glyphosate were sequentially extracted with hexane, chloroform, and acetone. Water extraction of the defatted and decolorised filter cake released 90 % of the available ^{14}C. The concentrated aqueous extract was investigated by cation exchange chromatography, resulting in an elution pattern of the radioactivity that was typical for glyphosate. There was no evidence for the presence

of
^{14}C-AMPA.

For analysis by ^{13}C-NMR and GC-MS a composite sample of untreated leaves from treated plants was sequentially extracted with hexane, chloroform, and acetone before water extraction of the defatted and decolorised filter cake. 87 % of the available ^{14}C were recovered in the aqueous extract. An equal amount of control calamondin leaves was purified in a similar fashion and the resulting extract was fortified with ^{13}C/^{14}C-glyphosate (2.45×10^6 dpm). The extract from leaves of treated plants contained 1.84×10^6 dpm, corresponding to 334 µg ^{13}C/^{14}C-glyphosate.

The concentrated extracts were purified by cation exchange chromatography, resulting in an elution pattern of the radioactivity that was typical for glyphosate in both the extracts from untreated leaves of treated plants and the fortified control extracts. There was no evidence of ^{14}C-AMPA in the extract of untreated leaves of treated plants.

After a further cation exchange clean-up of the radioactive fractions of the extracts of untreated leaves of treated plants and the fortified control extracts, ^{13}C-NMR was performed on the concentrated aqueous eluates. Spectra for both treated and fortified leaves showed signals well within the experimental error for the authentic glyphosate ^{13}C-standard.

After further purification by sequential anion / cation exchange, Bio-Gel chromatography and NTFA-butyl ester derivatisation, glyphosate was determined in the extracts of untreated leaves from treated plants via GC-MS.

Fruit from treated plants were sequentially extracted with acetone and diethyl ether and the extracted filter cake was then extracted with water, recovering 99 % of the radioactivity in the aqueous extract. An equal amount of control calamondin fruit was extracted in a similar fashion and the resulting extract was fortified with ^{13}C/^{14}C-glyphosate (2.38×10^6 dpm, corresponding to 440 µg). The extract of fruit from treated plants contained 2.17×10^6 dpm, corresponding to 395 µg ^{13}C/^{14}C-glyphosate.

Cation exchange chromatography of the concentrated extracts showed the absence of ^{14}C-AMPA in the extract of fruit from treated plants.

After additional anion exchange and cation exchange clean-up, the presence of glyphosate could be shown in the extracts of fruit from treated plants and fortified control extracts by ^{13}C-NMR.

Isotopic dilution was performed by fortifying aliquots of the extracts of fruit from treated plants and fortified control extracts with a 10 fold excess of ^{12}C-glyphosate compared to the estimated amount of ^{13}C/^{14}C-glyphosate. GC-MS analysis after NTFA-butyl ester derivatisation showed a good agreement of the calculated ^{13}C-enrichment for the mass ion pairs 106/107 and 433/434 with the theoretical values.
Confirmation of glyphosate was achieved by GC-MS after further clean-up and NTFA-butyl ester derivatisation in the extracts of fruit from treated plants.

Comparative glyphosate metabolism in commercial orange

The untreated leaves of the treated plants were extracted with water and 94 % of the available radioactivity was removed. In order to compare the glyphosate metabolism by commercial orange to that by calamondin citrus, the commercial orange extract was submitted to exactly the same chromatographic purification sequence as the water extract from calamondin citrus leaves: a sequence of two cation exchange chromatographies, an anion exchange chromatography, an additional cation exchange chromatography, and chromatography on Bio-Gel P-2. The chromatographic elution patterns for both commercial orange and calamondin fruit showed elution volumes that were smaller for the commercial orange extracts on the first three columns, while they were identical for the last two columns. High voltage electrophoresis of the final purified extracts showed that the electrophoretic mobility of the glyphosate metabolite fractions from commercial orange and calamondin citrus was identical.

C. Storage stability

No dates are reported for the experimental work from sampling to extraction and analysis of extracts. Thus, it is not possible to conclude on storage stability. However, a theoretical maximum storage period can be estimated from the study duration given in the report (February 1973 - October 1974) to be not longer than 21 months.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in fruit crops at the end of this chapter.

III. Conclusion

After soil treatment of calamondin citrus plants with ^{14}C-glyphosate at a rate of 2.24 kg a.s./ha, less than 0.1 % of the applied radioactivity was absorbed from treated soil and translocated into the leaves, stems, immature fruit and mature fruit, respectively. Comparable low rates of absorption were observed after soil application of ^{14}C-AMPA. Foliar treatment at the same rate led to higher translocation of the applied activity into untreated leaves of the same plants at 0.27 - 1.01 % between 1 - 4 months.

During hydroponic treatment for 1 week at 10 mg/L ^{14}C-glyphosate or ^{14}C-AMPA, respectively, in the nutrient solution, the percentage of radioactivity recovered was 1.3 % or 1.8 % in the leaves, 0.3 % in the stems both for ^{14}C-glyphosate or ^{14}C-AMPA treatment, and 4.2 % and 5.5 % in the roots, for ^{14}C-glyphosate or ^{14}C-AMPA, respectively. $^{14}CO_2$ amounted to 2.1 % and 1.4 % of the applied activity with ^{14}C-glyphosate or ^{14}C-AMPA, respectively. Replacement of the ^{14}C-glyphosate and ^{14}C-AMPA treatment solutions with fresh nutrient solution resulted in a decrease of $^{14}CO_2$ for the second week to 0.3 % and 0.2 % of the applied radioactivity for ^{14}C-glyphosate or ^{14}C-AMPA treatments, respectively.

After foliar application of 4 mg ^{14}C-glyphosate, treated leaves contained 76.6 % of the applied radioactivity after one week. The radioactivity in treated leaves declined to 10.3 % after 6 weeks. Non-treated leaves of the same plant showed 0.8 - 2.6 % and stems 1.3 - 2.2 % of the applied radioactivity at 1 - 8 weeks after treatment. In fruit, <0.1 to 1.4 % were recovered at 1 - 6 weeks, while 9.8 % of the applied radioactivity were found after 8 weeks. Thus, accidental treatment of the lower foliage in a citrus orchard could result in a detectable residue of glyphosate in mature fruit. A total of 79.7 % of the applied radioactivity was recovered after one week; the amount of recovered radioactivity declined during the course of the study, being 13.5 % of the applied radioactivity after 8 weeks.

^{13}C-NMR and GC-MS showed the presence of glyphosate in purified calamondin citrus extracts. No indications of AMPA could be found.

High voltage electrophoresis showed similar electrophoretic mobility of the glyphosate metabolite fractions from commercial orange and calamondin citrus.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in calamondin citrus has been previously evaluated at EU level. It was not performed under GLP (as in 1973 - 1975 GLP was not yet established at the test facility). The study is deemed to partly comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with major deficits (Radioactive residues in RAC are expressed in % of applied activity rather than in terms of mg/kg (TRR values), recalculation is possible for the 1 to 4 months untreated leaf samples from the soil and foliar application experiments; radioactive residues found in untreated leaves in the time course experiment were characterised by ion exchange chromatography, but not identified by two independent methods; radioactive residues found after hydroponic treatment were not characterised; radioactive counting data for soil and foliar uptake application experiment only; unextracted radioactive residues not precisely quantified; no release and characterisation and/or identification was attempted on the unextracted

radioactive residues; no description of conditions and length of storage of samples).

Quantitative information in terms of absolute amounts of radioactive residues in mg/kg is limited to the 1 to 4 months untreated leaf samples from the soil and foliar application experiments. However, relative amounts in terms of percentage of applied radioactivity, as reported in the study, allow for an assessment of the relative uptake and distribution of ^{14}C-glyphosate or ^{14}C-AMPA after soil or hydroponic treatment and of ^{14}C-glyphosate after foliar treatment.
Unambiguous identification by ^{13}C-NMR and GC-MS was achieved for untreated leaf and fruit samples from plants treated foliar with ^{13}C/^{14}C-glyphosate for metabolite production. Residues in fruit from plants treated foliar with ^{14}C-glyphosate to study uptake were first characterised by ion exchange chromatography; glyphosate was identified in combined collected fractions by co-electrophoresis with authentic standard. No indications of AMPA were found. These experiments show that relevant transformations of the parent compound applied did not occur in untreated fruit or leaves after foliar treatment. Therefore, the study data allow for a qualitative assessment of the nature of the residue in citrus leaves and fruit after foliar treatment.
The amount of unextracted residues can be estimated from the radioactivity reported in the water extracts (87 – 98 % of the radioactivity in water extracts of leaves and 99 % in water extracts of organic solvent extracted fruit) to be approximately 13 % or less in leaves and approximately 1 % in fruit.

Overall information given in the report can be considered to estimate a theoretical maximum storage period to be not longer than 21 months. A storage stability study is available [redacted] 2012, CA 6.1/002) showing the stability of glyphosate and its metabolite AMPA in commodities with high acid content over a storage period of 24 months. No degradation of glyphosate and its metabolites was found in matrices with high water content, like corn forage, fodder, cotton forage, soybean forage. Over an investigated storage duration of 215-393 days no degradation was observed [redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/022). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1). Moreover, analysis of extracts showed that glyphosate was the major residue and thus degradation during storage was negligible. Total residues in orange commodities were determined by LSC as total ^{14}C-derived radioactivity which is expected to be stable during the course of the study.
Thus, although the study does not comply with current guideline requirements in major aspects, it still gives relevant and consistent qualitative information on the uptake and distribution of glyphosate-derived residues after soil, foliar, and hydroponic application and on the nature of the residues in leaves and fruit from citrus plants treated accidentally with glyphosate in a citrus orchard.
Therefore, this study is considered to be supportive / additional data for the assessment of the metabolic behavior of glyphosate in fruit crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/002
Report author	[redacted]
Report year	1987
Report title	The nature of the residue of SC-0224 in citrus
Report No	PMS-158R

Document No	VV-497772
Guidelines followed in study	Pesticide Assessment Guideline Number 171-4(a) of Subdivision O: Nature of the Residues in Plants
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Radiochemical purity of MS label shortly below 95 %; • LOD of LSC is not specified; • Physical facility and environmental conditions are poorly described.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 3a

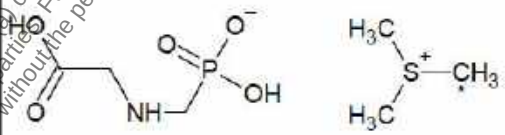
2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues, resulting from soil uptake after application of 3.918 kg glyphosate acid equivalents/ha to bare soil containing lemon trees. In total 5 lemon trees were used and maintained in large pots throughout the study: Two trees were treated with glyphosate radiolabelled on the TMS, or trimethylsulfonium, portion, and two were treated with glyphosate radiolabelled on the PMG, or phosphonomethylglycine, portion. The test substance consisted of an isotopic mixture of ^{12}C- and ^{14}C labelled glyphosate. The other tree remained as a control.
Aqueous solutions of the two treatments were applied to the soil around the base of the trees by spraying. The lemons, leaves of the trees, and soil around the trees were sampled directly after application, and two months and four months after treatment. The total radioactive residue (TRR) in mature lemons harvested four months after treatment was very low (≤0.01 mg/kg), although some residue was found in the leaves at that collection time. The average level of residue (expressed as PMG- or TMS-equivalents, depending on which portion was labelled) found in the mature lemons from ^{14}C-PMG-labelled glyphosate-trimesium treated trees ranged between 0.001 - 0.010 mg/kg PMG-equivalents, while that in mature lemons from ^{14}C-TMS-labelled glyphosate-trimesium treated trees ranged between 0.002 - 0.005 mg/kg TMS-equivalents. Due to the low level of residue in mature lemons, characterisation of metabolites was not pursued.

I. Materials and Methods

A. Materials

1. Test Material:	**1. N-(phosphono-^{14}C-methyl)glycine trimesium salt** (^{14}C-PMG-labelled glyphosate-trimesium) a) N-(phosphono-^{14}C-methyl)glycine (14.073 mg) b) N-(phosphono-^{12}C-methyl)glycine (76.35 mg) **2. N-(phosphonomethyl)glycine ^{14}C-trimesium salt** (^{14}C-TMS labelled glyphosate-trimesium) a) N-(phosphono-^{14}C-methyl)glycine (16.645 mg) b) N-(phosphono-^{12}C-methyl)glycine (73.948 mg)
Chemical structure:	1. ^{14}C-PMG-labelled glyphosate-trimesium  * Position of label 2. ^{14}C-TMS labelled glyphosate-trimesium  * Position of label
Radiochemical purity:	95.4 % (PMG label) 93.1 % (TMS label)

Specific activity:	^{14}C-PMG-labelled glyphosate-trimesium: 30 mCi/mM PMG label solution: 50 mL: 4.01 ×10^9 dpm (0.0574 mM or 14.0729 mg) 24 mL: 1.92 × 10^9 dpm/24 mL (actually applied) Specific activity of the treatment solution: 42,307 dpm/µg glyphosate equivalents 64,442 dpm/µg PMG equivalents ^{14}C-TMS labelled glyphosate-trimesium: 20 mCi/mM TMS label solution: 50 mL: 3.24 ×10^9 dpm (0.0679 mM or 16.6448 mg) 24 mL: 1.56 × 10^9 dpm/24 mL (actually applied) Specific activity of the treatment solution: 33,297 dpm/µg glyphosate equivalents 96,935 dpm/µg TMS-equivalents
CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate:	- 3.2, pH 7 at 25 °C (glyphosate)

2. Test system

Soil:	Supersoil® potting mix
Crop:	Lisbon lemon trees
Botanical name:	*Citrus limon*
Crop part(s):	Leaves, lemon (peel, pulp, juice)

B. Study design

1. In-life phase

For the investigation of the nature of residues of glyphosate in citrus, five Lisbon lemon trees were transplanted into plastic tubs, using Supersoil potting mix as backfill soil. The trees were immediately watered after transplantation and supplemented with Ortho Vitamin B-1 Plant Starter. Thereafter, the trees were supplemented with Romeo 18-18-18 soluble plant food at two- to four-week intervals. The soil treatment of the potted citrus plants was done with a hand pump sprayer to ensure even application of spray solution with a mixture of ^{12}C and ^{14}C glyphosate, labelled either in the phosphonomethyl-moiety or in the trimesium-moiety.

The treatment of the study was conducted in controlled environment growth chambers. For both labels, a rate equivalent to 3.918 kg glyphosate acid equivalents/ha was applied to the soil containing the lemon tree. Two potted lemon trees were used for each label and one lemon tree was the untreated control. After treatment, the plants were returned to the outdoor pen.

2. Sampling

Crop samples (lemons) were taken at immature states 3 days after treatment and 2 months after treatment. Mature lemons were collected 4 months after treatment. Leaf tissue was sampled as a comparison. The location of the lemon and leaf samples on the tree (i.e. "top", "middle" or "bottom" area of the branches) was noted.
All of the 3-day and one of the 2-month lemon samples were so small that no pulp had developed, so the lemons were ground whole and frozen under liquid nitrogen until analysis. The remaining lemons of the 2-month sample time and lemons of the 4-month sample time were cut in half, juiced and peels and pulps were separated. Only lemon samples weighing 41.6 g and more yielded juice, which was collected. Pulp remaining in the juicer was collected and added to the peel. The peel and pulp of each lemon were frozen separately using liquid nitrogen and pureed using a food processor. Aliquots of the samples were taken for combustion analysis. The volume of the juice was determined, and aliquots were analysed by LSC.

The soil was sampled by scooping several randomly located samples from the soil surface of the pots and combining the sample for each tree. Aliquots of a well-mixed soil sample were taken five times throughout the study and leachate was collected three times throughout the study.

The samples were stored frozen until analysis.

3. Analytical procedures

Total radioactive residues (TRR) in all plant and soil samples were determined by Liquid Scintillation Counting (LSC) following combustion.
Samples from control trees were used as background for all analyses. Amount of radiolabel in the lemons is reported as mg/kg 14C-PMG-equivalents or 14C-TMS-equivalents because it is not likely that the anion and cation stay together as a salt after application, uptake, and translocation.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in soil, leachate, citrus lemons and citrus leaves are summarised in the tables below.

Subsequent after treatment of the soils, the TRRs were the highest and amounted to 45.1 and 50.3 mg/kg for soil treated with ^{14}C-PMG-labelled glyphosate-trimesium and to 59.8 and 106.4 mg/kg for soil treated with ^{14}C-TMS-labelled glyphosate-trimesium. The expected rate for both treatments was between 4.07 and 4.20 kg/ha. The results of the soil analysis are considered rough estimates, and the 106.4 mg/kg value found for the ^{14}C-TMS-labelled glyphosate-trimesium treated soil must be due to a soil sample not taken to a deep enough depth.
However, throughout the study the data indicate that the amount of radiolabel on the soil surface decreases with time. By the end of the study, only an average of 7.1 mg/kg remained on the ^{14}C-PMG-labelled glyphosate-trimesium treated soils, while an average of 1.6 mg/kg remained on ^{14}C-TMS-labelled glyphosate-trimesium treated soils.

Very little radioactivity was recovered in the leachate throughout the study. The cumulative average total for the ^{14}C-PMG-labelled glyphosate-trimesium-treated trees was 1.31 %, and for the ^{14}C-TMS-labelled glyphosate-trimesium-treated trees was 0.65 % of the total applied radiolabel.

Lemons and leaves from the trees were sampled at 3 days, 2 months and 4 months after treatment. Early, immature samples were taken to determine whether the radiolabel was taken up quickly after treatment.
However, no significant difference or trend in the residue levels in the lemons (which were quite low) relative to the location from which the lemon sample was taken from the tree, was found. For leaf samples taken at 4 months after treatment, sample location (bottom, middle, or top of the tree/branch) did make a difference in the amount of residue seen, but the residues were not high.

The TRRs were the highest in leaf tissues and amounted in average to 0.003 mg/kg, 0.035 mg/kg and 0.030 mg/kg for samples at 3 days, 2 months and 4 months, respectively, of ^{14}C-PMG-labelled glyphosate-trimesium treated trees and to <0.001 mg/kg, 0.215 mg/kg and 0.037 mg/kg for samples at 3 days, 2 months and 4 months, respectively, of ^{14}C-TMS-labelled glyphosate-trimesium treated trees. This indicates that the labelled material was taken up by the trees. The leaf tissues were not analyzed further.

Immature whole lemons at 3 days after treatment had very low radioactivity amounting for 0.010 to 0.011 mg/kg in lemons of ^{14}C-PMG-labelled glyphosate-trimesium treated trees and for 0.006 to 0.016 mg/kg in lemons of ^{14}C-TMS-labelled glyphosate-trimesium treated trees.

For the ^{14}C-PMG-labelled glyphosate-trimesium treated trees, at 2 months, peel, pulp, and juice of three lemons were analysed for tree 1, while for tree 2 only two lemons could be separated into peel and pulp, and none of those provided juice for analysis. The average residues for the peel and pulp of the separated lemons were 0.006 and 0.009 mg/kg, respectively. One lemon was too small for separation into peel and pulp and hence was analysed as a whole, accounting for 0.019 mg/kg. Average residue in the juice of lemons from tree 1 at 2 months was 0.004 mg/kg. For the ^{14}C-TMS-labelled glyphosate-trimesium treated trees, at 2 months after treatment, average residues of 0.008 mg/kg, 0.007 mg/kg, and 0.004 mg/kg residue were found in the peel, pulp, and juice of lemons, respectively. These residue levels are equivalent to those in the ^{14}C-PMG-labelled glyphosate-trimesium treated trees, but the lemons were still quite immature.

Mature lemons were obtained at 4 months after treatment. The amount of residue was quite low, and between the two trees there was an average of 0.009 mg/kg in the peel, 0.010 mg/kg in the pulp, and only 0.001 mg/kg in the juice. For the ^{14}C-TMS-labelled glyphosate-trimesium treated trees, the residue levels of all mature lemon samples were very low (< 0.006 mg/kg). The average residue levels were 0.005 mg/kg, 0.003 mg/kg, and 0.002 mg/kg in the peel, pulp, and juice of the lemons, respectively.
The low residues in the mature lemons precluded further characterisation.

Table 6.2.1-7: Total radioactive residues in soils and leachate following soil application of ^{14}C-PMG and TMS-labelled glyphosate-trimesium treated lemon trees

	DAT	PMG-labelled glyphosate-trimesium treated lemon trees		TMS-labelled glyphosate-trimesium treated lemon trees	
		Soil[1]	Leachate[2]	Soil[1]	Leachate[2]
		mg/kg	% ^{14}C	mg/kg	% ^{14}C
Tree 1	0	50.318	---	106.449	---
	3	31.836	---	---	---
	10	25.218	---	38.66	---
	75	11.470	2.24	5.775	0.66
	111	---	0.10	---	0.09
	136	7.795	---	1.423	---
	144	---	0.06	---	0.03
Tree 2	0	45.098	---	59.812	---
	3	33.756	---	49.016	---
	10	33.577	---	27.378	---
	75	22.790	0.14	5.543	0.43
	111	---	0.06	---	0.14
	136	6.417	---	1.729	---
	144	---	0.12	---	0.03
Average	0	47.708	---	83.131	---

Table 6.2.1-7: Total radioactive residues in soils and leachate following soil application of ^{14}C-PMG and TMS-labelled glyphosate-trimesium treated lemon trees

	DAT	PMG-labelled glyphosate-trimesium treated lemon trees		TMS-labelled glyphosate-trimesium treated lemon trees	
		Soil[1]	Leachate[2]	Soil[1]	Leachate[2]
		mg/kg	% ^{14}C	mg/kg	% ^{14}C
	3	32.796	---	49.016	---
	10	29.397	---	33.019	---
	75	17.130	1.19	5.659	0.55
	111	---	0.08	---	0.12
	136	7.106	---	1.576	---
	144	---	0.09	---	0.03

DAT days after treatment
[1] For tree 1 and tree 2, the mg/kg of the soil sample represents the average from the combustion of 6 replicates of the composited soil sample from each tree.
[2] Leachate is presented as percent of the total ^{14}C applied.

Table 6.2.1-8: Total radioactive residues in lemons and leaves following soil application of ^{14}C-PMG-labelled glyphosate-trimesium treated lemon trees

Matrix		DAT		
		Day 3	Month 2	Month 4
		TRR (mg/kg)		
Tree 1	Whole lemon	0.010	---	---
	Peel	---	0.011	0.008
	Pulp	---	0.009	0.014
	Juice	---	0.004	0.001
	Leaves	<0.001	0.047	0.029
Tree 2	Whole lemon	0.011	0.019	---
	Peel	---	0.007	0.010
	Pulp	---	0.004	0.006
	Juice	---	---	0.002
	Leaves	0.006	0.023	0.031
Average	Whole lemon	0.010	0.019	---
	Peel	---	0.009	0.009
	Pulp	---	0.006	0.010
	Juice	---	0.004	0.001
	Leaves	0.003	0.035	0.030

Table 6.2.1-9: Total radioactive residues in lemons and leaves following soil application of ^{14}C-TMS-labelled glyphosate-trimesium treated lemon trees

Matrix		DAT		
		Day 3	Month 2	Month 4
		TRR (mg/kg)		
Tree 1	Whole lemon	0.016	---	---
	Peel	---	0.009	0.005
	Pulp	---	0.006	0.005
	Juice	---	0.004	0.002
	Leaves	<0.001	0.303	0.041
Tree 2	Whole lemon	0.006	---	---
	Peel	---	0.007	0.006
	Pulp	---	0.007	<0.001
	Juice	---	0.005	0.002
	Leaves	<0.001	0.127	0.033
Average	Whole lemon	0.011	---	---
	Peel	---	0.008	0.005
	Pulp	---	0.007	0.003
	Juice	---	0.004	0.002
	Leaves	<0.001	0.215	0.037

B. Extraction and characterisation of residues

Due to the very low radioactive residues obtained in the peel, pulp, and juice of mature lemons, no further extraction/ characterisation of the residues was performed in this study.

C. Storage stability

All samples of this study were taken and analysed within 6 months after treatment. Mature samples of lemons were harvested 144 days after treatment and were analysed within less than two months.
Hence, no storage stability analysis was performed.

III. Conclusions

The nature and magnitude of residues resulting from soil uptake was investigated after application of 3.98 kg glyphosate acid equivalents/ha to the bare soil around established lemon trees. Lemon fruit were collected in an immature state (three days after treatment and two months after treatment) and at a mature state at normal harvest (four months after treatment).

The average total radioactive residue (TRR) in mature lemons after soil treatment with ^{14}C-PMG-labelled glyphosate-trimesium amounted to 0.009 mg/kg PMG-equivalents in the peel, 0.010 mg/kg in the pulp, and only 0.001 mg/kg in the juice. Mature lemons harvested four months after treatment with ^{14}C-TMS-labelled glyphosate-trimesium had an average of 0.005 mg/kg TMS-equivalents in the peel, 0.003 mg/kg in the pulp, and 0.002 mg/kg in the juice.

In conclusion, the results from this study show that glyphosate and its residues do not accumulate to any appreciable extent in the mature fruit of lemon trees after the herbicide is applied under simulated field conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behavior of glyphosate in citrus has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with minor deficit: Radiochemical purity of TMS-labelled glyphosate-trimesium is slighty below 95 %, but as the deviation is very minor and no identification was done within this study, the reduced purity does not affect the quality of the study. Physical facility and environmental conditions are poorly described. However, the plants appear to be healthy throughout the study and thus no negative effect is expected. The limit of detection (LOD) of LSC measurements is not specified. It is assumed that the LOD was sufficient. Storage stability data is not provided within the report, but from the data provided (treatment on 30-11-1984; lab work completed May 1985) it can be calculated that all samples were taken and analyzed within 6 month.

Therefore, the study is considered reliable for the assessment of the metabolic behavior of glyphosate in citrus plants and in the whole group of fruits.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/003
Report author	[redacted]
Report year	1976
Report title	Absorption, translocation, and metabolism of Roundup® herbicide in walnut, almond, and pecan trees
Report No	403
Document No	Not available
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • No information of the storage stability for all major components of the total radioactive residues • No description of conditions and length of storage of samples • Application rate for foliar treatment is given as total amount of radioactivity applied per plant or per leaf and not as kg a.s./ha. • Edible commodity nuts have not been sampled and investigated. • Radioactive residues in RAC are expressed in % of applied activity. Recalculation in mg/kg expressed in glyphosate equivalents is not possible based on the info given in the report. For foliar and soil treatment the residues of glyphosate and metabolites found are expressed as % of extracted radioactivity. For this dossier the values were recalculated in % of TRR. • Residues after solvent extraction (RRR) were not further measured or examined (the RRRs were calculated assuming total equal to 100 % and that there were no losses during extraction and purification). • No full accountability reported.

	• For some matrices less than 90 % of TRR was identified and characterised
Previous evaluation	Yes, accepted the RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this study the uptake and metabolism of ^{14}C-glyphosate following soil treatment or foliar application to tree nut plants (almond, pecan and walnut) was investigated.

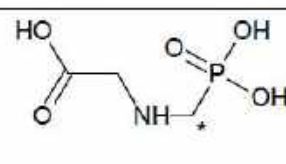
In the soil experiment ^{14}C-glyphosate was applied as an aqueous solution at the rate of 5.07 kg a.s./ha for pecan and walnut and at the rate of 2.43 kg a.s./ha for almonds. In the foliar application experiments, ^{14}C-glyphosate was formulated to simulate the commercial Roundup® formulation and applied at the rate of 100 µg ^{14}C-glyphosate (1.2×10 dpm^7) to the leaf surface of two trees per variety. Samples were collected after 16 weeks (113 days) for the soil treatment and after 14 days (walnut) and/or 35 days (walnut, almond and pecan) for the foliar treatment. Trees were separated from soil and the roots were washed with water.

After foliar treatment of single plants most of the radioactivity applied was located on the treated leaves. The translocation into untreated plant parts (roots, other tops) was minor. The results of the first foliar experiment with walnut, almond and pecan are difficult to interpret because of the residues in controls (artefact during the simultaneous conduction of the foliar and soil experiments in one cubicle). The results of the second foliar experiment with walnut only (DALT 14, in a cubicle that was not being used with any other 14C experiment; no significant residues in controls) indicate that these nut trees only slowly metabolize ^{14}C-glyphosate (81.6 to 94.8 % TRR), and the only recognizable product is ^{14}C-AMPA (<3 % TRR) in all tree commodities tested.

The soil application experiments yielded low residues in comparison to radioactivity applied, demonstrating low plant uptake of ^{14}C-glyphosate from soil.

I. Materials and methods

A. Materials

Test Material:	N-(phosphonomethyl-^{14}C)-glycine
Chemical structure:	 * Position of radiolabel
Radiochemical purity:	98-99 % [1]
Specific activity:	1.98 MBq/mg (9.07 mCi/mmol) for foliar application 0.41 MBq/mg (1.87 mCi/mmol) for soil application

CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate:	- 3.2

[1] prior to use the samples were re-purified by ion-exchange chromatography on AG-50-XS (H^+ form). The resulting materials were 98-99 % pure, the 1-2 % impurity being an unknown substance(s) which adheres to AG-1 (HCO_3^- form) resin until 0.4 M NH_4HCO_3 is used as eluant.

Test system:

Soil:	Ray silt loam
Crop:	Walnut Almond Pecan
Botanical name:	*Jurglans carpathian, Prunus amygdalus, Carya Illinoisis,* respectively
Crop part(s):	Leaves, other tops, tops, roots

B. Study design

1. In-life phase

In this study the uptake and metabolism of ^{14}C-glyphosate following soil treatment or foliar application to tree nut plants (almond, pecan and walnut) was investigated.
Walnut and pecan trees (50 cm height) and almond trees (1-1.5 cm height) were planted in individual containers.
In the soil experiment 12.9 mg ^{14}C-glyphosate (as an aqueous solution) were applied to the surface of pots (18 cm diameter for pecan and walnut, 26 cm diameter for almonds). Thus, the application rate corresponds to 5.07 kg a.s./ha for pecan and walnut and to 2.43 a.s kg/ha for almonds.
For the foliar application experiments, ^{14}C-glyphosate solutions were prepared which simulated the commercial Roundup® formulation, as follows: pure, neat ^{14}C-glyphosate 1.98 mBq/mg was dissolved in a solution made by combining water, isopropylamine, and G 3780A surfactant. Radioassay of the solution showed that each 225 µl contained 100 µg, 1.2 x 10 ^{7} dpm, of ^{14}C-glyphosate. For the foliar treatment an amount of 100 µg ^{14}C-glyphosate (1.2 × 10 dpm^7) was applied to the leaf surface of two trees per variety. It has to be noted, that the plants were grown in the same greenhouse cubicle that was used for the soil application experiment (foliar experiment 1).As the control tree plants contained a significantly high degree of radioactivity the foliar experiment was repeated in walnut trees in a cubicle that was not being used for any other C^{14} experiment (foliar experiment 2).

2. Sampling

Samples were collected after 16 weeks (113 days) for the soil treatment and after 35 days (walnut, almond and pecan) and/or 14 days (walnut) for the foliar treatments 1 and 2, respectively. The trees were separated from soil and the roots were washed with water. The trees were dissected in roots, treated leaves (for foliar treatment) and (other) tops by means of pruning shears and the samples were frozen, lyophilised, and ground.

3. Analytical procedures

Dry plant powders were quantitatively oxidised for total radioactivity determination. The quantification and identification following aqueous extraction was performed by liquid scintillation counting (LSC).

The samples were extracted three times with water at room temperature for 30 minutes; the aqueous extract aliquots were subjected to ion-exchange chromatography (both cation and anion), with subsequent

high voltage electrophoresis (HVE). Duplicate root samples from treated walnut trees of the second foliar application experiment were combined and extracted three times with water, and the extract was refined by anion followed by cation exchange chromatography. A portion of the radioactive residue was derivatised by successive treatment with trifluoroacetic acid/trifluoroacetic anhydride and ethereal diazomethane, and the derivative was purified on a micro column of silica gel.

For confirmation purposes the aliquots of interest were analysed by GC/MS.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residues were determined for each of the two treated trees individually. After the foliar application (35 DALT) the highest residues were found in treated leaves 47.7 - 71.3 % AR. The residues in roots and in other tops were significantly lower and ranged from 3.3 – 27.4 and 4.2 – 14.5 % AR, respectively.

Similarly, residues in walnut at 14 DALT were within the same range compared to the residues in walnut at 35 DALT and amounted to 60.5 and 45.0 % AR in treated leaves, 10.4 and 23.5 % AR in roots and 7.3 and 6.2 % AR in other tops.

For foliar treatment control tree parts contained a significantly high degree of radioactive residues. This was undoubtedly since these plants were grown in the same greenhouse cubicle that was currently housing the soil application experiment (discussed below). Much of the relatively high amount of ^{14}C-glyhosate that was applied to the soil surface was microbially degraded to $^{14}CO_2$ which was then available for photofixation by all the plants in the cubicle. This complicated interpretation of the results and therefore the foliar application experiment was repeated, although only on walnut trees (DALT 14), in a cubicle that was not used for any other experiment. As expected, the residues in controls within the second experiment were significantly lower 0.01 to 0.04 % AR) in comparison to 1.5 – 9.7 % AR in the first experiment (DALT 35).

Table 6.2.1-10: Total radioactive residues in tree nut plants following foliar treatment 35 and 14 days before sampling

Sample description	Days after treatment (DALT)	Tree 1	Tree 2
		% AR[2]	% AR[2]
Walnut, treated leaves	35	64.5	47.7
Walnut, other tops	35	5.8	8.2
Walnut tops (control)	35	(2.9)	-
Walnut, roots	35	27.4	14.8
Walnut, roots (control)	35	(4.4)	-
Walnut, treated leaves [1]	14	60.5	45.0
Walnut, other tops [1]	14	7.3	6.2
Walnut tops (control) [1]	14	(0.02)	(0.04)
Walnut, roots [1]	14	10.4	23.5
Walnut roots (control) 1[1]	14	(0.01)	(0.03)
Almond, treated leaves	35	71.3	61.1
Almond, other tops	35	14.5	12.6
Almond, tops (control)	35	(9.7)	-
Almond, roots	35	6.6	8.2
Almond, roots (control)	35	(2.6)	-
Pecan, treated leaves	35	54.0	53.1
Pecan, other tops	35	10.0	4.2
Pecan, tops (control)	35	(3.1)	-
Pecan, roots	35	16.6	3.3
Pecan, roots (control)	35	(1.5)	-

[1] For walnut the experiment was repeated in a cubicle that was not used by any other ^{14}C experiment. All other samples are from the first experiment, where plants from foliar treatment were grown in the same greenhouse cubicle that was used for the soil application experiment.
[2] The TRRs are given in the report as % applied radioactivity (% AR).
In brackets results of control experiments are given. For the control experiments the leaves and tops were sampled together representing sample material tops.
The TRR was determined after direct combustion.

After the soil treatment only 0.08 to 0.29 % of applied radioactivity was found in treetops and roots. Therefore, the further investigation was intended. The high residues in control tree parts are due to microbial degradation of ^{14}C-glyphosate applied to soil to $^{14}CO_2$ which was then available for photofixation by all the plants.

Table 6.2.1-11: Total radioactive residues in tree nut plants following soil treatment 113 days before sampling

Sample description	Days after treatment (DALT)	Tree 1	Tree 2
		% AR	% AR
Walnut, tops	113	0.13	0.14
Walnut, tops (control)	113	(0.09)	(0.05)
Walnut, roots	113	0.28	0.19
Walnut, roots (control)	113	(0.16)	(0.07)
Almond, tops	113	0.23	0.29
Almond, tops (control)	113	(0.29)	(0.29)
Almond, roots	113	0.14	0.08
Almond, roots (control)	113	(0.14)	(0.14)
Pecan, tops	113	0.36	0.19
Pecan, tops (control)	113	(0.07)	(0.07)
Pecan, roots	113	0.25	0.27
Pecan, roots (control)	113	(0.04)	(0.10)

The TRRs are given in the report as % of applied radioactivity (% AR)
The TRR was determined after direct combustion.

B. Extraction and characterisation of residues

The first foliar experiment yielded low extraction rates for some samples: after extraction with water about 54 to 82 % of TRR could be extracted from leaves, 26 to 70 % of TRR from other tops and 48 to 79 % of TRR from roots. During the second experiment (DALT 14) with walnut trees significantly higher extraction rates were achieved: 100 % of TRR could be extracted with water treated leaves, 85 % of TRR for other tops and 88 % for roots. Ion exchange chromatography and HVE of the extracts from the treated tree parts of the first foliar experiment, showed that the dominant radioactive species is unchanged ^{14}C-glyphosate, but significant radioactivity (1.2-6.56 % of TRR) occurred in the zones of ^{14}C-AMPA. Here again the results were clouded by the fact that control extracts contained relatively high ^{14}C-activity due to photofixation of $^{14}CO_2$, and in some cases much of this activity was to be found in the HVE zone of ^{14}C-glyphosate. Such was not a problem in the second foliar experiment where control trees contained no significant radioactivity. Here treated tree part extracts had 81.6 - 94.76 % of TRR in the ^{14}C-glyphosate zones, 1.70 - 3.09 % of the TRR in the ^{14}C-AMPA zones, and the rest in unidentifiable residue which had chromatographic properties identical to a 1 - 2 % impurity in the starting ^{14}C-glyphosate. Thus, it is apparent that these nut trees only slowly metabolize ^{14}C-glyphosate, and the only recognizable product is ^{14}C-AMPA. The identity of ^{14}C-glyphosate in the translocated material (walnut roots) was confirmed by derivatisation to ^{14}C-glyphosate-N-trifluoroacetyl-trimethyl ester and subsequent GC/MS analysis.

Table 6.2.1-12: Extracted radioactive residues in tree nut plants following foliar treatment 35 and 14 days before sampling

Sample description	**Days after treatment (DALT)**	**% TRR, extracted**
Walnut, treated leaves	35	82
Walnut, other tops	35	26
Walnut, roots	35	48
Walnut, treated leaves [1]	14	103
Walnut, other tops [1]	14	85
Walnut, roots [1]	14	88
Almond, treated leaves	35	54
Almond, other tops	35	26
Almond, roots	35	79
Pecan, treated leaves	35	72
Pecan, other tops	35	70
Pecan, roots	35	63

[1] For walnut the experiment was repeated (14 DALT). All other samples are from the first experiment (35 DALT).
Walnut 14 days: mean of tree 1 and tree 2 was calculated; for all crops taken after 35 days tree 1 and tree 2 corresponding parts were combined prior extraction.

Table 6.2.1-13: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in walnut trees following foliar application of glyphosate

	Walnut, treated leaves, % TRR	**Walnut, other tops, % TRR**	**Walnut, roots, % TRR**	**Walnut, treated leaves, % TRR**	**Walnut, other tops, % TRR**	**Walnut, roots , % TRR**
Days after treatment (DALT)	**35**	**35**	**35**	**14**	**14**	**14**
TRR	**100**	**100**	**100**	**100**	**100**	**100**
Parent (Glyphosate)	*63.22* (77.1)	*18.28* (70.3)	*41.76* (87.0)	*94.76* (92.0)	*81.60* (96.0)	*85.36* (97.0)
Metabolite (AMPA)	*6.56* (8.0)	*1.20* (4.6)	*1.92* (4.0)	*3.09* (3.0)	*1.70* (2.0)	*1.76* (2.0)
Total identified	***69.78*** **(85.1)**	***19.48*** **(74.9)**	***42.68*** **(91.0)**	***97.85*** **(95.0)**	***83.30*** **(98.0)**	***87.12*** **(99.0)**
Other	*7.38* (9.0)	-	-	*5.15* (5.0)	*0.85* (1.0)	*0.88* (1.0)
Neutral	-	-	-	-	-	-
Total character-rised	***7.38*** **(9.0)**	**-**	**-**	***5.15*** **(5.0)**	***0.85*** **(1.0)**	***0.88*** **(1.0)**
Non-retarded	*1.64* (2.0)	*6.79* (26.1)	*2.02* (4.2)	*1.03* (1.0)	*1.70* (2.0)	*0.88* (1.0)
ERR	**82**	**26**	**48**	**103**	**85**	**88**
RRR	***18***	***74***	***52***	***0***	***15***	***12***
Total sum	***100***	***100***	***100***	***103***	***100***	***100***

In brackets residues expressed as % of extracted radioactivity are given.
Values calculated upon dossier compilation and are given in *italics*.
DALT days after treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

Table 6.2.1-14: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in almond and pecan trees following foliar application of glyphosate

	Almond, treated leaves, % TRR	Almond, other tops, % TRR	Almond, roots, % TRR	Pecan, treated leaves, % TRR	Pecan, other tops, % TRR	Pecan, roots, % TRR
Days after treatment (DALT)	**35**	**35**	**35**	**35**	**35**	**35**
TRR	**100**	**100**	**100**	**100**	**100**	**100**
Parent (Glyphosate)	*41.58* (77.0)	*13.70* (52.7)	*62.65* (79.3)	*62.06* (86.2)	*61.74* (88.2)	*59.85* (95.0)
Metabolite (AMPA)	*4.32* (8.0)	*2.47* (9.5)	*4.66* (5.9)	*4.32* (6.0)	*1.4* (2.0)	-
Total identified	*45.90 (85.0)*	*16.17 (62.3)*	*67.31 (85.2)*	*66.38 (92.2)*	*63.14 (90.2)*	*59.85 (95.0)*
Neutral	n.a.	*1.23* (4.8)	-	n.a.	-	-
Other	*5.45* (10.1)	-	-	*4.54* (6.3)	-	-
Total character-rised	*5.45 (10.1)*	*1.23 (4.8)*	-	*4.54 (6.3)*	-	-
Non- retarded	0.59 (1.1)	9.83 (37.8)	9.24 (11.7)	0.72 (1.0)	6.37 (9.1)	1.01 (1.6)
ERR	**54**	**26**	**79**	**72**	**70**	**63**
RRR	***46***	***74***	***21***	***28***	***30***	***37***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***

In brackets residues expressed as % of extracted radioactivity are given.
Values calculated upon dossier compilation and are given in *italics*.
DALT days after treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
n.a. not applicable

C. Storage stability

Storage period is not specified within the study, the dates of sampling are 3rd July 1975, 13th October 1975 and 18th September 1975 for the first and second foliar experiments as well as for soil experiment, respectively. The study finalisation was in April 1976. Thus, as the worst case assumption, the storage time would be maximum 302 days (10 months). It has to be kept in mind that this is likely to be an overestimation. Within the study various water-rich matrices (leaves, tops and roots) were investigated. Storage stability of frozen samples of high water content has been shown in carrot tops for 15 months (McMullan_MSL_9810).

Therefore- the storage stability is covered.

D. Degradation pathway

Please refer to the pathway of glyphosate presented further below.

III. Conclusion

After foliar treatment of single plants most of the radioactivity applied was located on the treated leaves. The translocation into untreated plant parts (roots, other tops) was minor.

The results of the first foliar experiment with walnut, almond and pecan are difficult to interpret because of the residues in controls (artefact during the simultaneous conduction of the foliar and soil experiments in one cubicle). The results of the second foliar experiment with walnut only (DALT 14, in a cubicle that was not being used with any other ^{14}C experiment; no significant residues in controls) indicate that that nut trees only slowly metabolize ^{14}C-glyhosate (which represents 81.6 to 94.8 % TRR), and the only recognizable product is ^{14}C-AMPA (<3 % TRR) in all tree commodities tested.

Soil application experiment yielded low residues in comparison to radioactivity applied, demonstrating low plant uptake of ^{14}C-glyhosate from soil.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in tree nuts has been previously evaluated at EU level. It was not performed under GLP (GLP not established at the testing facility in 1976). The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with certain deficits:

- No information of the storage stability for all major components of the total radioactive residues; no description of conditions and length of storage of samples
- Edible commodity nuts have not been sampled and investigated.
- Radioactive residues in RAC are expressed in % of applied activity. Recalculation in mg/kg expressed in glyphosate equivalents is not possible based on the data given in the report. For foliar and soil treatment the residues of glyphosate and metabolites found are expressed as % of extracted radioactivity. For this dossier the values were recalculated in % of TRR. It was not possible to express residues as mg/kg glyphosate equivalents.
- Residues after solvent extraction (RRR) were not further measured or examined (the RRRs were calculated assuming total equal to 100 % and that there were no losses during extraction and purification).
- No full accountability reported.
- For some matrices less than 90 % of TRR was identified and characterised

Storage period is not specified within the study, the dates of sampling are 3rd July 1975, 13th October 1975 and 18th September 1975 for the first and second foliar experiments as well as for soil experiment, respectively. The study finalisation was in April 1976. Thus, as the worst case assumption, the storage time would be maximum 302 days (10 months). It has to be kept in mind that this is likely to be an overestimation. Within the study various water-rich matrices (leaves, tops and roots) were investigated. Storage stability of frozen samples of high water content has been shown in carrot tops for 15 months (McMullan_MSL_9810). Therefore, the storage stability is covered.

Despite the deficits listed above the study provides data on the distribution of glyphosate-derived radioactivity within the tree nuts plants and on the metabolism in leaves, other tops and roots. The study is considered to be supportive for the assessment of the metabolic behaviour of glyphosate in tree nuts.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/004
Report author	[redacted]
Report year	1974
Report title	CP 67573 residue and metabolism Part 23: The metabolism of CP 67573 in apple trees
Report No	342
Document No	M-649026-01-1
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Physical facility and environmental conditions insufficiently described. • No samples of RAC (apple fruit, edible commodity) were taken. • Radioactive residues in RAC are expressed in % of applied activity rather than in terms of TRR. Recalculation is only possible for foliar treatment experiments. • Recoveries of radioactivity in aqueous extract of foliar treated apple trees were below 90 % for leaves and stem (new growth above) at 7 days (87.94 %), leaves and stem (other new growth) at 21 (88.98 %) and 28 days (71.91 %), roots and trunk / branches at 28 days (71.51 and 79.47 %) • Unextracted radiolabel for each sample not precisely quantified. Overall percentages of total unextracted radioactivity in apple commodities are reported to be approximately 5 % in leaves and stem. Sampling time (DALT) and samples analysed referring to this are not specified. • No release and characterisation and/or identification was attempted on the non-aqueous extractable radioactive residue. • No full accountability reported. • No flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed. • No details on radioactive counting data. • No calculations or data for sample and reference R_f values on TLC. • No photographs or images of TLC plates critical to the identification. • No flowchart of metabolic pathway included in report • No description of length of storage of samples.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this study the uptake and metabolism of ^{14}C-labelled N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) and ^{14}C-labelled aminomethylphosphonic acid (^{14}C-AMPA) in dwarf Golden Delicious apple trees were investigated following soil, foliar or trunk application.

Soil treatments were performed with ^{14}C-glyphosate at a rate corresponding to 3.36 kg glyphosate/ha or with ^{14}C-AMPA at a rate corresponding to 1.68 kg amino-methylphosphonic acid/ha. For trunk uptake, 92.4 µg N-(phosphono-^{14}C-methyl)glycine/ tree was applied to the dwarfing section of the trunk of each apple tree. Foliar application was performed by applying approximately 10 µg N-(phosphono-^{14}C-methyl)glycine/leaf to selected leaves of a given tree.

The uptake of ^{14}C-glyphosate or ^{14}C-AMPA was very low after soil treatment with a maximum total uptake of 0.134 % of the applied radioactivity 12 weeks after treatment with N-(phosphono-^{14}C-methyl)glycine. Moreover, the untreated control samples grown as controls in the greenhouse with the treated pots also contained a considerable amount of ^{14}C, as compared to the treated samples.

After trunk treatment, uptake and translocation was minimal with 0.08 % of the applied activity recovered in leaves and stems and untreated trunk. 0.1 % of the applied radioactivity was recovered in roots, while 72.05 % of the applied radioactivity were found in treated trunk.

No calculation of the total radioactive residue was possible from the data provided in the report for soil and trunk treatment.

After foliar treatment at approximately 10 µg/ leaf, TRRs in treated leaves ranged between 98.06 and 144.3 mg/kg during the course of the study.

Foliar applied ^{14}C-glyphosate in formulation was rapidly and efficiently transported throughout the apple tree from the treated leaves. The greatest amount was observed in the growing stem and leaves immediately above the treatment. Significant amounts of compound could also be found in other new growth, trunk and roots.

In new growth above treatment, 1.075 – 2.052 mg/kg, and in other new growth 0.387 – 1.123 mg/kg were found, respectively. Branches and trunk contained a TRR of 0.022 mg/kg, while in roots 0.041 mg/kg were detected.

Extractabilities in apple tree commodities after foliar treatment ranged from 71.51 % of the TRR (0.029 mg/kg) in roots and 79.47 % (0.018 mg/kg) in trunk and branches, respectively, at 28 days after application, to 102.59 % TRR in new growth above at day 21. The aqueous extractability of the stem and leaf samples showed no significant pattern of change with time.

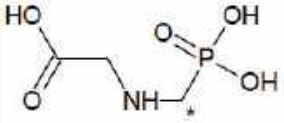
Chromatographic analysis of the aqueous extracts showed that the major residue in treated leaves, new growth above the treatment, other new growth, roots, and trunk of the apple trees was parent glyphosate, at amounts of 83.85 – 96.09 % TRR (94.23 – 128.1 mg/kg), 85.91 – 101.3 % TRR (1.021 – 1.842 mg/kg), 66.82 – 95.08 % TRR (0.346 - 0.980 mg/kg), 66.39 % TRR (0.027 mg/kg) and 64.43 % TRR (0.014 mg/kg), respectively.

A maximum of 6.45 % TRR of the ^{14}C-activity taken up by the apple trees behaved in a manner chromatographically identical to aminomethylphosphonic acid/N-methyl-aminomethylphosphonic acid. No other metabolites were identified.

I. Materials and Methods

A. Materials

Test Material:	a) N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) b) N-(phosphono-^{13}C-methyl)glycine (^{13}C-glyphosate) c) Amino-^{14}C-methylphosphonic acid (^{14}C-AMPA)

Chemical structure:	 * Position of radiolabel
Radiochemical purity:	a) for trunk and large scale foliar uptake experiment: 94.8 % with the presence of 3.7 % aminomethylphosphonic acid and 1.5 % N-methyl- aminomethylphosphonic acid; purified before application, radiochemical purity after purification not stated for trunk and preliminary foliar uptake experiment: 98.9 % with 0.7 % aminomethylphosphonic acid and 0.4 % $CH_3PO_3H_2$ present after purification for soil application experiment: 98.6 % c) for soil application experiment: 97 %
Specific activity:	a) N-(phosphono-^{14}C-methyl)glycine: for trunk and large scale foliar uptake experiment: 9.07 mCi/mmol (1.98 MBq/mg). for trunk and preliminary foliar uptake experiment: 8.03 mCi/mmol (1.76 MBq/mg), specific activity counted 8.06 mCi/mmol (1.78 MBq/mg)[1]. for soil application experiment: 1.87 mCi/mmol (0.41 MBq/mg) c) for soil application experiment: 8.90 mCi/mmol (2.96 MBq/mg)
CAS No:	Glyphosate: 1071-83-6 AMPA: 1066-51-9
Log $P_{o/w}$:	Glyphosate: -3.2 AMPA: -2.47

[1]During the first year after synthesis TLC and Beta Camera analysis indicated a radiochemical purity of 96.0 % with the presence of 3.3 and 0.6 % of AMPA and N-methyl AMPA, respectively. After storage for one year it was found that the solid material had decomposed partially so that its composition was 89.9 % glyphosate, 6.9 % AMPA and 1.7 % N-methyl AMPA. Column chromatographic purification was used to remove the latter two impurities.

Test system:

Crop:	Apple (Golden Delicious, dwarf)
Botanical name:	*Malus domestica*
Soil:	Ray silt loam (1.0 % organic matter, 0.6 % clay, 82.3 % silt, 6.0 % sand, pH 6.5) Drummer silty clay loam (6 %organic matter, 36.8 % clay, 55.4 % silt, 2.0 % sand, pH 7.0)
Crop part(s):	Leaves, branches, stems, trunk, roots

B. Study design

1. In-life phase

In this study the uptake and metabolism of ^{14}C-labelled glyphosate and ^{14}C-labelled aminomethylphosphonic acid in apple trees were investigated following soil, foliar or trunk application.
Dormant apple trees were planted into drained buckets of approximately 19 litres volume in either Ray silt loam or Drummer silty clay loam soil. The trees were watered as necessary daily and periodically supplemented with dilute Hoaglands nutrient solution. The trees were grown at a temperature of approximately 24 – 29 °C.

Soil uptake experiment:
Six buckets, each planted with one apple tree in Drummer silty clay loam were utilised for the soil uptake study six weeks after the end of dormancy. Two pots were treated on the soil surface with 12.5 mL of the ^{14}C-glyphosate treatment solution (^{14}C-glyphosate and unlabelled glyphosate at a ratio of 1.2:1 (w/w) in 0.1 N NH_4HCO_3), corresponding to an application rate of 3.36 kg glyphosate/ha. The radioactivity applied was 288850000 dpm (4.81 MBq), corresponding to 21.45 mg glyphosate.

Two additional pots were treated on the soil surface with 5 mL of the ^{14}C-AMPA treatment solution (^{14}C-AMPA and unlabelled aminomethylphosphonic acid at a ratio of 0.18:1 (w/w) in 0.1 N NH_4HCO_3), corresponding to an application rate of 1.68 kg/ha. The radioactivity applied was 296272000 dpm (4.94 MBq), corresponding to 10.67 mg amino-^{14}C-methyl-phosphonic acid. Two additional pots were left untreated and were grown as controls in the greenhouse with the treated pots. The pots were watered twice daily from the top for the duration of the experiment.
Trunk uptake experiment:
Two pots each planted with one apple tree in Ray silt loam were used for the trunk uptake study. 330 µL of the trunk treatment solution (0.5 mL (280 µg) ^{14}C-glyphosate stock solution in 0.1 M NH_4HCO_3 mixed with 25 mg isopropylamine, 50 mg G3780A surfactant and 14.7 mg unlabelled N-(phosphonomethyl)glycine) was applied to the dwarfing section of the trunk of each dwarf Golden Delicious apple tree. The amount of ^{14}C-glyphosate applied was 92.4 µg/tree or 9514880 dpm/tree (0.16 MBq/tree). The treated trunk section was shielded by wrapping an inverted plastic pot around the treated area to prevent splashing of the area during watering.

Foliar uptake experiments:
For the preliminary foliar uptake study two pots each containing one tree in Ray silt loam were treated at approximately six weeks after the end of dormancy. Both surfaces of four adjacent leaves of a new growth were treated with 10 µL (5 µg) ^{14}C-glyphosate in formulation (17.79 mL (10.0 mg) ^{14}C-glyphosate stock solution in 0.1 M NH_4HCO_3 mixed with 241 µL of 5 % (v/v) isopropylamine in water, 241 µL of 5 % (w/v) G3780A surfactant in water and water *ad* 20 mL). The solution was applied to the leaf surface with a syringe (1024000 dpm/leaf or 0.02 MBq/leaf). Five or six new growths were treated on each tree. Within one week stunting was observed in the immature leaves at the growing tip of those stems containing treated leaves. No significant phytotoxic symptoms were observed on the remaining stems or leaves.

The large scale foliar uptake study was done with thirty-two containers each containing one tree. The application was made five weeks after the end of dormancy. At the time of treatment each tree had three or more shoots, each of which contained a minimum of 4 or 5 leaves in various stages of development. For each tree three to six shoots were selected and foliar treatment was carried out on the four most fully developed adjacent leaves. Each side of each leaf was treated with 10 µL of formulated ^{14}C-glyphosate (8.52 mL (7.5 mg) ^{14}C-glyphosate stock solution mixed with 181 µL of 5 % (v/v) isopropylamine in water, 181 µL of 5 % (w/v) G3780A surfactant in water and water *ad* 15 mL) applied with a syringe. The radioactivity applied was 609000 dpm (0.01 MBq), corresponding to 5.356 µg N-(phosphono-^{14}C-methyl)glycine. Stunting of the immature leaves at the growing tip was observed on those stems containing treated leaves; no other phytotoxic symptoms were apparent on the remaining leaves and

stems. It was observed that the stunting effect was evident on only those leaves which had begun to unfold at the time of treatment; leaves which appeared later were not stunted.

The different experiments are summarised in the following table:

Table 6.2.1-15: Overview on soil, foliar or trunk application experiments in apple

Experiment	Duration of the experiment	Sampling
Soil application experiments		
2 apple trees in Drummer silty clay loam, 21.45 mg ^{14}C-glyphosate (288850000 dpm (4.81 MBq)), equivalent to 3.36 kg/ha	12 weeks	6 weeks (1 tree) 12 weeks (1 tree)
2 apple trees in Drummer silty clay loam, 10.67 mg ^{14}C-AMPA (296272000 dpm (4.94 MBq)), equivalent to 1.68 kg/ha	12 weeks	6 weeks (1 tree) 12 weeks (1 tree)
Trunk application experiments		
2 apple trees in Ray silt loam, 92.4 µg/tree ^{14}C-glyphosate (9514880 dpm/tree (0.16 MBq/tree))	42 days	8 days (1 tree) 42 days (1 tree)
Foliar uptake experiments		
Preliminary: 2 apple trees in Ray silt loam, 10 µg/leaf ^{14}C-glyphosate (1024000 dpm/leaf (0.02 MBq/leaf))	21 days	7 days (1 tree) 21 days (1 tree)
Large scale: 32 apple trees, 10.712 µg/leaf ^{14}C-glyphosate (1218000 dpm/leaf (0.02 MBq/leaf))	10 weeks	4 weeks (2 and 24 trees) 7 weeks (2 trees) 10 weeks (2 trees)

2. Sampling

The sampling time schedule for each experiment is listed in the table above.

<u>Soil uptake experiment:</u>
At 6 and 12 weeks the trees were harvested with the trunk cut off approximately 7.6 cm above the soil surface. At harvest the leaves and the stems were weighed as were the trunks and branches, after which the samples were frozen, lyophilised, the dry weight determined, ground to 40 mesh in a Wiley Mill and aliquots combusted to determine the total ^{14}C content.

<u>Trunk uptake experiment:</u>
At 8 and 42 days a tree was harvested and separated into four categories: leaves and stems, treated trunk, untreated trunk and branches and roots. The fresh weight was recorded for each sample and the samples were frozen, lyophilised, the dry weight determined, ground to 40 mesh with a Wiley Mill and aliquots combusted to determine the total ^{14}C content.

<u>Foliar uptake experiments:</u>
At 7 and 21 days a tree from the preliminary uptake study was harvested and separated into the following categories: treated leaves, new growth above treatment, other new growth, branches and trunk, and roots. At harvest the wet weight was determined, after which the sample was frozen, lyophilised, the dry weight determined, ground to 40 mesh in a Wiley Mill and aliquots combusted to determine the total ^{14}C content.

At 4, 7 and 10 weeks after treatment two trees from the large scale study were harvested and the plant parts separated into five categories: treated leaves, new growth above treatment, other new growth, branches and trunk, and roots. At harvest the wet weight was determined, the sample was frozen, lyophilised, the dry weight determined, ground to 40 mesh in a Wiley Mill and aliquots combusted to determine the total ^{14}C content. At four weeks 24 trees were harvested and the plant part separated into the above mentioned categories. Wet weights were recorded for each category and the treated leaf, new growth above, and other new growth samples were frozen lyophilised, weighed, ground to 40 mesh in a

Wiley Mill and aliquots were combusted to determine the total ^{14}C content. The branches and trunk and roots were frozen for storage.

3. Analytical procedures

Shoots and leaves samples were extracted three times with water for one hour at room temperature. Roots or branches and trunk samples were extracted three times with 0.5 N NH_4OH for one hour at room temperature. The plant residue was separated by centrifugation, and the extractable radioactive residue was assayed by LSC.
Plant extracts (shoots and leaves, roots or branches and trunk) were chromatographed on a cation exchange column (AG 50W-X4 /H^+). Fractions comprising the major ^{14}C-containing peak were pooled and assayed by LSC.

Standard compounds, chromatographic fractions and plant extracts were characterised using two dimensional TLCs on cellulose plates. Radioactive spots were quantitated by Beta-camera analysis. For amino acids and amino acid analogues detection Ninhydrin reagent was applied. Hanes reagent was applied to detect phosphorous-containing compounds under ultraviolet light.

Standard compounds used for characterisation of radioactive residues were N-(phosphonomethyl)glycine, N-methyl-N-(phosphonomethyl)glycine (CP-67205), amino-methylphosphonic acid, N-methyl-aminomethylphosphonic acid (CP-70948), N,N-dimethyl- aminophosphonic acid (CP-66283), hydroxymethylphosphonic acid, methylphosphonic acid, sarcosine, glycine, and N,N-dimethylglycine.

For spectral characterisation of the radioactive residues, extraction was performed on samples harvested 4 weeks after foliar treatment from 24 trees simultaneously. Dried 40 mesh apple stems and leaves were extracted with water for two hours. After centrifugation and filtering, aliquots of the extract were assayed by LSC. The extract was applied to a cation exchange column (AG 50W-X4, H^+ form), radioactive fractions were combined, assayed by LSC and adjusted to pH 7 with 1 N ammonium hydroxide. The recovery from the column was calculated to be equivalent to 108.0 µg ^{14}C-glyphosate. 1 mg N-(phosphono-^{13}C-methyl)glycine (^{13}C-glyphosate) was added and the solution was applied to an anion exchange column (AG 1-X8, HCO_3^- form), eluted with 0.2 N NH_4HCO_3. Radioactive fractions were combined and assayed by LSC. After evaporation of NH_4HCO_3 and dissolution of the residue in water, a cation exchange chromatography (AG 50W-X8, H^+ form) was performed, radioactive fractions were pooled, assayed by LSC, evaporated to dryness, redissolved in water and cleaned up on Bio-Gel P-2. The resulting radioactive fractions were pooled, assayed by LSC and evaporated to dryness. After dissolution in 0.1 N NH_4HCO_3, the material was frozen and lyophilised.
Quantification and identification were performed by GC/MS on glass columns packed with 1.5 % OV-17 on Chromosorb W-HP or 3 % OV-25 on Chromosorb W-HP after derivatisation to the n-butyl N-trifluoroacetyl derivatives using diazo-n-butane and trifluoroacetic acid/ trifluoroacetic anhydride.
Standards of unlabelled N-(phosphonomethyl)glycine, aminomethylphosphonic acid, N-methyl-aminomethylphosphonic acid, glycine and sarcosine were derivatised to the n-butyl N-trifluoroacetyl derivatives for use as reference substances, while n-butyl esters were derived from standards of N-methyl-N-(phosphonomethyl)glycine and methylphosphonic acid.

Isoptopic dilution techniques as well as NMR techniques were applied for verification.

II. Results and Discussion

A. Total radioactive residues (TRRs)

The recovered activity in apple tree leaves and stems as well as branches and trunks after soil treatment with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) at a rate equivalent to 3.36 kg/ha or ^{14}C-AMPA at a rate equivalent to 1.68 kg/ha is summarised in the table below. No calculation of the total radioactive residue was possible from the data provided in the report.

Uptake of ^{14}C-glyphosate or ^{14}C-AMPA was very low after soil treatment, with a maximum total uptake of 0.134 % of the applied radioactivity 12 weeks after treatment with N-(phosphono-^{14}C-methyl)glycine. Moreover, the untreated control samples also contained a considerable amount of ^{14}C, as compared to the treated samples, as a result of fixation of soil evolved $^{14}CO_2$.

After trunk treatment at 92.4 µg/tree, uptake and translocation was minimal with 0.08 % of the applied activity recovered in leaves and stems and untreated trunk. 0.1 % of the applied radioactivity were recovered in roots, while 72.05 % of the applied radioactivity were found in treated trunk. No calculation of the total radioactive residue was possible from the data provided in the report.

The recovered activity in apple tree treated leaves, new plant growth (leaves and stem), branches and trunk, and roots after foliar treatment with ^{14}C-glyphosate at 10 or 10.712 µg/leaf and sampled at intervals of 7 to 70 days is summarised below. In treated leaves, 45.06 – 75.06 % of the applied activity were recovered. In new growth above treatment 2.32 – 6.70 %, and in other new growth 0.33 – 3.87 % of the applied activity were found, respectively. Branches and trunk contained 0.89 – 2.70 % of the applied activity, while in roots 0.53 – 3.17 % were found. Minor phytotoxic symptoms of chlorosis and terminal bud growth inhibition were observed at these treatment rates and these rates of uptake.
The total radioactive residue (TRR) was calculated from the radioactivity measurement data reported and are expressed as glyphosate equivalents. In treated leaves, TRRs ranged between 98.06 and 144.3 mg/kg during the course of the study. In new growth above treatment 1.075 – 2.052 mg/kg, and in other new growth 0.387 – 1.123 mg/kg were found, respectively. At 28 DALT, branches and trunk contained a TRR of 0.022 mg/kg, while in roots 0.041 mg/kg were detected.

Table 6.2.1-16: Recovered radioactivity in apple matrices after soil treatment with ^{14}C-glyphosate at a rate equivalent to 3.36 kg/ha or amino-^{14}C-methyl-phosphonic acid at a rate equivalent to 1.68 kg/ha

Treatment	N-(phosphono-^{14}C-methyl)glycine		Amino-^{14}C-methyl-phosphonic acid		Control	
Sample	**6 Weeks**	**12 Weeks**	**6 Weeks**	**12 Weeks**	**6 Weeks**	**12 Weeks**
% AR						
Leaves and stems	0.0013	0.093	0.0016	0.059	0.0095	0.020
Branches and trunk	0.0020	0.041	0.0018	0.011	0.0043	0.009
Total Uptake	0.0033	0.134	0.0034	0.070	0.0138	0.029

% AR = percent of applied radioactivity (^{14}C-glyphosate initial: 288850000 dpm (21.45 mg); ^{14}C-AMPA initial: 296272000 dpm (10.67 mg)

Table 6.2.1-17: Recovered radioactivity in apple matrices after trunk treatment with ^{14}C-glyphosate at 92.4 µg/tree

Sample	DALT	Applied dose (dpm)	Recovered dose (dpm)	% AR
Leaves and stems	42	9514880	7550	0.08
Untreated trunk	42	9514880	8240	0.08
Roots	42	9514880	10080	0.10
Treated trunk	42	9514880	6872760	72.05
Total accountability	42	9514880	6898630	72.31

DALT = days after last treatment
% AR = percent of applied radioactivity (^{14}C-glyphosate initial: 9514880 dpm (92.4 µg per tree))

Table 6.2.1-18: Recovered radioactivity and total radioactive residue in apple matrices after foliar treatment with ^{14}C-glyphosate at 10 or 10.712 µg/leaf

Sample	7 DALT	21 DALT	28 DALT	28 DALT	49 DALT	70 DALT
% AR						
Treated leaves	64.86	75.06	45.06[1]	67.75[2]	60.03	60.51
New growth above treatment (leaves and stem)	3.24	2.32	3.56[1]	6.70[2]	4.13	5.03
Other new growth (leaves and stem)	0.33	3.87	1.68[1]	1.48[2]	2.84	1.47
Branches and trunk	0.89	1.78	1.68	-	2.38	2.70
Roots	0.94	0.53	3.17	-	2.19	2.47
Total accountability	70.26	83.56	55.25	-	71.61	72.18
TRR (mg equiv./kg)*						
Treated leaves	*44.31*	*131.2*	*98.06[1]*	*136.1[2]*	*119.3*	*123.2*
New growth above treatment (leaves and stem)	*1.824*	*1.466*	*1.075[1]*	*2.052[2]*	*1.227*	*1.323*
Other new growth (leaves and stem)	-	*1.123*	*0.517[1]*	*0.929[2]*	*0.393*	*0.387*
Trunk and branches	-	-	*0.022*	-	-	-
Roots	-	-	*0.041*	-	-	-

DALT = days after last treatment
TRR = total radioactive residue (*calculated upon dossier compilation from reported dpm and weight data, expressed as glyphosate equivalents; based on specific activity of treatment solution)
% AR = percent of applied radioactivity (^{14}C-glyphosate initial: For 7 and 21 DALT samples 1024000 dpm/leaf (10 µg/leaf), for 28, 49 and 70 DALT 1218000 dpm/leaf (10.712 µg/leaf)
[1] Data based on harvest of 2 trees
[2] Data based on harvest of 24 trees
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded

B. Extraction and characterisation of residues

The plant contained ^{14}C-activity resulting from the foliar treatment studies was analysed for extractability using water as the solvent for all matrices except roots and trunk and branches which were extracted with 0.5 M NH_4OH in order to remove bound N-(phosphono-^{14}C-methyl)glycine.

Extractabilities in apple tree commodities ranged from 71.51 % of the TRR (0.029 mg/kg) in roots and 79.47 % (0.018 mg/kg) in trunk and branches, respectively, at 28 days after application, to 102.59 % TRR in new growth above at day 21. The aqueous extractability of the stem and leaf samples showed no significant pattern of change with time. Non-extractable radioactive resides amounted to approximately 5 % in leaves and stems, 28 in roots and 21 % in trunk. The non-extractable residue was not further examined.

Chromatographic analysis of the aqueous extracts showed that the major residue in treated leaves, new growth above the treatment, other new growth, roots, and trunk of the apple trees was parent glyphosate, at amounts of 83.85 – 96.09 % TRR (94.23 – 128.1 mg/kg), 85.91 – 101.3 % TRR (1.021 – 1.842 mg/kg), 66.82 – 95.08 % TRR (0.346 – 0.980 mg/kg), 66.39 % TRR (0.027 mg/kg) and 64.43 % TRR (0.014 mg/kg), respectively.

The elution volume of the major ^{14}C-containing fraction after cation exchange chromatography was comparable in each case with the elution volume of ^{14}C-glyphosate standard. The major ^{14}C-containing fractions were also characterised as ^{14}C-glyphosate by TLC/ Beta-Camera analysis.

A maximum of 6.45 % TRR of the ^{14}C-activity taken up by the apple trees behaved in a manner chromatographically identical to amino-methylphosphonic acid/ N-methyl-aminomethylphosphonic acid. No other metabolites were identified.

Spectral and chromatographic identification was performed on leaves and stems from 24 apple trees harvested simultaneously in order to provide sufficient quantities of material. The TLC/ Beta Camera and column chromatographic analysis of these samples agreed well with analogous small scale uptake experiment (Table 6.2.1-21 and Table 6.2.1-23).

After aqueous extraction and cation exchange chromatography, isotopic dilution was performed by fortifying the pooled sample with ^{13}C-glyphosate to give a theoretical enrichment of 80.82 %. Sequential chromatography of the fortified sample on anion exchange resin (AG 1-X8), cation exchange resin (AG 50W-X8) and Bio-Gel P-2 gave elution volumes for the radioactivity that were comparable with those of authentic ^{14}C-glyphosate standard and with those of spiked grape extract that had been previously characterised in study 95-01191 [redacted] *et al.*, 1974, report no. 335, CA 6.2.1/006).

GC-MS after derivatisation of the sample to give n-butyl N-trifluoroacetyl derivates showed a fragmentation pattern that was identical to that of derivatised standards of ^{13}C-glyphosate and ^{12}C-glyphosate. Visual comparison of the mass spectral data of derivatised ^{13}C-glyphosate standard and of the ^{14}C-labelled material in the sample showed a decrease in ^{13}C enrichment for ion pairs 106/107, 247/248, and 433/434. Enrichments were calculated to be 81.4, 77.8, and 78.6 % for the ion pairs 106/107, 247/248, and 433/434, respectively.

Correcting for the natural abundance ^{13}C content of 1.1 % resulted in an actual enrichment of 82.5, 78.9, and 79.7 % or an average of 80.4 %, which agreed excellently with the theoretical value of 80.8 %. No other metabolites were identified.

Table 6.2.1-19: Extraction and distribution of the radioactive residues of ^{14}C-glyphosate in apple treated leaves and new growth at 7 days following foliar treatment at 10 µg/ leaf

	Treated leaves				New growth above (leaves and stem)			
DALT	**7**				**7**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***144.3***			***100***	***1.824***
Aqueous extract	90.49	*100*	*90.49*	*130.6*	87.94	*100*	*87.94*	*1.604*
Glyphosate		98.06	*88.73*	*128.1*		97.69	*85.91*	*1.567*
AMPA+ N-methyl-AMPA		0.76	*0.69*	*0.99*		0.54	*0.47*	*0.009*
Total identified		***98.82***	***89.42***	***129.0***		***98.23***	***86.38***	***1.576***
Other		-	-	-		-	-	-
Total characterised		-	-	-		-	-	-
ERR			***90.49***	***130.6***			***87.94***	***1.604***
RRR			**n r.[1]**	**n.r.[1]**			**n r.[1]**	**n r.[1]**
Total sum			***90.49***	***130.6***			***87.94***	***1.604***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[1] Non-extractable radioactivity is reported to be approximately 5 % of the ^{14}C-activity in leaves and stems. Sampling time (DALT) and samples analysed are not specified and therefore this fraction is not presented in the table

Table 6.2.1-20: Extraction and distribution of the radioactive residues of ^{14}C-glyphosate in apple treated leaves and new growth at 21 days following foliar treatment at 10 µg/leaf

	Treated leaves				New growth above (leaves and stem)				Other new growth (leaves and stem)			
DALT	**21**				**21**				**21**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***131.2***			***100***	***1.466***			***100***	***1.123***
Aqueous extract	92.35	*100*	*92.35*	*121.1*	102.59	*100*	*102.59*	*1.504*	88.98	*100*	*88.98*	*0.999*
Glyphosate		98.71	*91.16*	*119.6*		98.75	*101.3*	*1.485*		98.11	*87.30*	*0.980*
AMPA+ N-methyl-AMPA		0.78	*0.72*	*0.94*		0.65	*0.67*	*0.010*		0.78	*0.69*	*0.008*
Total identified		***99.49***	***91.88***	***120.5***		***99.40***	***101.97***	***1.495***		***98.89***	***87.99***	***0.988***
Other		-	-	-		-	-	-		-	-	-
Total characterised		-	-	-		-	-	-		-	-	-
ERR			***92.35***	***121.1***			***102.59***	***1.504***			***88.98***	***0.999***
RRR			**n r.[1]**	**n r.[1]**			**n.r.[1]**	**n r.[1]**			**n.r.[1]**	**n r.[1]**
Total sum			***92.35***	***121.1***			***102.59***	***1.504***			***88.98***	***0.999***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[1] Non-extractable radioactivity is reported to be approximately 5 % of the ^{14}C-activity in leaves and stems. Sampling time (DALT) and samples analysed are not specified and therefore this fraction is not presented in the table

Table 6.2.1-21: Extraction and distribution of the radioactive residues of ^{14}C-glyphosate in apple treated leaves and new growth at 28 days following foliar treatment at 10.712 µg/leaf

	Treated leaves[1]				New growth above[1] (leaves and stem)				Other new growth[1] (leaves and stem)			
DALT	**28**				**28**				**28**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***98.06***			***100***	***1.075***			***100***	***0.517***
Aqueous extract	101.43	*100*	*101.43*	*99.47*	97.13	*100*	*97.13*	*1.044*	71.91	*100*	*71.91*	*0.372*
Glyphosate		94.74	*96.09*	*94.23*		97.77	*94.96*	*1.021*		92.92	*66.82*	*0.346*
AMPA+ N-methyl-AMPA		3.99	*4.05*	*3.97*		0.22	*0.21*	*0.002*		1.43	*1.03*	*0.005*
Total identified		***98.73***	***100.14***	***98.20***		***97.99***	***95.18***	***1.023***		***94.35***	***67.85***	***0.351***
Other		-	-	-		-	-	-		-	-	-
Total characterised		-	-	-		-	-	-		-	-	-
ERR			***101.43***	***99.47***			***97.13***	***1.044***			***71.91***	***0.372***
RRR			**n r.[2]**	**n.r.[2]**			**n.r.[2]**	**n.r.[2]**			**n.r.[2]**	**n.r.[2]**
Total sum			***101.43***	***99.47***			***97.13***	***1.044***			***71.91***	***0.372***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
[1] Data based on harvest of 2 trees
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[2] Non-extractable radioactivity is reported to be approximately 5 % of the ^{14}C-activity in leaves and stems. Sampling time (DALT) and samples analysed are not specified and therefore this fraction is not presented in the table

Table 6.2.1-22: Extraction and distribution of the radioactive residues of [14]C-glyphosate in apple roots and trunk and branches at 28 days following foliar treatment at 10.712 µg/leaf

	Roots				Trunk and branches			
DALT	**28**				**28**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***0.041***			***100***	***0.022***
Aqueous extract	71.51	*100*	*71.51*	*0.029*	79.47	*100*	*79.47*	*0.018*
Glyphosate		92.84	*66.39*	*0.027*		81.08	*64.43*	*0.014*
AMPA+ N-methyl-AMPA		-	-	-		-	-	-
Total identified		***92.84***	***66.39***	***0.027***		***81.08***	***64.43***	***0.014***
Other		-	-	-		-	-	-
Total characterised		-	-	-		-	-	-
ERR			***71.51***	***0.029***			***79.47***	***0.018***
RRR			***28***[1]	***0.011***[1]			***21***[1]	***0.005***[1]
Total sum			***99.51***	***0.041***			***100.47***	***0.022***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[1] Non-extractable radioactivity is reported to be greater or equal to 28 % of the [14]C-activity in roots and 21 % of the [14]C-activity in trunk. No analysis data are reported.

Table 6.2.1-23: Extraction and distribution of the radioactive residues of ^{14}C-glyphosate in apple treated leaves and new growth at 28 days following foliar treatment at 10.712 µg/leaf

	Treated leaves[1]				New growth above[1] (leaves and stem)				Other new growth[1] (leaves and stem)			
DALT	**28**				**28**				**28**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***136.1***			***100***	***2.052***			***100***	***0.929***
Aqueous extract	96.86	*100*	*96.86*	*131.8*	97.13	*100*	*97.13*	*1.993*	91.45	*100*	*91.45*	*0.849*
Glyphosate		95.69	*92.69*	*126.1*		92.44	*89.79*	*1.842*		92.20	*84.32*	*0.783*
AMPA+ N-methyl-AMPA		2.84	*2.75*	*3.74*		2.28	*2.21*	*0.045*		0.91	*0.83*	*0.008*
Total identified		***98.53***	***95.44***	***129.9***		***94.72***	***92.00***	***1.888***		***93.11***	***85.15***	***0.791***
Other		-	-	-		-	-	-		-	-	-
Total characterised		-	-	-		-	-	-		-	-	-
ERR			***96.86***	***131.8***			***97.13***	***1.993***			***91.45***	***0.849***
RRR			**n r.[2]**	**n r.[2]**			**n r.[2]**	**n.r.[2]**			**n.r.[2]**	**n r.[2]**
Total sum			***96.86***	***131.8***			***97.13***	***1.993***			***91.45***	***0.849***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
[1] Data based on harvest of 24 trees
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[2] Non-extractable radioactivity is reported to be approximately 5 % of the ^{14}C-activity in leaves and stems. Sampling time (DALT) and samples analysed are not specified and therefore this fraction is not presented in the table

Table 6.2.1-24: Extraction and distribution of the radioactive residues of ^{14}C-glyphosate in apple treated leaves and new growth at 49 days following foliar treatment at 10.712 µg/leaf

	Treated leaves				New growth above (leaves and stem)				Other new growth (leaves and stem)			
DALT	**49**				**49**				**49**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***119.3***			***100***	***1.227***			***100***	**0.393**
Aqueous extract	98.15	*100*	*98.15*	*117.1*	93.86	*100*	*93.86*	*1.151*	100.4	*100*	*100.4*	*0.395*
Glyphosate		92.31	*90.60*	*108.1*		93.14	*87.42*	*1.072*		91.83	*92.20*	*0.363*
AMPA+ N-methyl-AMPA		5.70	*5.59*	*6.68*		2.62	*2.46*	*0.030*		2.88	*2.89*	*0.011*
Total identified		**98.01**	***96.20***	***114.8***		**95.76**	***89.88***	***1.103***		**94.71**	***95.09***	***0.374***
Other		-	-	-		-	-	-		-	-	-
Total characterised		-	-	-		-	-	-		-	-	-
ERR			***98.15***	***117.1***			***93.86***	***1.151***			***100.4***	***0.395***
RRR			**n.r.[1]**	**n r.[1]**			**n r.[1]**	**n r.[1]**			**n r.[1]**	**n r.[1]**
Total sum			***98.15***	***117.1***			***93.86***	***1.151***			***100.4***	***0.395***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[1] Non-extractable radioactivity is reported to be approximately 5 % of the ^{14}C-activity in leaves and stems. Sampling time (DALT) and samples analysed are not specified and therefore this fraction is not presented in the table

Table 6.2.1-25: Extraction and distribution of the radioactive residues of ^{14}C-glyphosate in apple treated leaves and new growth at 70 days following foliar treatment at 10.712 µg/leaf

	Treated leaves				New growth above (leaves and stem)				Other new growth (leaves and stem)			
DALT	**70**				**70**				**70**			
	% extr. total	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**	**% extr. total**	**% of extr.**	**% TRR**	**mg/kg**
TRR			***100***	***123.2***			***100***	***1.323***			***100***	***0.387***
Aqueous extract	91.39	*100*	*91.39*	*112.6*	94.65	*100*	*94.65*	*1.252*	99.91	*100*	*99.91*	*0.387*
Glyphosate		91.75	*83.85*	*103.3*		92.07	*87.14*	*1.153*		95.17	*95.08*	*0.368*
AMPA+ N-methyl-AMPA		7.06	*6.45*	*7.949*		5.32	*5.04*	*0.067*		2.72	*2.72*	*0.011*
Total identified		***98.81***	***90.30***	***111.2***		***97.39***	***92.18***	***1.219***		***97.89***	***97.80***	***0.379***
Other		-	-	-				-		-	-	-
Total characterised		-	-	-			-	-		-	-	-
ERR			***91.39***	***112.6***			***94.65***	***1.252***			***99.91***	***0.387***
RRR			**n r.[1]**	**n r.[1]**			**n r.[1]**	**n.r.[1]**			**n.r.[1]**	**n r.[1]**
Total sum			***91.39***	***112.6***			***94.65***	***1.252***			***99.91***	***0.387***

DALT = days after last treatment
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
n.r. = not reported
Residues are expressed as mg/kg glyphosate equivalents
Values in *italics* were calculated from reported values upon dossier compilation, final results are rounded
Minor deviations may occur due to rounding
[1] Non-extractable radioactivity is reported to be approximately 5 % of the ^{14}C-activity in leaves and stems. Sampling time (DALT) and samples analysed are not specified and therefore this fraction is not presented in the table

C. Storage stability

No dates are reported for the experimental work from sampling to extraction and analysis of extracts. Thus, it is not possible to conclude on storage stability. However, a theoretical maximum storage period can be estimated from the study duration given in the report (December 1973 – May 1974) to be not longer than 6 months.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in fruit crops at the end of this chapter.

III. Conclusion

In this study the uptake of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) or amino-^{14}C-methylphosphonic acid (^{14}C-AMPA) was very low after soil treatment at a rate equivalent to 3.36 kg N-(phosphonomethyl)glycine/ha or at a rate equivalent to 1.68 kg aminomethylphosphonic acid/ha, respectively, with a maximum total uptake of 0.134 % of the applied radioactivity 12 weeks after treatment with N-(phosphono-^{14}C-methyl)glycine. Moreover, the untreated control samples grown as controls in the greenhouse with the treated pots also contained a considerable amount of ^{14}C, as compared to the treated samples.

After trunk treatment at 92.4 µg ^{14}C-glyphosate/tree, uptake and translocation was minimal with 0.08 % of the applied activity recovered in leaves and stems and untreated trunk. 0.1 % of the applied radioactivity were recovered in roots, while 72.05 % of the applied radioactivity were found in treated trunk.

No calculation of the total radioactive residue was possible from the data provided in the report for soil and trunk treatment.

After foliar treatment at approximately 10 µg/ leaf, TRRs in treated leaves ranged between 98.06 and 144.3 mg/kg during the course of the study.

Foliar applied ^{14}C-glyphosate in formulation was rapidly and efficiently transported throughout the apple tree from the treated leaves. The greatest amount was observed in the growing stem and leaves immediately above the treatment. Significant amounts of compound could also be found in other new growth, trunk and roots.

In new growth above treatment, 1.075 – 2.052 mg/kg, and in other new growth 0.387 – 1.123 mg/kg were found, respectively. Branches and trunk contained a TRR of 0.022 mg/kg, while in roots 0.041 mg/kg were detected.

Extractabilities in apple tree commodities after foliar treatment ranged from 71.51 % of the TRR (0.029 mg/kg) in roots and 79.47 % (0.018 mg/kg) in trunk and branches, respectively, at 28 days after application, to 102.59 % in new growth above at day 21. The aqueous extractability of the stem and leaf samples showed no significant pattern of change with time.

Chromatographic analysis of the aqueous extracts showed that the major residue in treated leaves, new growth above the treatment, other new growth, roots, and trunk of the apple trees was parent glyphosate, at amounts of 83.85 – 96.09 % TRR (94.23 - 128.1 mg/kg), 85.91 - 101.3 % TRR (1.021 – 1.842 mg/kg), 66.82 - 95.08 % TRR (0.346 - 0.980 mg/kg), 66.39 % TRR (0.027 mg/kg) and 64.43 % TRR (0.014 mg/kg), respectively.

A maximum of 6.45 % TRR of the ^{14}C-activity taken up by the apple trees behaved in a manner chromatographically identical to aminomethylphosphonic acid/N-methyl-aminomethylphosphonic acid. No other metabolites were identified.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in apple has been previously evaluated at EU level. It was not performed under GLP (as in 1973 - 1974 GLP was not yet established at the test facility). The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with major deficits: Physical facility and environmental conditions insufficiently described; data for soil and trunk application data insufficient to calculate TRRs; no samples of RAC (apple fruit) were taken; recoveries of radioactivity in aqueous extract of foliar treated apple trees were below 90 % for leaves and stem (new growth above) at 7 days (87.94 %), leaves and stem (other new growth) at 21 (88.98 %) and 28 days (71.91 %), roots and trunk / branches at 28 days (71.51 and 79.47 %); unextracted radiolabel for leaves and stem samples not precisely quantified; no release and characterisation and/or identification on the non-aqueous extractable radioactive residue; no full accountability reported; no details on radioactive counting data; no calculations or data for sample and reference Rf values on TLC; no photographs or images of TLC plates critical to the identification; no information of the storage stability for all major components of the total radioactive residues, no description of length of storage of samples.

Quantitative information in terms of absolute amounts of radioactive residues in mg/kg is limited to the 7 to 70 days apple tree commodities from the foliar application experiments. However, relative amounts in terms of percentage of applied radioactivity, as reported in the study, allow for an assessment of the relative uptake and distribution of ^{14}C-glyphosate or ^{14}C-AMPA after soil treatment and of ^{14}C-glyphosate after trunk treatment.

Identification by GC-MS was achieved 28 DALT leaf and stem samples from plants treated foliar with ^{14}C-glyphosate. Residues in treated leaves, new growth above, (leaves and stem), other new growth (leaves and stem), root and trunk/branches sampled 7 to 70 days from plants treated foliar with ^{14}C-glyphosate to study uptake were characterised by ion exchange chromatography and TLC/Beta Camera analysis. The major residue in treated leaves, new growth above the treatment, other new growth, roots, and trunk of the apple trees was parent glyphosate, at amounts of 83.85 – 96.09 % TRR, 85.91 – 101.3 % TRR, 66.82 – 95.08 % TRR, 66.39 % TRR and 64.43 % TRR, respectively. A maximum of 6.45 % TRR, found in treated leaves at 70 DALT, behaved in a manner chromatographically identical to amino-methylphosphonic acid/ N-methyl-aminomethylphosphonic acid. These experiments show that the major fraction of the total radioactive residue in apple trees after foliar treatment consists of the parent compound, while formation of AMPA and / or N-methyl AMPA is also observed. Therefore, the study data allow for a qualitative assessment of the nature of the residue in apple trees after foliar treatment.

The amount of unextracted residues is reported to approximately 5 % in leaves and stem. Sampling time (DALT) and samples analysed referring to this are not specified.

Overall information given in the report can be considered to estimate a theoretical maximum storage period to be not longer than 6 months, therefore it is not necessary to further investigate storage stability. Moreover, no degradation of glyphosate and its metabolites was found in matrices with high water content, like corn forage, fodder, cotton forage, soybean forage over an investigated storage duration of 215-393 days (7-13 months) ([redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/024). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1).

Thus, although the study does not comply with current guideline requirements in major aspects, it still gives relevant and consistent qualitative information on the uptake and distribution of glyphosate-derived residues in apple plants after soil, foliar, and hydroponic application.

Therefore, this study is considered to be supportive for the assessment of the metabolic behaviour of glyphosate in fruit crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/005
Report author	[redacted]
Report year	1991
Report title	Glyphosate – Trimesium: Uptake and metabolism in USA grape vines
Report No	RJ1002B
Document No	VV-323412
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • No detailed information of the storage stability for all major components of the total radioactive residues • No description of conditions and length of storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The nature of the residues in plants following the use of glyphosate-trimesium was studied in grape vine. N-(phosphono-methyl)glycine trimesium salt, the trimethylsulfonium salt of glyphosate, labelled either in the glyphosate- or the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively) was applied at a rate of 8.1 or 7.8 kg a.s./ha, respectively (corresponding to 5.6 or 5.4 kg glyphosate equivalents/ha), to the soil around mature vines 14 days or one year prior to sampling. In an additional experiment, ten bunches of grapes were deliberately oversprayed with ^{14}C-PMG and ^{14}C-TMS-labelled glyphosate-trimesium, at amounts of 14.3 mg and 13.2 mg (9.9 mg and 9.1 mg expressed as glyphosate equivalents), respectively, per vine.
The calculated total radioactive residues (TRR) in grapes (fruit) accounted for 0.0072 mg/kg (^{14}C-PMG-label) and 0.0029 mg/kg (^{14}C-TMS-label) at 14 days after soil treatment and for 0.007 mg/kg (^{14}C-PMG-label) and 0.0013 mg/kg (^{14}C-TMS-label) for samples taken at maturity one year later. Overspray application resulted in TRRs of 1.25 mg/kg (^{14}C-PMG-label) and 1.15 mg/kg (^{14}C-TMS-label) at 14 days after treatment.
For the ^{14}C-PMG-labelled glyphosate-trimesium oversprayed grapes, 96.4 % of the residue was extractable with water and for the ^{14}C-TMS-labelled glyphosate-trimesium oversprayed grapes, 87.5 % of the residue was extractable with water.

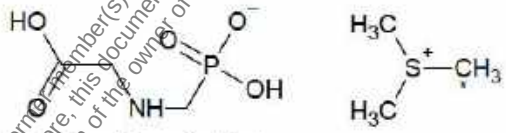
The unaltered glyphosate anion, named as phosphonomethyl glycine (PMG) was the major residue detected in ^{14}C-PMG-labelled treated grapes (fruit) accounting for 77.1 % of the TRR (0.964 mg/kg). AMPA was detected in fruit accounting for 2.5 % of the TRR (0.031 mg/kg)

The unaltered cation, trimethylsulfonium ion (TMS) was the only major residue detected in the ^{14}C-TMS-labelled treated grapes (fruit) and accounted for 83.4 % of the TRR (0.959 mg/kg). These were the only compounds detected.
These results were confirmed by HPLC or GC-analysis in a separate laboratory.

I. Materials and Methods

A. Materials

Test Material: N-(phosphono-methyl)glycine trimesium salt, (ICIA0224; glyphosate-trimesium), radiolabelled (^{14}C) in either the N-phosphonomethylglycine (PMG) anion or the trimethylsulfonium (TMS) cation

Chemical structure:	a) ^{14}C-PMG label  * Position of the radio label b) ^{14}C-TMS label  * Position of the radio label
Radiochemical purity:	a) ^{14}C-PMG label (determined before each application): 95.0 % (first soil treatment); 96.7 % (second soil treatment) and 97.6 % (overspray application) b) ^{14}C-TMS label (determined before each application): 99.2 % (first soil treatment); 98.6 % (second soil treatment) and 98.6 % (overspray application)
Specific activity (in radiodiluted treatment solution):	a) ^{14}C-PMG-labelled glyphosate-trimesium: used for 1st and 2nd soil treatment: 0.2997 MBq/mg for the first and 0.2872 MBq/mg for the second treatment (both expressed as PMG). used for overspray application: 5.364 MBq/mg (expressed as PMG) b) ^{14}C-TMS labelled glyphosate-trimesium: used for 1st soil treatment: 0.5099 MBq/mg (expressed as TMS) used for 2nd soil treatment and overspray application: 0.4898 MBq/mg (expressed as TMS) used for overspray application: 12.156 MBq/mg (expressed as TMS)
CAS No:	81591-81-3

Log $P_{o/w}$ for glyphosate-trimesium:	-2.9

Test system:

Soil:	Foster fine sandy loam, pH 7.5
Crop:	Grape (Chenin Blanc)
Botanical name:	*Vitis vinifera*
Crop part(s):	Grapes

B. Study design

1. In-life phase

In this study conducted in California (USA), N-(phosphono-methyl)glycine trimesium salt, the trimethylsulfonium salt of glyphosate formulated as an aqueous concentrate, was applied to the soil below vine grapes (Chenin Blanc variety) or used as overspray on selected parts of the plants.
The active substance was ^{14}C-radiolabelled either in the glyphosate- or in the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively). For both labels the radiolabelled test compound was diluted with an aqueous concentrate of the non-radiolabelled test compound (YF7712, consisting of surfactant
AL-2042 (blend of glucosides (67 %), amine ethoxylate (5 %) and water) and water). The study was conducted using an outdoor test plot.

Soil treatment
Prior to the first soil treatment the vines were pruned and the soil beneath the vines was cleared of weeds by hand. A 2 m^2 (1 m × 2 m) treatment area around the vine was marked and remained in place for the duration of both applications. Based on previous irrigation patterns this 2 m^2 treatment area was selected to enclose the majority of the vines feeding roots, maximising the potential for uptake of glyphosate-trimesium into the vine. The soil around the vine was irrigated for approximately 1 hour prior to the application to ensure dampness of the soil, facilitating penetration of glyphosate-trimesium to reach the roots more easily. Polythene sheeting was wrapped around the base of each vine to prevent accidental contamination during application. Prior to the 2nd soil application the above procedures were repeated.
The first soil applications were made when the grapes were in early to mid-bloom, well established with numerous leaves and good vigour. The 2nd soil applications and the overspray applications were made seven days later when the grapes were in late reproductive stage, again well established with numerous leaves and good vigour, leaf surfaces were dry.
The specific activity of the first application solution for soil treatment of the PMG-label contained 0.2066 Bq/mg glyphosate trimesium (0.2997 MBq/mg expressed as PMG), the second solution contained 0.1980 MBq/mg glyphosate trimesium (0.2872 MBq/mg expressed as PMG).
The specific activity of the first application solution for soil treatment of the TMS-label contained 0.1604 MBq/mg glyphosate trimesium (0.5099 MBq/mg expressed as TMS), the second solution contained 0.541 MBq/mg glyphosate trimesium (0.4898 MBq/mg expressed as TMS).
For the soil treatment two sprayings with a total target rate equivalent to 8.0 kg a.s./ha were applied. The actual total application rates were 8.1 kg a.s./ha for the PMG-label and 7.8 kg a.s./ha for the TMS-label (corresponding to 5.6 or 5.4 kg glyphosate equiv./ha, respectively).
The amount of radioactivity applied was 728.3 MBq in the first and 696.0 MBq in the second soil treatment with the ^{14}C-PMG-label or 544.6 MBq in the first and 510.9 MBq in the second soil treatment of the ^{14}C-TMS-label.

Overspray application

In addition to the soil treatment 10 bunches of grapes on two additional vines were oversprayed with ^{14}C-PMG- and ^{14}C-TMS-labelled glyphosate-trimesium.
Prior to the over spray applications, the 10 best bunches of grapes per vine were selected. A polythene cone was taped around each bunch of grapes, so that the grapes could be sprayed without contaminating of the leaves or shoots. Finally, adsorbent pads were placed on the ground below the selected bunches to prevent accidental contamination of the soil beneath the vine.
The overspray treatment solutions were applied using aerosol sprayers. Each treatment solution was sprayed evenly between the 10 selected bunches of grapes per vine. The polythene cones protecting the leaves and shoots from contact with the over spray treatment solution were removed after application.
The specific activity of the overspray application solution of the PMG-label contained 3.698 MBq/mg glyphosate trimesium (5.364 MBq/mg expressed as PMG) and the overspray application solution of the TMS-label contained 3.825 MBq/mg glyphosate trimesium (12.156 MBq/mg expressed as TMS).
A target amount of 12.2 mg of glyphosate-trimesium per vine applied in 30 mL of 0.1 % surfactant was selected as a treatment rate which, protection of leaves and stems provided, was unlikely to damage the grapes.
Actual amounts were 14.3 mg and 13.2 mg of glyphosate-trimesium (corresponding to 5.6 and 5.4 kg/ha) applied in the experiment for the PMG and TMS-label respectively which were applied per vine corresponding to an amount of radioactivity applied of 52.9 MBq of the ^{14}C-PMG-label and 50.5 MBq of the ^{14}C-TMS-label.
The overspray applications were made at the same time as the second soil treatment and the grapes harvested with the first mature grape sampled at a PHI of 14 days.

The study report also includes a method validation part using grapes with incurred residues after treatment with ^{14}C-PMG-glyphosate trimesium and ^{14}C-TMS-glyphosate trimesium. The relevant methods were successfully validated. Details are not relevant to the metabolism section and therefore are not summarised here.

2. Sampling

Samples of mature grapes were collected 14 days after the application (soil and overspray treatments) and also one year later for the soil treatment.
At each harvest interval between 7 to 10 of the best bunches of grapes per vine were harvested. The bunches were removed from the vine by cutting the stems behind the bunch using a pair of scissors. All bunches of grapes harvested from an individual vine were combined in a bag. After harvest, the bunches of grapes were transferred to the processing area, where the grapes were removed from the stalks, and separated into good and bad grapes based on appearance.
Samples were stored frozen, held frozen during shipment and stored frozen until analyses.

3. Analytical procedures

Samples of grapes were homogenised using an ultra turrax, followed by ultrasonication and filtration (using a cellulose acetate or cellulose nitrate filter). The grape pulp was washed with water and the washing was combined with the grape juice filtrate. The radioactivity in the grape juice filtrate quantified directly by liquid scintillation counting (LSC). The grape pulp and the filter were re-extracted (2-3 x) with water. The remaining grape pulp debris was dried, divided into pulp and pips, and the remaining un-extracted activity quantified by combustion. In general, grapes were extracted until the level of activity recovered in the last extract fell below 5 % of the total activity extracted to that point, or the level of activity being extracted became so low it could no longer be quantified by LSC.
Total radioactive residues were determined in liquid samples using liquid scintillation counting (LSC). Radioactivity in solid samples was measured by combustion in a Biological Oxidiser. After combustion ^{14}C-labelled CO_2 was trapped in a mixture of scintillation cocktail/2-methoxyethylamine/water (1500/500/40, v/v/v) followed by LSC.
Thin layer chromatography (TLC) was used to measure the purity of the radiochemicals prior to application, and to characterise radioactive compounds in sample extracts against standard reference compounds (N-phosphonomethylglycine trimethylsulfonium salt, phosphonomethylglycine,

aminomethylphosphonic acid (AMPA), methylphosphonic acid, N-methyl-N-phosphonomethylgycine, hydroxymethylphosphonic acid, trimethylsulfonium iodide, trimethylsulfoxonium iodide).
Aqueous plant extracts were applied to tracks individually and admixed with appropriate reference compounds. Three to four different TLC systems were used for characterisation/identification. Co-extractives in some samples interfered with chromatography, and it was necessary to clean-up these samples (using Bio Rad AG 50W-X2/Amberlite XAD-2 resin to remove co-extractives from the ^{14}C-PMG-labelled glyphosate-trimesium overspray application grape extract or Bio Rad AG 50W-X2 to remove co-extractives from the combined ^{14}C-TMS-labelled glyphosate-trimesium overspray application grape extract) before meaningful data could be obtained from TLC analysis.
Reference compounds were visualised by reaction with suitable spray reagents, i.e. molybdenum blue, ninhydrin (0.2 % ninhydrin in ethanol), potassium iodoplatinate (5 % aqueous platinic chloride solution (5 mL), mixed with of 10 % potassium iodide solution, (45 mL) diluted to 100 mL with water) or Dragendorff's reagent.
In addition, the radioactive areas on plates were located and quantified using an Isomess 6800 Automatic Linear Analyser or an AMBIS Beta Scanning System.
Autoradiograms of the developed plates were prepared using Hyperfilm β-max. The position and shape of radioactive areas on the film was compared against the results of linear analysis, and the visualisation of references compounds for the same plate.
Three chromatographic systems were always used to determine purity (except for one occasion where only two systems were used). Radiochemical purity was measured both prior to shipment to the USA and immediately prior to application.
High performance liquid chromatography (HPLC) was used to determine the specific activity of the ^{14}C-PMG-labelled glyphosate-trimesium application solutions. Gas chromatography (GC) was carried out to determine the specific activity of the ^{14}C-TMS-labelled glyphosate-trimesium application solutions.
Subsamples of ^{14}C-PMG and ^{14}C-TMS-labelled glyphosate-trimesium oversprayed grapes were sent to a second laboratory (ICI Americas Western Research Center, Richmond, CA, USA) for repeat radiochemical and residue analysis. Residue analysis confirmed the presence of PMG by derivatisation and HPLC while TMS was confirmed by demethylation and GC.

II. Results and Discussion

A. Total radioactive residues (TRRs)

Residue levels detected in mature grapes harvested after soil application were low. The total radioactive residue (TRR) in grapes (fruit) was determined by summation of the extracted radioactivity plus the radioactivity remaining in the solids. All the residue levels were either determined as N-phosphonomethylglycine (PMG) anion (glyphosate anion) or trimethylsulfonium (TMS) cation equivalents.

Residue levels in grapes treated with ^{14}C-PMG labelled glyphosate-trimesium and sampled 14 days after last treatment were 0.0072 mg/kg and 0.0029 mg/kg after application of the ^{14}C-TMS-labelled glyphosate-trimesium.
Residues ^{14}C-PMG-labelled glyphosate-trimesium treated vines sampled at maturity one year later - accounted for 0.0070 mg/kg and in the ^{14}C-TMS-labelled glyphosate-trimesium treated vines residues for 0.0013 mg/kg.

In addition to the soil treatment, 10 bunches of grapes on two additional vines were oversprayed with ^{14}C-PMG- and ^{14}C-TMS-labelled glyphosate-trimesium. Residue levels detected in these mature grape samples were significantly higher. ^{14}C-PMG-labelled glyphosate-trimesium treated grapes showed residues of 1.25 mg/kg and ^{14}C-TMS-labelled glyphosate-trimesium treated grapes showed residues of 1.15 mg/kg at 14 days after application.
The total radioactive residues (TRR) in grape samples are summarised in the table below.

Table 6.2.1-26: Total radioactive residues in grapes

Sample description	Days after last treatment (DALT)	Experiment	TRR_{calc} (calculated as sum of ERR + RRR)	
			(mg ^{14}C-PMG anion equiv./kg)	(mg ^{14}C-TMS cation equiv./kg)
			^{14}C-PMG-label	^{14}C-TMS-label
Grape	14	Soil treatment	0.0072	0.0029
		Overspray application	1.25	1.15
	~ 1 year after last treatment	Soil treatment	0.0070	0.0013

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

B. Extraction and characterisation of residues

The ^{14}C-levels found in fractions of grapes (fruit) are shown in the tables below.
Due to the low levels of activity (<0.01 mg/kg) present in grape samples after soil application with ^{14}C-PMG- or ^{14}C-TMS-labelled glyphosate-trimesium, these samples were not further characterised.

For the samples from the overspray treatment three aqueous extracts were combined and the washing, the juice and the combined aqueous extracts analysed separately by TLC using different chromatographic systems. Due to the high levels of activity in these extracts it was possible to analyse them directly by TLC without prior concentration. However, only the washing extract gave well resolved chromatography. High levels of co-extractives, mainly sugars, in the juice and combined aqueous extracts caused broadening and streaking of radioactive area, making it difficult to conclusively characterise radioactive components against reference compounds. To overcome this, equivalent sub-samples of the three extracts were combined, and co-extractives removed by the clean-up method. The combined, cleaned and concentrated extract was then re-analysed by TLC in different chromatographic systems.
For the ^{14}C-PMG-labelled glyphosate-trimesium oversprayed grapes, 96.4 % of the residue was extractable with water.
After combination, clean-up and concentration, 77.1 % (0.964 mg/kg, expressed as PMG equivalents) of the residue was identified by TLC co-chromatography as PMG and 2.5 % (0.031 mg/kg) as AMPA.
For the ^{14}C-TMS-labelled glyphosate-trimesium oversprayed grapes 87.5 % of the residue was extractable with water. After combination, clean-up and concentration 83.4 % (0.959 mg/kg) of the residue was identified by TLC-co-chromatography as TMS. These were the only compounds detected.

Sub-samples of ^{14}C-PMG- and ^{14}C-TMS-labelled glyphosate-trimesium oversprayed grapes were sent to another laboratory, for repeated radiochemical and residue analysis. Residue analysis confirmed the presence of PMG by derivatisation and HPLC, while TMS was confirmed by demethylation and GC-analysis.

Table 6.2.1-27: Extraction of the radioactive residues of ^{14}C-PMG or ^{14}C-TMS-label in grape vine following soil application of glyphosate-trimesium at a dose rate of 8.1 kg a.s./ha (PMG-label) and 7.8 kg a.s./ha (TMS-label) (corresponding to 5.6 or 5.4 kg glyphosate equiv./ha respectively)

	Soil treatment							
	Grape vine fruit							
Label	**^{14}C-PMG-label**				**^{14}C-TMS-label**			
Growth stage	Maturity (14 DALT)		Maturity (after 1 year)		Maturity (14 DALT)		Maturity (after 1 year)	
	mg/kg[1]	**% TRR**	**mg/kg[1]**	**% TRR**	**mg/kg[2]**	**% TRR**	**mg/kg[2]**	**% TRR**
TRR	0.0072	100	0.0070	100	0.0029	100	0.0013	100
Extract 1 (juice)	0.0034	47.8	0.0010	13.8	0.0012	41.0	0.0005	36.6
Extract 2 (water)	0.001	14.7	0.0034	48.8	0.0007	24.6	0.0003	21.1
Extract 3 (water)	0.0005	6.6	-	-	0.0003	9.8	-	-
ERR	***0.0049***	***69.1***	***0.0044***	***62.6***	***0.0022***	***75.4***	***0.0008***	***57.7***
Pulp	0.0016	21.7	-		0.0004	13.0	-	-
Pips	0.0007	9.3	-		0.0003	11.5	-	-
RRR	***0.0023***	***31.0***	0.0026	37.4	***0.0007***	***24.5***	0.0005	42.3
Accountability	***0.0072***	***100.1***	***0.0070***	***100.0***	***0.0029***	***99.9***	***0.0013***	***100.0***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
Accountability Sum of extractable radioactive residue and residual radioactive residue
[1] Residues calculated as mg ^{14}C-PMG anion equiv./kg
[2] Residues calculated as mg ^{14}C-TMS cation equiv./kg
Minor deviations may occur due to rounding
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-28: Extraction of the radioactive residues of ^{14}C-PMG or ^{14}C-TMS-label in grape vine following overspray application of glyphosate-trimesium (14.3 mg and 13.2 mg of the PMG- and TMS-label; 9.9 mg and 9.1 mg expressed as glyphosate equiv., respectively)

	Overspray treatment			
	Grape vine fruit			
Label	^{14}C-PMG-label		^{14}C-TMS label	
Growth stage	Maturity (14 DALT)		Maturity (14 DALT)	
	mg/kg[1]	% TRR	mg/kg[2]	% TRR
TRR	**1.25**	**100**	**1.15**	**100**
Washings	0.319	25.5	0.127	11.0
Extract 1 (juice)	0.575	46.0	0.505	43.9
Extract 2 (water)	0.218	17.4	0.258	22.4
Extract 3 (water)	0.068	5.4	0.104	9.0
Extract 4 (water)	0.025	2.0	0.014	1.2
Combined extracts	1.161	92.9	1.018	88.5
Combined extracts (clean up)	1.085	86.8	1.007	87.6
Combined extracts (concentrated)	1.024	81.9	0.966	84.0
ERR	**1.20**	**96.4**	**1.010**	**87.5**
Pulp	-	-	0.128	11.1
Pips	-	-	0.016	1.4
RRR	**0.045**	**3.6**	***0.144***	***12.5***
Accountability	***1.25***	***100***	***1.15***	***100***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (named as debris in the report; in case of TMS-label the two fractions of debris (pulp and pips) were analysed separately.
Accountability Sum of extractable radioactive residue and residual radioactive residue
[1] Residues calculated as mg ^{14}C-PMG anion equiv./kg
[2] Residues calculated as mg ^{14}C-TMS cation equiv./kg
Minor deviations may occur due to rounding
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-29: Distribution of the radioactive residues of ^{14}C-PMG or ^{14}C-TMS-label in grape vine following overspray application of glyphosate-trimesium (14.3 mg and 13.2 mg of the PMG and TMS-label, 9.9 mg and 9.1 mg expressed as glyphosate equiv., respectively)

	Overspray treatment			
	Grape vine fruit			
Label	**^{14}C-PMG label**		**^{14}C-TMS label**	
Growth stage	Maturity (14 DALT)		Maturity (14 DALT)	
	mg/kg[1]	**% TRR**	**mg/kg[2]**	**% TRR**
TRR	**1.25**	**100**	**1.15**	**100**
ERR	**1.20**	**96.4**	**1.010**	**87.5**
PMG	0.964	77.1	-	-
AMPA	0.031	2.5	-	-
TMS	-	-	0.959	83.4
Total identified[3]	***0.995***	***79.6***	***0.959***	***83.4***
Total characterised[4]	***0.21***	***16.80***	***0.05***	***4.10***
RRR	**0.045**	**3.6**	***0.144***	**12.5**

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
1 Residues calculated as mg ^{14}C-PMG anion equiv./kg
2 Residues calculated as mg ^{14}C-TMS cation equiv./kg
3 Identification of analytes was done by TLC-co-chromatography as well as different analytical technique (HPLC or GC) in a separate laboratory.
4 Characterised by extraction.
Values in *italics* were recalculated upon dossier compilation based on available values in the report.

C. Storage stability

No exact dates are reported for the experimental work from extraction to analysis of extracts, thus it is not possible to conclude on storage stability. However, it is stated that analyses of grape samples stored frozen was initiated within 7 months of harvest.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in fruits at the end of this chapter.

III. Conclusion

The nature of the residues in plants following the use of glyphosate-trimesium was studied in grape vine. N-(phosphono-methyl)glycine trimesium salt, labelled either in the glyphosate- or the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively) was applied at a rate of 8.1 or 7.8 kg a.s./ha, respectively (corresponding to 5.6 or 5.4 kg glyphosate equiv./ha respectively), to the soil around mature vines or by overspray to bunches at rates of 14.3 mg and 13.2 mg (9.9 mg and 9.1 mg expressed as glyphosate equiv.), respectively per vine.
The calculated total radioactive residues (TRR) in grapes (fruit) accounted for 0.0072 mg/kg (^{14}C-PMG-label) and 0.0029 mg/kg (^{14}C-TMS-label) at 14 days after soil treatment and for 0.007 mg/kg (^{14}C-PMG-

label) and 0.0013 mg/kg (^{14}C-TMS-label) for samples taken at maturity one year later. Overspray application resulted in TRRs of 1.25 mg/kg (^{14}C-PMG-label) and 1.15 mg/kg (^{14}C-TMS-label) at 14 days after treatment.
The unaltered anion, phosphonomethyl glycine (PMG) accounted for 77.1 % of the TRR (0.964 mg/kg) in ^{14}C-PMG-labelled oversprayed grapes (fruit) AMPA was detected in oversprayed grape fruit accounting for 2.5 % of the TRR (0.031 mg/kg). The unaltered cation, trimethylsulfonium ion (TMS) was the only major residue detected in the ^{14}C-TMS-labelled oversprayed grapes (fruit) and accounted for 83.4 % of the TRR (0.959 mg/kg). These were the only compounds detected.
These results were confirmed by HPLC or GC-analysis in a separate laboratory.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in grapes (fruit) has been previously evaluated at EU level. It was not performed under GLP but is considered to be reliable. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with major deficits (no detailed information of the storage stability for all major components of the total radioactive residues, no description of conditions and length of storage of samples).
As there are no detailed information on storage duration, whole information given in the report may be considered: The overspray application was done on 1989-08-25. Samples of grapes (fruit) were taken after 14 days and analysed for glyphosate related residues. It is stated that analyses of grape samples stored frozen was initiated within 7 months of harvest and referred to a storage stability of up to two years. Therefore it is considered that this duration covers the duration of the present study which is supported by detailed data given in the report (within the study conduct of the lab phase the laboratory was inspected by quality assurance unit at three different interval, the latest one dated at 1991-06-26; duration sampling until last inspection: 656 days (~ 22 months).
A storage stability study is available ([redacted] 2012, CA 6.1/002) showing the stability of glyphosate and its metabolite AMPA in commodities with high acid content over a storage period of 24 months.
Therefore, the study is considered reliable in context of current guideline requirements and may be used to support the uses in the crop category fruits.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/006
Report author	[redacted]
Report year	1990
Report title	ICIA0224: Uptake and metabolism in grape-vines
Report No	RJ 0815B
Document No	Not available
Guidelines followed in study	Not specified

Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Soil characteristics are not reported • Samples of grape vine leaves, stems and stalks were not extracted. No characterisation of the radioactive residues was performed in samples of grape vine leaves, stems and stalks • No information of the storage stability for all major components of the total radioactive residues • No description of length of storage of sample
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this study grape vines were treated with ^{14}C-glyphosate-trimesium labelled either in the N-phosphono-methylglycine (PMG) anion (^{14}C-PMG label) or the trimethylsulfonium (TMS) cation (^{14}C-TMS label). The application was made as a soil drench to the bases of the trunks at rates equivalent to 8.3 kg as/ha (^{14}C-PMG label) (corresponding to 5.7 kg glyphosate equivalents/ha) or 7.1 kg/ha (^{14}C-TMS label) (corresponding to 4.9 kg glyphosate equivalents/ha). Samples of grapes, leaves and stems were collected 7 days after the application.
The calculated total radioactive residues in grapes (fruit) after treatment accounted for <0.006 mg/kg (^{14}C-PMG-label) and <0.003 mg/kg (^{14}C-TMS-label).
For the ^{14}C-PMG-label, the TRR was <0.023 mg/kg in grape stems, ≤0.023 mg/kg in grape leaves and <0.023 mg/kg in grape stalks. For the ^{14}C-TMS-label, the TRR was <0.009 mg/kg in grape stems, 0.031 mg/kg in grape leaves and 0.010 mg/kg in grape stalks.

Within seven days after ground treatment no significant uptake of glyphosate residues into grape vines was observed.

No characterisation of the residue was attempted.

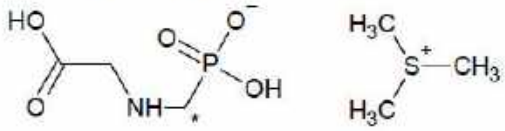
I. Materials and Methods

A. Materials

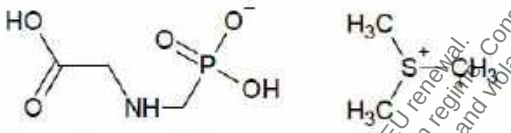
Test Material: N-(phosphonomethyl)-glycine trimesium salt (ICIA0224; glyphosate-trimesium), radiolabelled (^{14}C) in either
a) the N-phosphonomethylglycine anion (^{14}C-PMG) or
b) the trimethylsulfonium cation (^{14}C-TMS)

Chemical structure:

a) ^{14}C-PMG label



b) ^{14}C-TMS label



* Position of the radio label

Radiochemical purity: a) ^{14}C-PMG: 97.4 % (mean percentage of formulated test substance)
b) ^{14}C-TMS: 98.0 % (mean percentage of formulated test substance)

Specific activity: a) ^{14}C-PMG-labelled glyphosate-trimesium, Batch No: 88-J30, quoted specific activity 2.07 GBq/mmol (8.4 MBq/mg)
b) ^{14}C-TMS- labelled glyphosate-trimesium, Batch No: 88-J19, quoted specific activity 2.06 GBq/mmol (8.4 MBq/mg)
Unlabelled N-phosphonomethylglycine trimethylsulfonium salt (ICIA0224 technical aqueous concentrate (57.6 %), WF1002/Lot WHC2501] was used to dilute the radiochemical to the required specific activity

CAS No: 81591-81-3

Log $P_{o/w}$ for glyphosate-trimesium: -2.9

Test system:

Soil:	Type not stated
Crop:	Grape vine (variety: Muller Thurgau)
Botanical name:	*Vinis vitifera*
Crop part(s):	Leaves, stems, stalks and grapes

B. Study design

1. In-life phase

The study was conducted between August 1988 and March 1989, at Jealott's Hill Research Station, Bracknell, Berkshire, UK. Ten year old vines were treated with ^{14}C-glyphosate-trimesium, the trimethylsulfonium salt of glyphosate, labelled either at the N-phosphonomethylglycine- or the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively).
For both labels the radiolabelled test compound was diluted with an aqueous concentrate of the non-radiolabelled test compound, a surfactant (AL-2042 (72 %)) and water.
Two mature vines were treated, one with ^{14}C-PMG-labelled glyphosate-trimesium and the other with ^{14}C-TMS-labelled glyphosate-trimesium.

The application of trimethylsulfonium salt of glyphosate was made as a soil drench to the bases of the trunks within an 2.5 m x 1.0 m (2.5 m^2) treatment area around the base of each vine, corresponding to rates equivalent to 8.3 kg/ha (^{14}C-PMG) (corresponding to 5.7 kg glyphosate equivalents/ha) or 7.1 kg/ha (^{14}C-TMS) (corresponding to 4.9 kg glyphosate equivalents/ha). The amount of radioactivity applied was 744.7 MBq (^{14}C-PMG) or 735.8 MBq (^{14}C-TMS).

Samples of grapes, leaves and stems were collected 7 days after the application.

2. Sampling

Prior to application of ^{14}C-PMG- and ^{14}C-TMS-labelled ^{14}C-glyphosate-trimesium, representative samples were taken of the soil, leaves, stems and grapes, for each vine, in order to assess the ^{14}C background levels.
The grapes from both the ^{14}C-PMG- and ^{14}C-TMS-labelled glyphosate-trimesium treated vines were harvested after a 7 days interval (7 DALT), by cutting the grape stalks at the point of attachment to the vine stem.
Samples of leaves and stem were also taken from each vine.
The grapes were separated from the stalks, and the leaves from the stems, and the samples stored at -l8 °C ± 5 °C prior to analysis.

3. Analytical procedures

The grapes were homogenised sequentially twice with methanol and twice with ultra-pure water using an ultra-turrax homogeniser for a period of 20 minutes. The extraction vessel was cooled in an ice/water bath. The liquid and solid phases were separated by centrifugation and the supernatant filtered under vacuum.
The filtrate was quantitatively transferred to a standard volumetric flask and diluted to volume with the appropriate solvent. The amount of activity contained in each extract was analysed by liquid scintillation counting (LSC).
Extracts from the same label were combined and concentrated by rotary evaporation and re-analysed by LSC.
The unextracted activity remaining in the grape debris was quantified by combustion analysis followed by LSC.

Samples of stalk, stem and leaf were air dried and homogenised using a blender mill prior to combustion analysis.
The amount of activity present in the stalk, stem and leaf for each label was quantified by combustion analysis followed by LSC.

All liquid scintillation counting was carried out using an LKB Wallac-1219 Rackbeta "Spectral" liquid scintillation counter. The amount of radioactivity in solid samples was measured by sample oxidation, using a Harvey OX300 Biological Oxidiser linked to a Zymark (II) robotics system, followed by LSC.

Analysis of the test solutions was performed applying thin layer chromatography (TLC) using standards of N-phosphonomethylglycine trimethylsulfonium salt, N–phosphono-methylglycine (PMG), aminomethylphosphonic acid (AMPA), methylphosphonic acid, N-methyl-N-phosphonomethylglycine, hydroxymethylphosphonic acid, trimethylsulfonium (TMS) iodide, trimethylsulfoxonium iodide and trimethylsulfoxonium chloride.
The specific activity of the ^{14}C-PMG-labelled glyphosate-trimesium application solution was determined by high performance liquid chromatography (HPLC) in combination with LSC.
The specific activity of the ^{14}C-TMS-labelled glyphosate-trimesium application solution was determined by gas chromatography (GC) in combination with LSC.

II. Results and Discussion

A. Total radioactive residues (TRRs)

Representative samples of soil, leaves, stems and grapes for each vine taken before application were assessed for ^{14}C-background levels. These samples showed no significant background activity levels. Results were only considered significant if the determined activity was greater than twice the background activity level.
All the residue levels were determined as N-phosphonomethylglycine (PMG) anion and trimethyl-sulfonium (TMS) cation equivalents.

The total radioactive residue (TRR) in grapes (fruit) was determined by summation of the extracted radioactivity in the methanol and water extracts plus the radioactivity remaining in the solids (debris). The calculated total radioactive residues in grapes (fruit) after treatment accounted for <0.006 mg/kg (^{14}C-PMG-label) and <0.003 mg/kg (^{14}C-TMS-label). Insignificant amounts of activity were found in the aqueous extract and grape debris for both labels. The residue levels quoted for these fractions correspond to theoretical values calculated based on the activity level equivalent to twice the background activity level (see Table 6.2.1-27 and Table 6.2.1-32).

The total radioactive residue (TRR) in grape vine stems, leaves and stalks was determined by sample oxidation. For the ^{14}C-PMG-label, the TRR was <0.023 mg/kg in grape stems, ≤0.023 mg/kg in grape leaves and <0.023 mg/kg in grape stalks. Insignificant amounts of activity were found in the vine stems and grape stalks. The residue levels quoted correspond to theoretical values calculated based on the activity level equivalent to twice the background activity level.

For the ^{14}C-TMS-label, the TRR was <0.009 mg/kg in grape stems, 0.031 mg/kg in grape leaves and 0.010 mg/kg in grape stalks. Insignificant amounts of activity were found in the vine stems. The residue levels quoted correspond to theoretical values calculated based on the activity level equivalent to twice the background activity level.

Table Error! Use the Home tab to apply Überschrift 3 to the text that you want to appear here.**-30:**
Total radioactive residues in grape vine

Sample description	Days after last treatment (DALT)	TRR_{calc} (calculated as sum of ERR + RRR)	
		(mg ^{14}C-PMG anion equiv./kg)	(mg ^{14}C-TMS cation equiv./kg)
		^{14}C-PMG	^{14}C-TMS
Grapes	7	<0.006	<0.003
		TRR_{calc} (direct combustion)	
		(mg ^{14}C-PMG anion equiv./kg)	(mg ^{14}C-TMS cation equiv./kg)
		^{14}C-PMG	^{14}C-TMS
Stems	7	<0.023	<0.009
Leaves	7	≤0.023	0.031
Stalks	7	<0.023	0.010

DALT Days after last treatment
ERR Extractable radioactive residue
RRR Residual radioactive residue

B. Extraction and characterisation of residues

No extraction was conducted with samples of grape vine stems, leaves and stalks.

The ^{14}C-levels found in fractions of grape fruit are shown in Table 6.2.1-27 and Table 6.2.1-32.
The residue levels were calculated based on the significance of the results obtained. Results were only considered significant if the determined activity was greater than twice the background activity level.

Significant results were only obtained for the methanol extract, with 0.003 mg/kg or 50 % of the TRR for the ^{14}C-PMG label and 0.001 mg/kg or 33.33 % of the TRR for the ^{14}C-TMS-label.
The aqueous extract and grape debris fractions, showing low, insignificant levels, were considered with theoretical values based on twice the background radioactivity level. Calculated results for the aqueous extract were <0.002 mg/kg or <33.33 % of the TRR for the ^{14}C-PMG-label and <0.001 mg/kg or <33.33 % of the TRR for the ^{14}C-TMS-label. For grape debris, <0.001 mg/kg or <16.67 % of the TRR were calculated for the ^{14}C-PMG-label and <0.001 mg/kg or <33.33 % of the TRR for the ^{14}C-TMS-label.

Table 6.2.1-31: Extraction of the radioactive residues of ^{14}C-PMG in grape vine following application of glyphosate-trimesium at a dose rate of 1 x 8.3 kg/ha (corresponding to 5.7 kg glyphosate equivalents/ha)

	Grape vine fruit		**Grape vine stems[1, 2]**		**Grape vine leaves[1, 2]**		**Grape vine stalks[1, 2]**	
Growth stage	Maturity (7 DALT)		Maturity (7 DALT)		Maturity (7 DALT)		Maturity (7 DALT)	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**<0.006**	***100***	**<0.023**	***100***	**≤0.023[4]**	***100***	**<0.023**	***100***
Methanol Extract[2]	0.003	*50.00*	-	-	-	-	-	-
Aqueous Extract[3]	<0.002	*<33.33*	-	-	-	-	-	-
Grape Debris[3]	<0.001	*<16.67*	-	-	-	-	-	-
ERR	**<0.005**	***<83.33***	-	-	-	-	-	-
RRR	**<0.001**	***<16.67***	-		-	-	-	-
Total sum	**<0.006**	***100***	-	-	-	-	-	-

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
mg/kg = mg ^{14}C-PMG anion equiv./kg
[1] Activities were determined on a dry weight basis
[2] The methanol extract residue level was the only significant result obtained
[3] Insignificant amounts of activity were found in the aqueous extract, grape debris, vine stems and grape stalks. The residue levels quoted correspond to theoretical values calculated based on the activity level equivalent to twice the background activity level
[4] The determined activity level in the vine leaves was found to be equivalent to twice the background activity level.
The residue levels were calculated based on the significance of the results obtained. Results were only considered significant if the determined activity was greater than twice the background activity level
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-32: Extraction of the radioactive residues of ^{14}C-TMS in grape vine following application of glyphosate-trimesium at a dose rate of 1x 7.1 kg/ha (corresponding to 4.9 kg glyphosate equivalents/ha)

	Grape vine fruit		**Grape vine stems[1, 2]**		**Grape vine leaves[1, 2]**		**Grape vine stalks[1, 2]**	
Growth stage	Maturity (7 DALT)		Maturity (7 DALT)		Maturity (7 DALT)		Maturity (7 DALT)	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**<0.003**	***100***	**<0.009**	***100***	**0.031[4]**	***100***	**0.010[4]**	***100***
Methanol Extract[2]	0.001	*33.33*	-	-	-	-	-	-
Aqueous Extract[3]	<0.001	*<33.33*	-	-	-	-	-	-
Grape Debris[3]	<0.001	*<33.33*	-	-	-	-	-	-
ERR	**<0.002**	***<66.67***	-	-	-	-	-	-
RRR	**<0.001**	***<33.33***	-	-	-	-	-	-
Total sum	**<0.003**	***100***	-	-	-	-	-	-

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
mg/kg = mg ^{14}C-TMS cation equiv./kg
[1] Activities were determined on a dry weight basis
[2] The methanol extract residue level was the only significant result obtained
[3] Insignificant amounts of activity were found in the aqueous extract, grape debris and vine stems. The residue levels quoted correspond to theoretical values calculated based on the activity level equivalent to twice the background activity level.
[4] The activity levels in the vine leaves and the grape stalks were determined on a dry weight basis, the quoted residue levels are therefore artificially high
The residue levels were calculated based on the significance of the results obtained. Results were only considered significant if the determined activity was greater than twice the background activity level
Values in *italics* were calculated from reported values upon dossier compilation

The TRR in grapes for ^{14}C-PMG- and ^{14}C-TMSlabelled glyphosate-trimesium were found to be <0.006 mg/kg and <0.003 mg/kg, respectively, and therefore no characterisation was attempted.

C. Storage stability

No exact dates are reported for the experimental work from extraction to quantitative analysis of extracts. However, it is stated in the report that the study was conducted between August 1988 and March 1989. A theoretical maximum storage period can be estimated from the time period between the date of harvest (7 days after treatment on 1988-10-20) and end of March 1989 to be not longer than 155 days, or approximately 5.2 months. Hence, the samples were not stored longer than 6 months and storage stability data are not required.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in fruit crops at the end of this chapter.

III. Conclusion

The nature of the residues in plants following the use of glyphosate-trimesium was studied in grape vine. ^{14}C-glyphosate-trimesium, the trimethylsulfonium salt of glyphosate, labelled either at the glyphosate- or the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively) was applied at a rate of

8.3 or 7.1 kg a.s./ha (corresponding to 5.7 kg glyphosate equivalents/ha or 4.9 kg glyphosate equivalents/ha), respectively, to mature vines 7 days prior to sampling.

The calculated total radioactive residues in grapes (fruit) after treatment accounted for <0.006 mg/kg (^{14}C-PMG-label) and <0.003 mg/kg (^{14}C-TMS-label).
For the ^{14}C-PMG-label, the TRR was <0.023 mg/kg in grape stems, ≤0.023 mg/kg in grape leaves and <0.023 mg/kg in grape stalks. For the ^{14}C-TMS-label, the TRR was <0.009 mg/kg in grape stems, 0.031 mg/kg in grape leaves and 0.010 mg/kg in grape stalks.

Within seven days after ground treatment no significant uptake of glyphosate residues into grape vines was observed.

No characterisation of the residue was attempted.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate-trimesium in grape has been previously evaluated at EU level. It was not performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with some deficits (samples of grape vine leaves, stems and stalks were not extracted; no characterisation of the radioactive residues in samples of grape vine leaves, stems and stalks; no detailed information of the storage stability for all major components of the total radioactive residues, no description of length of storage of samples).
As there are no detailed data on storage duration, overall information given in the report may be considered. It is stated in the report that the study was conducted between August 1988 and March 1989. A theoretical maximum storage period can be estimated from the time period between the date of harvest (7 days after treatment on 1988-10-20) and end of March 1989 to be not longer than 155 days, or approximately 5.2 months. Hence, the samples were not stored longer than 6 months and storage stability data are not required. In addition, a storage stability study is available ([redacted] 2012, CA 6.1/012) showing the stability of glyphosate and its metabolite AMPA in commodities with high acid content over a storage period of 24 months.
Residues in grape commodities were determined by LSC as total ^{14}C-derived radioactivity which is expected to be stable during the course of the study. Moreover, the TRR in vine grapes (fruit) after soil drench application of ^{14}C-PMG labelled glyphosate-trimesium, calculated as the sum of extractable and residual radioactive residues, was only <0.006 mg/kg. The amounts of radioactivity determined after soil drench application of ^{14}C-PMG labelled glyphosate-trimesium in vine stems and grape stalks were less than twice the background; the radioactivity in the vine leaves was equal to twice the background level. The value reported in each case was calculated based on the radioactivity equivalent to twice the background, which is <0.023 mg PMG eq./kg dry weight for vine stems and grape stalks and ≤0.023 mg PMG eq./kg dry weight for vine leaves. Considering the fact that the calculated TRR values for vine stems and grape stalks is an overestimate and also with a view to the high water content of stalks, stems and leaves, it can be expected that the trigger for extraction and characterisation of the radioactive residues (TRR of 0.01 mg eq./kg) would actually not be exceeded if the values were referred to wet weight.
Thus, although the study does not comply with current guideline requirements in some aspects, it still gives relevant and consistent quantitative information on the uptake and distribution of glyphosate-derived residues in grape vine leaves and stems and grape stalks and fruit after soil drench application.
Therefore, this study is considered to be reliable for the assessment of the metabolic behaviour of glyphosate in fruit crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/007
Report author	[redacted]
Report year	1974
Report title	CP 67573 residue and metabolism Part 20: The metabolism of CP 67573 in grape plants
Report No	335
Document No	M-649025-01-1
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Radioactive residues in RAC are expressed in % of applied activity rather than in terms of TRR. Recalculation in mg/kg only possible for experiments where sample wet weights are available. • Residues in samples after soil, trunk or hydroponic treatment were neither characterised nor identified. • Unextracted radioactive residue for each sample not precisely quantified. • In foliar uptake experiments relevant amounts of non-extractable residues were not investigated further. No exhaustive extraction procedures were applied; unknown radioactivity was not investigated further. • No information of the storage stability for all major components of the total radioactive residues. • No description of conditions and length of storage of samples. • Foliar, trunk and hydroponic treatment are not relevant to the GAP. • Physical facility and environmental conditions insufficiently described.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this study the uptake and metabolism of ^{14}C-labelled N-(phosphono-^{14}C-methyl)glycine (glyphosate) in grapevines was investigated following soil, trunk, hydroponic or foliar application as well as uptake of ^{14}C-labelled aminomethylphosphonic acid after soil application. Different grape varieties (Concord, Thompson Seedless, and Sauvignon Blanc) were used as representatives of juice, table, and wine grapes.

Soil treatments were performed with N-(phosphono-^{14}C-methyl)glycine at a rate corresponding to 3.36 kg N-(phosphonomethyl)glycine/ha or with amino-^{14}C-methyl-phosphonic acid at a rate corresponding to 1.68 kg amino-methylphosphonic acid/ha (corresponding to 2.56 kg glyphosate/ha).
For trunk uptake, 0.41 mg N-(phosphono-^{14}C-methyl)glycine per pot was applied (corresponding to 0.17 kg glyphosate/ha).
Foliar applications were performed by applying 120 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 6 leaves (12 surfaces, top and bottom) over different time ranges.

The maximum uptake of N-(phosphono-^{14}C-methyl)glycine or its metabolite amino-^{14}C-methyl-phosphonic acid after soil treatment was 0.12 % of the applied radioactivity 12 weeks after treatment of either Concord or Sauvignon Blanc varieties. The maximum uptake into leaf was 0.087 % of the applied radioactivity (0.617 mg/kg) after application of glyphosate and up to 0.006 % of the applied radioactivity (0.118 mg/kg) after application of AMPA. In vines the maximum uptake was 0.083 % of the applied radioactivity (0.098 mg/kg) after application of glyphosate and up to 0.12 % of the applied radioactivity (0.091 mg/kg) after application of AMPA. After treatment with AMPA, 0.0053 % of the applied radioactivity were present in grapes (0.058 mg/kg expressed as glyphosate-equivalents). Moreover, the untreated control samples also contained a considerable amount of ^{14}C, as compared to the treated samples, as a result of fixation of soil evolved $^{14}CO_2$.
After trunk treatment, uptake and translocation was minimal with 1.57 % of the applied activity recovered in vines (leaves and stems), while up to 93.3 % of the applied radioactivity were found in treated trunk.
After hydroponical treatment significant ^{14}C-activity was observed in or on the roots of the grapevines; between 4.7 and 18.7 % of the applied ^{14}C-activity (0.83 – 4.10 mg/kg) was associated with the roots. Markedly less activity was observed in the aerial portions of the grapevines; the maximum uptake (sum of trunk, stem and leaf) at 10, 21, and 42 days was 0.26, 0.43 and 0.67 % of the applied radioactivity, respectively.
After foliar treatment the majority of the treatment remained on the treated leaves substantial uptake and translocation has occurred. The majority of the translocated ^{14}C-activity was associated with the stems and leaves (new growth) above the treated leaves and with the roots ^{14}C-activity translocation to the fruit was observed whenever fruit was present.

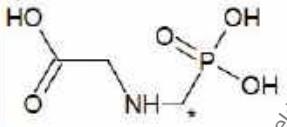
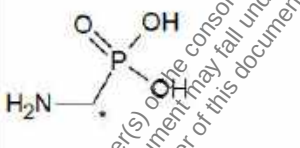
Extractabilities in treated leaves ranged between 72.0 and 101.1 % of the contained ^{14}C-activity which was solubilised by a single water extraction at room temperature; the extractabilities in new growth for the three varieties ranged between 65.1 and 112.1 %. The aqueous extractability of the treated leaf and new growth samples shows no significant pattern of change with time upon examination of the data.
Grapes were produced on some of the treated plants and also analysed for extractability with water yielding extractabilities of 64.6 and 88.0 % of the TRR for Concord and Sauvignon Blanc, respectively.
In root samples, the water extractability was significantly decreased compared to the corresponding aerial samples; however, use of 0.5 M NH_4OH under the same mild conditions (room temperature, 2 hrs) gave efficient extraction of the root samples of all three varieties. A single ammoniacal extraction released 87.6, 90.2, and 87.7 % of the ^{14}C-activity from Concord, Sauvignon Blanc and Thompson seedless varieties, respectively.

Chromatographic analysis of the aqueous extracts after foliar treatment showed that the major residue in treated leaves, new growth above the treatment, roots and old stock and grapes was parent glyphosate, at amounts of 70.5 – 97.1 % of the TRR, 58.5 – 103.1 %, 87.6 – 90.2 %, 64.6 – 79.5 %, respectively.

In root and old stock only glyphosate was present, while in treated leaf, new growth and grapes the metabolite aminomethylphosphonic acid (AMPA) was indicated accounting for 1.5 – 9.2 % of the TRR, 1.0 – ≤2.0 %, <1.0 %, respectively.
In new growth as well as grape unknown radioactivity was present accounting for 1.0 – 9.66 % of the TRR and 6.9 % of the TRR, respectively. Traces of N-methyl AMPA are stated to be found in grapevine leaves and stem (<1.0 % of TRR) and are discussed in context of impurity in the test item.

I. Materials and Methods

A. Materials

Test Material:	a) N-(phosphono-^{14}C-methyl)glycine b) Amino-^{14}C-methyl-phosphonic acid
Chemical structure:	a)  b)  * Position of the radio label
Radiochemical purity:	a) N-(phosphono-^{14}C-methyl)glycine for soil and preliminary foliar uptake experiment: initial 96.0 % with the presence of 3.3 % aminomethylphosphonic acid and 0.6 % N-methyl- aminomethylphosphonic acid; after storage for one year 89.9 % with the presence of 6.9 % aminomethylphosphonic acid and 1.7 % N-methyl-aminomethylphosphonic acid after storage followed by purification for large scale foliar uptake experiment: 98.9 % with 0.7 % aminomethylphosphonic acid and 0.4 % $CH_3PO_3H_2$ for hydroponic uptake experiment: 97 % b) Amino-^{14}C-methyl-phosphonic acid: > 97 % (TLC and ^{1}H-NMR)

Specific activity[1]:	a) N-(phosphono-^{14}C-methyl)glycine: for soil and preliminary foliar uptake experiment: reported: 8.03 mCi/mmol (1.76 MBq/mg) counted: 8.06 mCi/mmol (1.76 MBq/mg) for hydroponic uptake experiment: 2.05 mCi/mmol (0.45 MBq/mg) b) Amino-^{14}C-methyl-phosphonic acid reported: 9.15 mC/mmol (3.05 MBq/mg) obtained: 9.23 mC/mmol (3.08 MBq/mg)
CAS No:	Glyphosate: 1071-83-6 Aminomethylphosphonic acid: 1066-51-9
Log $P_{o/w}$:	Glyphosate: -3.2 Aminomethylphosphonic acid: -2.47

[1] specific activity in MBq/mg calculated based on a molecular mass of 169.07 g/mol for N-(phosphono-^{14}C-methyl)glycine and of 111.04 g/mol for amino-^{14}C-methyl-phosphonic acid

Test system:

Crop:	Grape vine (Varieties Concord, Sauvignon Blanc, Thompson Seedless)
Botanical name:	*Vitis vinifera*
Soil:	Norfolk loamy sand (1.0 % organic matter, 2.3 % clay, 11 % silt, 86.0 % sand, pH 5.7) Ray silt loam (1.0 % organic matter, 0.6 % clay, 82.3 % silt, 6.0 % sand, pH 6.5)
Crop part(s):	Vines, leaves, stems, trunk, roots, fruit (grapes)

B. Study design

1. In-life phase

The experiments were conducted under greenhouse conditions.
Two rooted cuttings of either Concord or Sauvignon Blanc grape vine plants were planted in plastic pots using Ray silt loam soil. For soil uptake experiments, cuttings were grown in Norfolk loamy sandy soil. The plants were watered as necessary daily and supplemented with dilute Hoagland's nutrient solution two times a week. The grapevines were grown at a temperature of 24 - 27 °C. Thompson Seedless grapevines were grown in soil in the same manner after first rooting the cuttings in sand.
Two rooted cuttings of Concord or Sauvignon Blanc or four cuttings of Thompson Seedless grape vine plants were planted in sand in plastic pots. The plants were watered twice daily, and nutrient was provided by application of dilute Hoagland's solution several times a week. The grapevines were grown at a temperature of 24 - 29 °C.

Soil and trunk uptake experiment:
Four pots, each containing with two rooted cuttings of either Concord or Sauvignon Blanc variety in Norfolk loamy sandy soil were utilised for the soil uptake study approximately four weeks after the end of dormancy. One pot was treated on the soil surface with 8.2 mg N-(phosphono-^{14}C-methyl)glycine in formulation (prepared by mixing of the radioactive test substance with isopropylamine and water and Atlas G-3780A adjuvant), corresponding to an application rate of 3.36 kg

N-(phosphonomethyl)glycine/ha. The radioactivity applied was 861000000 dpm (14.35 MBq). A second pot was treated on the soil surface with amino-^{14}C-methyl-phosphonic acid (1 mg/mL in 0.1 M NH_4HCO_3), corresponding to an application rate of 1.68 kg/ha (2.56 kg/ha expressed as parent glyphosate eqivalents). The radioactivity applied was 750000000 dpm (12.50 MBq), corresponding to 4.1 mg amino-^{14}C-methyl-phosphonic acid.
The trunks of two cuttings growing in a third pot were each treated with N-(phosphono-^{14}C-methyl)glycine in formulation as above. The radioactivity applied was 42000000 dpm (0.70 MBq), corresponding to 0.41 mg N-(phosphono-^{14}C-methyl)glycine corresponding to 0.17 kg glyphosate/ha.
The treated trunks were covered with a slitted polyethylene tube and sealed with tape to minimise loss of sample during watering.
The remaining pot was used as a control in order to monitor for $^{14}CO_2$ evolution from the treatment. The pots were watered from the top twice daily for the duration of the experiment.
Hydroponic uptake experiment:
Three weeks after planting in sand culture, 15 rooted cuttings of Concord variety were removed, and the roots rinsed with distilled water. One rooted cutting was placed in each hydroponic chamber with 2 L of grape nutrient medium, aerated with an air pump.
To the hydroponic solution of each chamber, 2 mg (50000000 dpm, 0.83 MBq) of N-(phosphono-^{14}C-methyl)glycine was added. Immediately thereafter, 8, 18, 38, and 78 mg of unlabelled N-(phosphonomethyl)glycine (1 mg/mL in grape nutrient at pH 6.0) were each added in sets of three to the ^{14}C treated hydroponic solution, resulting in five sets of three grape hydroponics which contained 0, 5, 10, 20, and 40 mg/kg concentrations of N-(phosphonomethyl)glycine. The volume of nutrient solution was maintained by daily adding fresh nutrient solution.

Foliar uptake experiments (large scale uptake and metabolism experiments):
Five different treatment schedules were applied in the large scale foliar uptake experiments.
For experiment 1, six containers (12 plants) of Concord variety one year old rooted cuttings growing in sand culture were treated three weeks after the end of dormancy. For experiment 2, 7 pots (14 plants) of Sauvignon Blanc rooted cuttings which had been non-dormant in sand culture for 8 weeks were used; 6 pots were treated, and one pot was used as a control.
For each plant two growing shoots were selected, and foliar treatment was carried out on the three largest and most developed leaves. Top and bottom of the leaf surface were both treated with 20 µL (10 µg) of N-(phosphono-^{14}C-methyl)glycine in formulation (prepared by mixing of the radioactive test substance with isopropylamine and water and Atlas G-3780A adjuvant) using a 25 µL syringe. Each plant received a total of 120 µg (12250000 dpm, 0.204 MBq) of N-(phosphono-^{14}C-methyl)glycine distributed over six leaves and twelve surfaces (top and bottom). After the applied solution had evaporated from the leaf surface, the treated leaves were protected by covering with small plastic bags cut open at the lower end to allow normal leaf respiration. All treated pots were placed on a cart containing sand along with the untreated control pot. Plants were continuously exposed via the treated leaves to the labelled herbicide for a total of 28 days.

Experiment 3 was carried out in the same manner as experiments #1 and #2. Three pots each containing 3 Thompson Seedless grapevines were treated and a fourth pot was used as an untreated control. One shoot (three leaves) per plant was treated with the aforementioned formulated N-(phosphono-^{14}C-methyl)glycine; each plant received 60 µg (6130000 dpm, 0.102 MBq) of herbicide distributed over three leaves.
Plants were continuously exposed via the treated leaves to the labelled herbicide for a total of 28 days.

Experiment 4 was carried out using 12 pots (24 plants) of Concord variety grown for 3 weeks in sand culture. N-(phosphono-^{14}C-methyl)glycine was applied to plants of 6 pots at the same rate and in the same manner as in experiments 1 and 2. Six pots (12 plants) were maintained as untreated controls and equally divided between two carts containing the treated plants. The duration of the experiment was 70 days; exposure to labelled herbicide was terminated seven days after treatment by detaching the treated leaves.

For experiment 5, 150 Concord variety rooted cuttings growing in 75 pots of Ray silt loam soil were treated with N-(phosphono-^{13}C-methyl)glycine and N-(phosphono-^{14}C-methyl)glycine. The application solution was prepared by mixing of the radioactive test substance with isopropylamine and water and Atlas G-3780A adjuvant. A total of 202800000 dpm (3.38 MBq, 27.0 mg) was applied to 150 plants. Plants were continuously exposed via the treated leaves to the labelled herbicide for a total of 28 days.

2. Sampling

The sampling time schedule for each experiment is listed in the table above.

Soil and trunk uptake experiment:
At 6 and 12 weeks, one plant from each treated and non-treated pot was cut off approx. 2.54 cm above the soil level. In the case of the trunk treatment, the treated area was analysed separately as were any grapes present. At harvest, the wet weight of each sample was determined after which the sample was frozen, lyophilised, the dry weight determined, ground to 40 mesh in a Wiley Mill.
Foliar uptake experiments (model study):
At 7 and 26 days, one of the plants was harvested to give the following samples: treated leaves, leaves and stem above treatment, stem connecting treated leaves, leaves and stem below treatment, other new growth and the trunk, and roots. At harvest, the wet weight of each sample was determined after which the sample was frozen, lyophilised, the dry weight determined, ground to 40 mesh in a Wiley Mill.

Hydroponic uptake experiment:
At 10, 21 and 42 days, one grapevine at each treatment rate (excluding controls) were harvested. Plants were removed, root, trunk, stem, and leaves of each sample separated, and the wet weights determined. In the case of root samples, the roots were rinsed with approximately 1000 mL of water and both the rinse and appropriate hydroponic solution assayed by LSC. After freezing, the samples were lyophilised, the dry weight determined, ground to 40 mesh in a Wiley mill.

Foliar uptake experiments (large scale uptake and metabolism experiments):
In experiments 1, 2 and 3, one third of the treated plants each time was harvested at 7 and 14 days. The experiments were terminated after 28 days with harvest of the remaining plants. The plants were separated into treated leaves, leaves and stem above treatment, remaining leaves and stem, grapes, and trunk and roots.

In experiment 4, one sixth of both the treated and untreated plants each time was harvested at 7, 14, 28, 42, 56 and at termination of the experiment at 70 days. The plants were separated into treated leaves, stems (shoots) and leaves, and trunk and roots.

Plants from experiment 5 were harvested at termination after 28 days. The harvested plant parts were weighed, frozen and lyophilised. The dry weight was determined, and each plant sample ground to 40 mesh in a Wiley Mill.

Table 6.2.1-33: Overview on soil, trunk, hydroponic and foliar application experiments in grape

Experiment		Grape variety	Duration of experiment (days)	Sampling (days)
Soil uptake experiments				
	Norfolk loamy sandy soil N-(phosphono-^{14}C-methyl)glycine 3.36 kg/ha	Concord Sauvignon Blanc	84	42, 84
	Norfolk loamy sandy soil Amino-^{14}C-methyl-phosphonic acid 1.68 kg/ha (2.56 kg/ha expressed as glyphosate equivalents)	Concord Sauvignon Blanc	84	42, 84

Table 6.2.1-33: Overview on soil, trunk, hydroponic and foliar application experiments in grape

	Experiment	Grape variety	Duration of experiment (days)	Sampling (days)
Trunk application experiments				
	N-(phosphono-^{14}C-methyl)glycine 0.41 mg per pot with two trunks corresponding 0.17 kg glyphosate/ha	Concord Sauvignon Blanc	84	42, 84
Hydroponic treatment experiments				
	N-(phosphono-^{14}C-methyl)glycine 5, 10, 20, 40 mg/kg hydroponic solution	Concord	42	10, 21, 42
Foliar uptake experiments				
1	12 plants in sand culture, in total 120 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 6 leaves and 12 surfaces (top and bottom) Treatment continuously	Concord	28	7, 14, 28
2	12 plants in sand culture, in total 120 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 6 leaves and 12 surfaces (top and bottom) Treatment continuously	Sauvignon Blanc	28	7, 14, 28
3	9 plants in sand culture, in total 60 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 3 leaves and 6 surfaces (top and bottom) Treatment continuously	Thompson Seedless	28	7, 14, 28
4	Pulse experiment 12 plants in sand culture, 120 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 6 leaves and 12 surfaces (top and bottom) Treatment for 7 days	Concord	70	7, 14, 28, 42, 56, 70
5	Large scale uptake experiment 150 plants in Ray silt loam, 180 µg mixture of phosphono-^{13}C-methyl)glycine and N-(phosphono-^{14}C-methyl)glycine (12.5:1) per plant distributed over 6 leaves (12 surfaces) Treatment for 28 days	Concord	28	28

3. Analytical procedures

All foliage samples were extracted with distilled water by stirring for two hours at room temperature. The plant residue was removed by centrifugation, and the extractable radioactivity was assayed by liquid scintillation counting (LSC).

Grape samples were homogenised with distilled water in a blender for 5 minutes. The plant residue was removed by centrifugation, and the extractable radioactivity was assayed by LSC. The plant residue was lyophilised and non-extractable radioactivity was assayed by PACA combustion.

Root samples were extracted by stirring 0.5 M NH_4OH for two hours. The residue was removed by centrifugation, and the extractable radioactivity was assayed by LSC.

Plant extracts (foliage, grapes and roots) were chromatographed on a cation exchange column (AG 50W-X4 /H^+). Fractions comprising the major ^{14}C-containing peak were pooled and assayed by LSC.

The pooled fractions of grape and roots from the AG-50 column were chromatographed for further identification on AG l-X8 / HCO_3^- and additionally on AG-50W-X8 /H^+.

The major ^{14}C-containing fractions of roots were further characterised after pooling and concentration by TLC/ Beta-camera analysis.
As the final purification step prior to derivatisation of plant metabolites, a gel filtration column (Bio-Gel P-2) has been developed.
Standard compounds, chromatographic fractions and plant extracts were characterised using two-dimensional TLCs on cellulose plates. Radioactive spots were quantitated by Beta-camera analysis. Amino acids and amino acid analogues were detected with Ninhydrin reagent and ammonium molybdate-perchloric acid. Hanes reagent was applied to detect phosphorous-containing compounds under ultraviolet light. Identification/characterisation was done by co-chromatography with reference standards.
For spectral characterisation of the radioactive residues, extraction was performed with water on samples of grape forage (stems and leaves above treatment) from the four weeks foliar application experiment (experiment #5). Extracts were assayed by LSC. The extract was then purified sequentially by different columns (AG 50W-X4, AG 1-X8, AG 50W-X8 and Bio-Gel P-2) and analysed by NMR (^{1}H-, ^{13}C and ^{31}P-NMR) and GC-MS/COM on glass columns packed with 1.5 % OV-17 on Chromosorb W-HP or 3 % OV-25 on Chromosorb W-HP after derivatisation to the n-butyl N-trifluoroacetyl derivatives using diazo-n-butane and trifluoroacetic acid/ trifluoroacetic anhydride.

II. Results and Discussion

A. Total radioactive residues (TRRs)

Soil and trunk uptake experiment:
After soil treatment the maximum uptake of N-(phosphono-^{14}C-methyl)glycine or its metabolite amino-^{14}C-methyl-phosphonic acid was 0.12 % of the applied radioactivity 12 weeks after treatment of either Concord or Sauvignon Blanc varieties. The maximum uptake into leaf was 0.087 % of applied radioactivity (0.617 mg/kg) after application of glyphosate and up to 0.006 % of the applied radioactivity (0.118 mg/kg) after application of AMPA. In vines the maximum uptake was 0.083 % of the applied radioactivity (0.098 mg/kg) after application of glyphosate and up to 0.12 % of the applied radioactivity (0.091 mg/kg) after application of AMPA. After treatment with AMPA, 0.0053 % of the applied radioactivity were present in grapes corresponding to 0.058 mg/kg expressed as glyphosate-equivalents.
Moreover, the untreated control samples also contained a considerable amount of ^{14}C, as compared to the treated samples, as a result of fixation of soil evolved $^{14}CO_2$.

After trunk treatment at 0.4 mg N-(phosphono-^{14}C-methyl)glycine per two trunks, uptake and translocation was minimal with 1.57 % of the applied activity recovered in vines (leaves and stems) (0.086 mg/kg), while up to 93.3 % of the applied radioactivity were found in treated trunk, 0.0026 % of the applied radioactivity was found in grapes after 42 days corresponding to 0.016 mg/kg.
The results are summarised in the table below. The calculation of the total radioactive residue in mg/kg was done based on data provided in the report.

Hydroponic uptake experiment:
Phytotoxic effects were observed in the hydroponic experiments. After 8 days treatment the 40 mg/kg treatments showed chlorotic leaves while 20 mg/kg treatments had lesser effects and 5 and 10 mg/kg treatments were normal. Wilted leaves were also observed on the 40 mg/kg grape treatments and after two weeks, no new root growth was evident on both the 20 and 40 mg/kg treatments. At 42 days, the grapevine treated with 40 mg/kg N-(phosphonomethyl)glycine was nearly dead. Very minor if any phytotoxicity was observed at 42 days with 5 and 10 mg/kg treatments.

The hydroponic uptake in hydroponic solution was investigated for 10, 21, and 42 days. Significant ^{14}C-activity was observed in or on the roots of the grapevines; between 4.7 and 18.7 % of the applied ^{14}C-activity (0.83 – 4.10 mg/kg) was associated with the roots.
Markedly less activity was observed in the aerial portions of the grapevines; the maximum uptake (sum of trunk, stem and leaf) at 10, 21, and 42 days was 0.26, 0.43 and 0.67 % of the applied radioactivity respectively.

Hydroponic uptake was not examined further due to the low aerial uptake, the excessive amounts of ^{13}C-methane labelled glyphosate that would have been required, the potential formation and incorporation of artefacts of metabolism in the hydroponic solution and the fact that any glyphosate that gets into a grapevine is not likely to come from root uptake via the soil. Results of the hydroponical treatments are summarised in the table below.

Foliar uptake experiments:
In experiments 1 - 3 comparing the different grapevine varieties, the uptake between the varieties was quite comparable. The main part of radioactivity was found in the treated stem accounting for 57.8 to 72.2 % of the applied radioactivity at day 7 and for 46.7 to 54.9 % of the applied radioactivity at day 28.
Radioactivity in the new growth region accounted for 0.8 to 8.7 % at day 7 and 1.6 to 4.9 % of the applied radioactivity at day 28 and for 12.8 to 33.4 % and 11.8 to 18.8 % in the stems and roots at day 7 and 28 respectively.
In the pulse experiment (experiment 4) the radioactivity in treated leaves remained relatively constant and accounted for 71.5 % after 7 days, 80.9 % after 56 days and 71.3 % after 70 days. In new growth 4.9 % of the applied radioactivity was present after 7 days and 8.1 % after 70 days, while in stems and roots 14.4 % were present after 7 days and 8.2 % after 70 days.
In the large scale experiment (experiment 5) where samples were taken after 28 days, also the main amount of radioactive residues was present in treated leaves (37.2 % of applied radioactivity), 9.4 % in new growth (leaves and stem) above treatment, and 3.8 % in other new growth regions, while 0.004 % of the applied radioactivity was present in grapes.
In an additional experiment the translocation of radioactive residues from painted leaves was investigated after 7 and 26 days. The main part of translocated radioactive residues was found in leaves and stem above the treated leaves (accounting for 16.2 % and 9.24 % of the applied radioactivity after 7 and 26 days respectively), while 8.0 % were present in roots after 26 days.
The different foliar uptake experiments showed that although the majority of the treatment remained on the treated leaves substantial uptake and translocation has occurred. The majority of the translocated ^{14}C-activity was associated with the stems and leaves (new growth) above the treated leaves and with the roots. ^{14}C-activity translocation to the fruit was observed whenever fruit was present.
Phytotoxic symptoms of enhanced secondary budding, chlorosis, and terminal bud growth inhibition were observed at these treatment rates and these rates of uptake.
Results of the different foliar treatments are summarised in the tables below.

Table 6.2.1-34: Recovered radioactivity in grape vine matrices after soil treatment with N-(phosphono-^{14}C-methyl)glycine at a rate equivalent to 3.36 kg/ha or amino-^{14}C-methyl-phosphonic acid at a rate equivalent to 1.68 kg/ha (2.56 kg/ha expressed as glyphosate equivalents) or trunk treatment with N-(phosphono-^{14}C-methyl)glycine at a rate of 40 µg per tree

Treatment	N-(phosphono-^{14}C-methyl)glycine		Amino-^{14}C-methyl-phosphonic acid		Control	
DALT	**42**	**84**	**42**	**84**	**42**	**84**
Soil treatment						
%AR[1]						
Vine (Concord)	0.067	0.083	0.068	0.12	0.031[3]	0.041[3]
Dead leaves (Concord)	0.087	NP	0.006	NP	-	-
Vine (Sauvignon Blanc)	0.075	0.078	0.094	0.049	0.061[3]	0.066[3]
Grapes (Sauvignon Blanc)	-	-	0.0053	NP	-	-
TRR (mg/kg)[2]						
Vine (Concord)	*0.098*	*0.095*	*0.056*	*0.091*	*0.082*[3]	*0.045*[3]
Dead leaves (Concord)	*0.617*	-	*0.118*	-	-	-

Table 6.2.1-34: Recovered radioactivity in grape vine matrices after soil treatment with N-(phosphono-^{14}C-methyl)glycine at a rate equivalent to 3.36 kg/ha or amino-^{14}C-methyl-phosphonic acid at a rate equivalent to 1.68 kg/ha (2.56 kg/ha expressed as glyphosate equivalents) or trunk treatment with N-(phosphono-^{14}C-methyl)glycine at a rate of 40 µg per tree

Treatment	N-(phosphono-^{14}C-methyl)glycine		Amino-^{14}C-methyl-phosphonic acid		Control	
DALT	**42**	**84**	**42**	**84**	**42**	**84**
Vine (Sauvignon Blanc)	*0.139*	*0.103*	*0.091*	*0.065*	*0.078*[4]	*0.107*[3]
Grapes (Sauvignon Blanc)	-	-	*0.058*	-	-	-
Trunk treatment						
%AR[1]						
Vine (Concord)	0.738	0.98				
Treated stem (Concord)	82.72	67.12				
Dead leaves (Concord)	0.13	NP				
Vine (Sauvignon Blanc)	0.267	1.57				
Treated stem (Sauvignon Blanc)	93.3	34.31				
Grapes (Sauvignon Blanc)	0.0026	NP				
TRR (mg/kg)[2]						
Vine (Concord)	*0.037*	*0.034*				
Treated stem (Concord)	*29.96*	*32.78*				
Dead leaves (Concord)	*0.257*	*NP*				
Vine (Sauvignon Blanc)	*0.021*	*0.086*				
Treated stem (Sauvignon Blanc)	*28.30*	*6.18*				
Grapes (Sauvignon Blanc)	*0.016*[4]	*NP*				

DALT = days after last treatment

1 % AR: Percent of applied radioactivity (N-(phosphono-^{14}C-methyl)glycine initial: 8.61 x 10^8 dpm (8.2 mg) (soil treatment) and 0.42 x 10^8 dpm (0.4 mg) (trunk treatment); amino-^{14}C-methyl-phosphonic acid initial: 7.62 x 10^8 dpm (4.1 mg) (soil treatment)

2 TRRs were calculated upon dossier compilation from reported dpm, wet weight data and specific activity. Results were expressed as glyphosate-equivalents. A conversion factor of 1.52 was applied for calculation of TRRs of aminomethylphosphonic acid (molecular weight of glyphosate (169.07 g/mol)/molecular weight of aminomethylphosphonic acid (111.04 g/mol)

3 Based on target activity applied

4 The calculation of TRR in mg/kg was checked for each sample also by calculation using % incorporated, amount applied as well as wet weight. In all cases the results of different calculation techniques were in good accordance with the exception of TRR for grapes of sauvignon Blanc with a difference of factor of 10. The calculation based on % incorporated as given in the report table resulted in a TRR of only 0.001 mg/kg.

NP: not performed

Values in *italics* were calculated upon dossier compilation

Table 6.2.1-35: Recovered radioactivity and total radioactive residues in Concord grape vine matrices after hydroponic treatment with N-(phosphono-^{14}C-methyl)glycine at 5 – 40 mg/L in solution

Sample	10 DALT		21 DALT		42 DALT	
	% AR	**TRR (mg/kg)**	**% AR**	**TRR (mg/kg)**	**% AR**	**TRR (mg/kg)**
5 mg/L in hydroponic solution						

Table 6.2.1-35: Recovered radioactivity and total radioactive residues in Concord grape vine matrices after hydroponic treatment with N-(phosphono-^{14}C-methyl)glycine at 5 – 40 mg/L in solution

	10 DALT		21 DALT		42 DALT	
Sample	**% AR**	**TRR (mg/kg)**	**% AR**	**TRR (mg/kg)**	**% AR**	**TRR (mg/kg)**
Root	9.82	*2.327*	15.03	*2.686*	12.37	*2.724*
Trunk	0.04	*0.038*	0.09	*0.155*	0.09	*0.107*
Stem	0.05	*0.068*	0.13	*0.120*	0.37	*0.598*
Leaf	0.12	*0.117*	0.21	*0.129*	0.21	*0.167*
Hydroponic solution	70.14	*0.708*	42.30	*0.387*	56.9	*0.570*
Rinse	2.51	*0.047*	2.60	*0.048*	2.5	*0.029*
Total	82.68	-	60.36	-	72.44	-
10 mg/L in hydroponic solution						
Root	6.45	*2.075*	7.77	*1.940*	18.66	*4.102*
Trunk	0.04	*0.068*	0.04	*0.065*	0.07	*0.106*
Stem	0.03	*0.069*	0.07	*0.113*	0.29	*0.498*
Leaf	0.07	*0.128*	0.16	*0.149*	0.21	*0.169*
Hydroponic solution	75.69	*0.742*	67.5	*0.638*	41.4	*0.385*
Rinse	1.42	*0.026*	2.6	*0.048*	4.5	*0.036*
Total	83.7		78.14	-	64.83	-
20 mg/L in hydroponic solution						
Root	6.91	*1.596*	6.00	*1.643*	9.12	*2.562*
Trunk	0.02	*0.028*	0.13	*0.202*	0.07	*0.121*
Stem	0.04	*0.058*	0.06	*0.113*	0.13	*0.284*
Leaf	0.09	*0.109*	0.13	*0.157*	0.19	*0.240*
Hydroponic solution	78.4	*0.751*	78.3	*0.706*	67.9	*0.601*
Rinse	2.32	*0.042*	1.3	*0.024*	1.7	*0.032*
Total	87.78	-	85.92	-	79.11	-
40 mg/L in hydroponic solution						
Root	4.73	*0.833*	10.15	*1.523*	14.67	*2.901*
Trunk	0.09	*0.080*	0.06	*0.075*	0.19	*0.175*
Stem	0.06	*0.093*	0.10	*0.114*	0.14	*0.435*
Leaf	0.11	*0.148*	0.20	*0.155*	0.30	*0.536*
Hydroponic solution	87.17	*0.802*	64.2	*0.628*	63.4	*0.640*
Rinse	2.11	*0.039*	3.6	*0.067*	2.9	*0.050*
Total	94.27	-	78.31	-	81.60	-

DALT = days after last treatment
% AR = Percent of applied radioactivity (N-(phosphono-^{14}C-methyl)glycine initial: 10.05 x 10^6dpm (approximately 95.76 µg per plant)
TRRs were calculated upon dossier compilation from reported radioactivity and wet weight data, expressed as glyphosate-

Table 6.2.1-35: Recovered radioactivity and total radioactive residues in Concord grape vine matrices after hydroponic treatment with N-(phosphono-^{14}C-methyl)glycine at 5 – 40 mg/L in solution

	10 DALT		21 DALT		42 DALT	
Sample	**% AR**	**TRR (mg/kg)**	**% AR**	**TRR (mg/kg)**	**% AR**	**TRR (mg/kg)**

equivalents.
Values in *italics* were calculated upon dossier compilation

Table 6.2.1-36: Recovered radioactivity in Concord, Sauvignon Blanc and Thompson seedless grape vine matrices after foliar treatment with a total of 120 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 6 leaves and 12 surfaces (top and bottom) for Concord and Sauvignon variety and 3 leaves and 12 surfaces (top and bottom) for Thompson variety, each continuously

	Concord grape vine matrices experiment 1			Sauvignon Blanc grape vine experiment 2			Thompson seedless experiment 3		
DALT	**7**	**14**	**28**	**7**	**14**	**28[1]**	**7**	**14**	**28**
Sample	**% AR**			**% AR**			**% AR**		
Treated leaves	72.2	60.9	54.9	57.8	62.2	46.7	65.4	57.7	43.3
New growth (leaves and stem) above treatment	8.7	6.8	4.9	0.8	3.1	1.6	1.1	5.0	0.7
Others	0.5	0.5	0.7	0.8	3.5	0.7	0.4	0.8	0.3
Stems and roots	15.9	7.9	18.8	33.4	12.8	16.2	12.8	12.1	11.8
Accountability	97.3	76.1	79.3	92.8	81.6	65.2	79.7	75.6	56.1
Control treated plant	n.a.	n.a.	0.63	n.a.	n.a.	0.69	n.a.	n.a.	0.38

DALT = days after last treatment
% AR = Percent of applied radioactivity
n.a. = not analysed
No calculation of the total radioactive residue in mg/kg was possible from the data provided in the report.
[1] In Sauvignon Blanc at day 28 grapes contained 0.7 %AR.

Table 6.2.1-37: Recovered radioactivity in Concord grape vine matrices after foliar treatment with a total of 120 µg N-(phosphono-^{14}C-methyl)glycine per plant distributed over 6 leaves and 12 surfaces (top and bottom) over 7 days

	Concord grape vine matrices experiment 4					
DALT	**7**	**14**	**28**	**42**	**56**	**70**
Sample	**% AR**					
Treated leaves	71.5	70.1	62.7	77.1	80.9	71.3
New growth (leaves and stem) above treatment	4.9	11.5	3.9	3.6	4.1	8.1
Stems and roots	14.4	23.4	16.2	11.7	6.7	8.2
Accountability	90.8	105.0	82.8	92.4	91.7	88.1
Control treated plant	0.12	0.28	0.48	0.35	0.25	2.03

DALT = days after last treatment
% AR = Percent of applied radioactivity

No calculation of the total radioactive residue in mg/kg was possible from the data provided in the report.

Table 6.2.1-38: Recovered radioactivity in Concord grape vine matrices after foliar treatment with a total of 180 µg N-(phosphono-$^{13/14}$C-methyl)glycine per plant distributed over 6 leaves and 12 surfaces over 28 days

	Concord grape vine matrices experiment 5
DALT	**28**
Sample	**% AR**
Treated leaves	37.2
New growth (leaves and stem) above treatment	9.4
Other phytotoxic new growth	3.8
Grapes	0.004
Other new growth	n.a.
Old stem and trunk	n.a.
Roots	n.a.

DALT = days after last treatment
n.a. = not analysed
% AR = Percent of applied radioactivity

Table 6.2.1-39: Recovered radioactivity in Concord grape vine matrices after painted leaf treatment with 95.8 µg N-(phosphono-^{14}C-methyl)glycine over 7 days

	Concord grape vine matrices	
DALT	**7**	**26**
Sample	**% AR**	
Treated leaves and connecting stems	100	101.86
Leaves and stem above treatment	16.2	9.24
Stem connecting treated leaves	-	1.24
Leaves and stem below treatment	0.5	0.41
Other new growth including trunk	1.4	5.74
Roots	-	8.00

DALT = days after last treatment
% AR = Percent of applied radioactivity
No calculation of the total radioactive residue in mg/kg was possible from the data provided in the report.

B. Extraction and characterisation of residues

The plant contained ^{14}C-activity resulting from the foliar treatment studies was analysed for extractability using water as the solvent for all matrices except roots which were extracted with 0.5 M NH_4OH in order to remove bound N-(phosphono-^{14}C-methyl)glycine.

The different experiments investigating the foliar uptake as a function of time as well as for different grape varieties did not show any difference.

As shown in the following tables, extractabilities in treated leaves ranged between 72.0 and 101.1 % of the contained ^{14}C-activity which was solubilised by a single water extraction at room temperature; the extractabilities in new growth ranged between 65.1 and 112.1 %. No significant variation in extractability was observed as a function of different varieties of grapevines. The aqueous extractability of the treated leaf and new growth samples shows no significant pattern of change with time upon examination of the data.

Grapes were produced on some of the treated and analysed for extractability with water yielding extractabilities of 64.6 and 88.0 % of the TRR for Concord and Sauvignon Blanc respectively.

In root samples, the water extractability was significantly decreased compared to the corresponding aerial samples; however, use of 0.5 M NH_4OH under the same mild conditions (room temperature, 2 hrs) gave efficient extraction of the root samples of all three varieties. A single ammoniacal extraction released 87.6, 90.2, and 87.7 % of the ^{14}C-activity from Concord, Sauvignon Blanc and Thompson seedless varieties respectively (experiments 1, 2, and 3).

Chromatographic analysis of the aqueous extracts after foliar treatment showed that the major residue in treated leaves, new growth above the treatment, roots and old stock and grapes was parent glyphosate, at amounts of 70.5 – 97.1 % of the TRR, 58.5- 103.1 %, 87.6 - 90.2 %, 64.6 – 79.5 %, respectively.

For the most samples, sample weights were only available in mg/kg dry matter in the report. A recalculation of values in mg/kg dry matter was of limited value for dietary purposes. Recalculation was only done in cases where wet weights were available from the report and given in the following tables.
For grape sample taken after 28 days in the Concord grapevine experiment (experiment 5), wet weights were available allowing recalculation in mg/kg. Residues of glyphosate accounting for 64.6 % of the TRR corresponded to an amount of 0.01 mg/kg wet weight.
In root and old stock only glyphosate was present, while in treated leaf, new growth and grapes the metabolite aminomethylphosphonic acid (AMPA) was identified accounting for 1.5 – 9.2 % of the TRR, 1.0 – ≤2.0 %, <1.0 %, respectively.

In new growth as well as grape unknowns were present in the void volume accounting for 1.0 – 9.66 % of the TRR and 6.9 % of the TRR, respectively.

The results of the pulse experiment (Table 6.2.1-47) show that grapevines do not rapidly degrade glyphosate after incorporation. Over a time span of 70 days glyphosate decreased from 98.5 % of the TRR to 58.5 % of the TRR only. The metabolite AMPA was formed up to 1.0 % of the TRR and remained constant during the time course pulse treatment experiment.

Traces of N-methyl AMPA are stated to be found in grapevine leaves and stem (< 1.0 % of TRR) and are discussed in context of impurity in the test item and thus may not represent actual plant metabolism.

Table 6.2.1-40: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in various parts of Concord grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces)

	Concord grape vine matrices experiment 1						
DALT	**7**		**14**		**28**		
Sample	**Treated leaves**	**New growth**	**Treated leaves**	**New growth**	**Treated leaves**	**New growth**	**Roots and old stock**
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
Aqueous extract[1]	78.7	78.3	72.0	82.9	83.2	105.1	87.6
Glyphosate	74.8	74.3	70.5	78.1	78.8	100.5	87.6
AMPA	3.9	-	1.5	-	4.4	-	-
Total identified	*78.7*	*74.3*	*72.0*	*78.1*	*83.2*	*100.5*	*87.6*

Table 6.2.1-40: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in various parts of Concord grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces)

	Concord grape vine matrices experiment 1						
DALT	**7**		**14**		**28**		
Sample	**Treated leaves**	**New growth**	**Treated leaves**	**New growth**	**Treated leaves**	**New growth**	**Roots and old stock**
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
Others	-	3.27	-	4.15	-	1.0	-
Total charac-terised	-	3.27	-	4.15	-	1.0	-
ERR	78.7	78.3	72.0	82.9	83.2	105.1	87.6
RRR[2]	*21.3*	*21.7*	*28.0*	*17.1*	*16.8*	-	*12.4*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] aqueous extract refers to water extraction except for roots where the sample material was extracted with 0.5 M NH_4OH.
[2] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation.

Table 6.2.1-41: Extraction and distribution of the radioactive residues of N-(phosphono-^{13} and ^{14}C-methyl)glycine in various parts of Concord grapevines following foliar treatment at 180 µg per 6 leaves (12 surfaces)

	Concord grape vine matrices experiment 5		
DALT	**28**		
Sample	**New growth**	**Grapes**	
	% TRR	**% TRR**	**mg/kg wet weight[1]**
Aqueous extract	83.2	64.6	*0.01*
Glyphosate	74.6	64.6	*0.01*
AMPA	-	-	-
Total identified	74.6	64.6	*0.01*
Others	4.3	-	-
Total characterised	4.3	-	-
ERR	83.2	64.6	*0.01*
RRR[2]	*16.8*	*35.4*	*0.004*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] TRR in mg/kg wet weight was calculated based on a specific activity and wet weight available. The measured dpm values expected (= 100 % TRR) were taken as reference for calculation.
[2] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation.

Table 6.2.1-42: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in treated leaves of Sauvignon Blanc grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces)

	Sauvignon Blanc grape vine matrices experiment 2		
DALT	**7**	**14**	**28**
Sample	**Treated leaves**		
	% TRR	**% TRR**	**% TRR**
Aqueous extract	90.5	91.1	93.5
Glyphosate	87.7	88.2	84.3
AMPA	2.8	2.9	9.2
Total identified	*90.5*	*91.10*	*93.50*
Others	-	-	
Total characterised	-	-	-
ERR	90.5	91.1	93.5
RRR[1]	*9.5*	*8.9*	*6.5*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation.

Table 6.2.1-43: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in new growth of Sauvignon Blanc grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces)

	Sauvignon Blanc grape vine matrices experiment 2				
DALT	**7**	**14**	**28**		
Sample	**New growth**			**Grape**	**Roots and old stock**
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**
Aqueous extract[1]	112.1	95.5	103.3	88.0	90.2
Glyphosate	103.1	80.1	84.3	79.5	90.2
AMPA	-	1	2	< 1.0	-
Total identified	*103.1*	*81.1*	*86.3*	*80.5*	*90.2*
Others	7.93	7.79	7.08	6.9	-
Total characterised	7.93	7.79	7.08	6.9	-
ERR	112.1	95.5	103.3	88.0	90.2
RRR[2]	-	*4.5*	-	*12.0*	*9.8*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] Aqueous extract of grape and roots and old stock refers to water extraction except for roots where the sample material was extracted with 0.5 M NH_4OH.
[2] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-44: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in treated leaves of Thompson seedless grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces)

	Thompson seedless grape vine matrices experiment 3		
DALT	**7**	**14**	**28**
Sample	**Treated leaves**		
	% TRR	**% TRR**	**% TRR**
Aqueous extract	101.1	89.6	72.4
Glyphosate	97.1	89.6	70.6
AMPA	-	-	1.8
Total identified	*97.1*	*89.6*	*72.4*
Others	-	-	-
Total characterised	-	-	-
ERR	101.1	89.6	72.4
RRR[1]	-	*10.4*	*27.6*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-45: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in new growth and root and old stock of Thompson seedless grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces)

	Thompson seedless grape vine matrices experiment 3			
DALT	**7**	**14**	**28**	
Sample	**New growth**			**Roots and old stock**
	% TRR	**% TRR**	**% TRR**	**% TRR**
Aqueous extract[1]	98.0	102.1	94.4	87.7
Glyphosate	88.5	89.3	74.4	87.7
AMPA	1.2	≤2.0	≤1.0	-
Total identified	*89.7*	*91.3*	*75.4*	*87.7*
Others	1.7	6.26	9.66	-
Total characterised	1.7	6.26	9.66	-
ERR	*98.0*	102.1	94.4	87.7
RRR[2]	*2.0*	-	*5.6*	*12.3*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] Aqueous extract refers to water extraction except for roots where the sample material was extracted with 0.5 M NH_4OH.
[2] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-46: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in treated leaves of Concord grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces) for 7 days

	Concord grape vine matrices experiment 4	
DALT	7	14
Sample	Treated leaves	
	% TRR	% TRR
Aqueous extract	89.0	96.3
Glyphosate	85.6	93.8
AMPA	3.4	2.5
Total identified	*89.0*	*96.3*
Others	-	-
Total characterised	-	-
ERR	89.0	96.3
RRR[1]	*11.0*	*3.7*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-47: Extraction and distribution of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine in new growth of Concord grapevines following foliar treatment at 120 µg per 6 leaves (12 surfaces) for 7 days

	Concord grape vine matrices experiment 4					
DALT	**7**	**14**	**28**	**42**	**56**	**70**
Sample	**New growth**					
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
Aqueous extract	107.6	89.8	90.5	89.7	82.0	65.1
Glyphosate	98.5	82.9	77.1	82.8	70.4	58.5
AMPA	1.0	1.0	1.0	1.0	1.0	1.0
Total identified	*99.5*	*83.9*	*78.1*	*83.8*	*71.4*	*59.5*
Others	5.7	4.1	5.3	1.2	5.0	4.8
Total characterised	5.7	4.1	5.3	1.2	5.0	4.8
ERR	107.6	89.8	90.5	89.7	82.0	65.1
RRR[1]	-	*10.2*	*9.5*	*10.3*	*18.0*	*34.9*

DALT = days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] Residual radioactive residues were not reported. The values recalculated are considered only indicative.
Values in *italics* were calculated from reported values upon dossier compilation

C. Storage stability

Storage intervals for frozen samples and extracts are not reported. No information on storage stability is reported. However, the study was performed between March 1973 and December 1973 (~10 months).

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in fruit crops at the end of this chapter.

III. Conclusion

In this study the uptake and metabolism of ^{14}C-labelled N-(phosphono-^{14}C-methyl)glycine (glyphosate) in grapevines was investigated following soil, trunk, hydroponic or foliar application as well as uptake of ^{14}C-labelled aminomethylphosphonic acid after soil application.

The uptake of N-(phosphono-^{14}C-methyl)glycine or its metabolite amino-^{14}C-methyl-phosphonic acid after soil treatment was 0.12 % of the applied radioactivity 12 weeks after treatment of either Concord or Sauvignon Blanc varieties. The maximum uptake into leaf was 0.087 % of the applied radioactivity (0.617 mg/kg) after application of glyphosate and up to 0.006 % of the applied radioactivity (0.118 mg/kg) after application of AMPA. In vines the maximum uptake was 0.083 % of the applied radioactivity (0.098 mg/kg) after application of glyphosate and up to 0.12 % of the applied radioactivity (0.091 mg/kg) after application of AMPA. After treatment with AMPA, 0.0053 % of the applied radioactivity were present (0.058 mg/kg expressed as glyphosate-equivalents).

After trunk treatment, uptake and translocation was minimal with 1.57 % of the applied activity recovered in vines (leaves and stems), while up to 93.3 % of the applied radioactivity were found in treated trunk, 0.0026 % of the applied radioactivity was found in grapes after 42 days corresponding to 0.016 mg/kg.

After hydroponical treatment significant ^{14}C-activity was observed in or on the roots of the grapevines; between 4.7 and 18.7 % of the applied ^{14}C-activity (0.83 – 4.10 mg/kg) was associated with the roots. Markedly less activity was observed in the aerial portions of the grapevines; the maximum uptake (sum of trunk, stem and leaf) at 10, 21, and 42 days was 0.26, 0.43 and 0.67 % of the applied radioactivity, respectively.

Although, after foliar treatment the majority of the treatment remained on the treated leaves, substantial uptake and translocation has occurred. The majority of the translocated ^{14}C-activity was associated with the stems and leaves (new growth) above the treated leaves and with the roots ^{14}C-activity translocation to the fruit was observed whenever fruit was present.

High extractabilities were yielded after aqueous extraction of treated leaves, new growth and grapes. In root samples, the water extractability was significantly decreased compared to the corresponding aerial samples; however, use of 0.5 M NH_4OH under the same mild conditions (room temperature, 2 hrs) gave efficient extraction.

Chromatographic analysis of the aqueous extracts after foliar treatment showed that the major residue in treated leaves, new growth above the treatment, roots and old stock and grapes was parent glyphosate, at amounts of 70.5 – 97.1 % of the TRR, 58.5 – 103.1 %, 87.6 – 90.2 %, 64.6 – 79.5 %, respectively.
In root and old stock only glyphosate was present, while in treated leaf, new growth and grapes the metabolite aminomethylphosphonic acid (AMPA) was indicated accounting for 1.5 – 9.2 % of the TRR, 1.0 – ≤2.0 %, <1.0 %, respectively.

In new growth as well as grape unknown radioactivity was present accounting for 1.0 – 9.7 % of the TRR and 6.9 % of the TRR, respectively. Traces of N-methyl AMPA are stated to be found in grapevine leaves and stem (<1.0 % of the total) and are discussed in context of impurity in the test item and thus may not represent actual plant metabolism.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in grape has been previously evaluated at EU level. It was not performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with major deficits (radioactive residues in RAC are expressed in % of applied activity rather than in terms of TRR; recalculation in mg/kg only possible for experiments where sample wet weights are available; residues after soil, trunk or hydroponic treatment were neither characterised nor identified; unextracted radioactive residue for each sample not precisely quantified; in foliar uptake experiments relevant amounts of non-extractable residues were not investigated further. No exhaustive extraction procedures were applied; unknown radioactivity was not investigated further; no information of the storage stability for all major components of the total radioactive residues; no description of conditions and length of storage of samples; foliar, trunk and hydroponic treatment are not relevant to the GAP; physical facility and environmental conditions insufficiently described).

It is considered that the study was performed in a reasonable timeframe below two years (on the front page of the report the timeframe of March 1973 to December 1973 is stated; the report date is given with June 1974) and therefore the qualitative and quantitative results of the present study are considered valid in context of storage stability.

A high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies. No degradation of glyphosate and its metabolites was found in matrices with high water content (corn forage, fodder, cotton forage, soybean forage). Over an investigated storage duration of 215-393 days no degradation was observed in the metabolic profile ([redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/022). For commodities with high acid content storage stability was shown for glyphosate and AMPA for up to 727 days in orange fruit ([redacted] and [redacted] 2012, CA 6.1/002). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1.

The study is considered reliable for the assessment of the metabolic behaviour of glyphosate in grapes because it provides data on the distribution of glyphosate and the formation of AMPA as a possible metabolite in grapes. Therefore, the present study is considered reliable for the uses in the crop category fruits.

Assessment and conclusion by RMS:

Root and tuber vegetables

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/008
Report author	[redacted]
Report year	1975
Report title	CP 67573 residue and metabolism Part 26: The metabolism of CP 67573 in potato plants- February 1974 - December 1974
Report No	376
Document No	M-649161-01-1

Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • The radiochemical purity of the test item(s) is not clearly specified. • No data on storage stability are available for this study. However, all samples of the soil propensity experiments were stored for a maximum of up to 6 months and no storage stability analysis was required. • Developmental stages of the crop at harvesting are not reported. • Only water has been used for extraction. Not extracted residues after solvent extractions were high (31.0 – 49.0 %) and no release and characterisation and/or identification was attempted. • Data to account for or track the loss of radioactivity in each subsequent step of the fractionation and isolation procedure are not provided. Total recovery (ERR + RRR) was partly < 90 % (The radioactivity balances are below 90 %) • Physical facility and environmental conditions are poorly described • For foliar application experiment the application rate is given as amount of glyphosate in mg per plant. Recalculation to g a.s./ha could be done if needed. • Radioactive residues in RACs are expressed in dpm/µg try weight and were therefore not presented in the summary. • Limit of detection (LOD) for LSC analysis is not provided. • No flow sheet or diagram depicting the overall extraction and fractionation strategies available.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Invalid
Category study in AIR 5 dossier (L docs)	Category 2b

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the degree to which ^{14}C-glyphosate is taken up from the soil and to determine the nature and magnitude of glyphosate-derived residues in potato tubers. Moreover, the objective of the study was to define what metabolic products are formed after foliar application of potato plants with Roundup® herbicide. The test substance consisted of ^{14}C labelled glyphosate or a mixture of ^{14}C and ^{12}C labelled AMPA with ^{14}C located at the carbon atom between the nitrogen and phosphonate moieties.

For the soil propensity experiments, potato plants with non-radioactive Roundup® herbicide were grown at a rate of 8.967 kg/ha and experienced no detrimental effects as compared to controls. For the foliar application experiments, the potato plants developed phytotoxic symptoms ranging from almost no effect at 50 µg per plant to complete cessation of new growth at 200 µg per plant, the highest level used. Special methods were devised to eliminate from the atmosphere of the growth chamber, $^{14}CO_2$ which was being evolved from the treated soils to avoid the possibility of $^{14}CO_2$ photofixation. In one part of the soil

propensity experiment the glyphosate was applied to a stand of weeds and after the weeds had died, the weeds and soil were thoroughly mixed, and from that point on the experiment was conducted as with the other plants in the soil propensity study. This method of incorporating the radioactive herbicide into the soil produced results, which were no different from the case where the herbicide was incorporated directly into the soil.

The nature of residues resulting from soil uptake was investigated by a pre-emergence application of 4.483 kg glyphosate acid equivalents/ha to bare soil immediately before planting of young potato shoots which were pre-grown in soil. Potato foliage was collected 9, 15 and 25 days after treatment, respectively. Potato plants were collected at 67 and 121 days and separated into tubers, tops and roots.

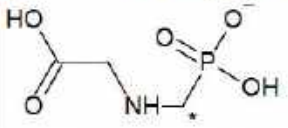
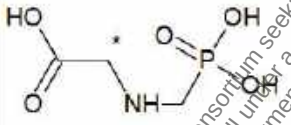
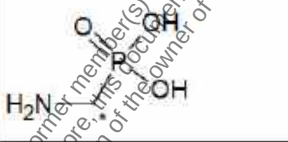
The nature of residues resulting from foliar uptake was investigated by a post-emergence treatment, involving a single application of 100 µL application solution containing ^{14}C-glyphosate (108 µg, 1.28×10^7 dpm) applied 40 days after planting (estimated approx. BBCH 50). For the foliar application experiment, replicate pairs of plants were harvested 1, 3, 14 and 34 DALT and separated into tubers, roots and tops. For all foliar applications, glyphosate was applied as the isopropylamine salt formulated as Roundup® herbicide, which is a water soluble commercial glyphosate formulation.

Analysis of soils after harvesting the plants indicated that in the case of the glyphosate treatments only 22 % of the radioactivity remained and in the case of the AMPA treatments 51 % of the starting radioactivity remained in the soil. The only identifiable soil metabolite was AMPA. N-methyl-AMPA, which exists as trace impurity in certain samples of ^{14}C-glyphosate, was also detected. Post-harvest soils, which had undergone glyphosate treatment, contained no detectable amounts of the parent compound.

The radioactivity in tubers treated pre-emergence by glyphosate or AMPA is characterised as ca. 25 % neutral, non-ionic compounds and about 50 % non-extractable (with water). AMPA was the only detected and identified metabolite accounting for about 25 % of the radioactivity. Extractability of treated leaves and of tubers from foliar post-emergence treatments was high accounting for ≥ 74 % and ≥ 86 %, respectively. Moreover, radioactivity isolated from treated leaves or tubers from the foliar experiment was determined to be practically exclusively parent compound glyphosate.

I. Materials and methods

A. Materials

1. Test Material:	N-(phosphonomethyl-^{14}C)glycine
Chemical structure:	**N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labelled glyphosate-trimesium):**  *Position of Radiolabel = ^{14}C **N-(phosphonomethyl)-^{14}C-methyl-glycine (^{14}C-glyphosate)**  **Amino-^{14}C-methylphosphonic acid (AMPA):** 
Radiochemical purity:	not stated within the report
Specific activity:	**^{14}C-Glyphosate:** Foliar application experiments: 1.98 MBq/mg (53.07 mCi/mmol or 119000 dpm/µg) Soil propensity phase experiments: 0.41 MBq/mg (1.87 mCi/mmol or 24600 dpm/µg) **^{14}C-AMPA** (3.84 mCi/mmol or 76800 dpm/µg): Diluted for soil propensity phase experiments with ^{12}C-AMPA: 0.37 MBq/mg (1.11 mCi/mmol or 22200 dpm/µg) ^{14}C-Sodium bicarbonate 1.37 MBq/mg (3.1 µCi/mol)
CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate	- 3.2 (glyphosate)

2. Test system

Soil:	Ray silt loam (pH: 6.5; organic matter 1 %, clay 0.6 %, silt: 82.3 %, sand 6.0 %, pH 6.5)
Crop:	Potato
Botanical name:	*Solanum tuberosum* (Katahdin variety)
Crop part(s):	Foliage (immature plants), tubers, tops and roots (mature plants)

B. Study design

1. In-life phase

The metabolism and uptake of ^{14}C-glyphosate labelled in the phosphonomethyl-moiety in potatoes was investigated following soil (pre-emergence) and foliar treatment (post-emergence) application. Glyphosate was applied as the isopropylamine salt formulated as Roundup@ herbicide, which is a commercial glyphosate formulation. The study was conducted in controlled environment growth chambers.

For Kathadin potato plants a rate equivalent to 8.97 kg non-radioactive glyphosate acid equivalents/ha was applied to bare soil immediately before planting. These plants experienced no detrimental effects. For the soil propensity experiment, either ^{14}C-glyphosate (23.8 mg per pot, 5.75×10^8 dpm), ^{14}C-AMPA (23.4 mg per pot, 5.2×10^9 dpm) or ^{14}C-$NaHCO_3$ (8.23×10^8 dpm per pot) were directly applied to the soils and thoroughly mixed. In addition, into two of the pots ^{14}C-glyphosate treated weeds were incorporated to simulate ploughing (23.3 mg to the weeds, 3 weeks before incorporation, 5.75×10^8 dpm). Seed potatoes were pre-grown in sand and transferred after approx. 10 days (BBCH 09) to grow in two planting pots per label each containing 10 kg radioactive soil at a rate equivalent to 4.48 kg glyphosate acid equivalents/ha.

In parallel control plots were treated with ^{12}C-glyphosate. The plants were either kept in the same growth chamber with ^{14}C-treated plants to investigate the uptake of $^{14}CO_2$ formed by degradation in soil or in separate chambers as control.

For the foliar application experiment, eight 40 day old potato plants (blooming stage, approx. BBCH 50) received each 100 µL application solution containing ^{14}C-glyphosate (108 µg, 1.28×10^7 dpm). The application solution was administered with a syringe in 2 µL droplets to the middle leaf cluster of each plant. Two untreated controls were additionally grown.

2. Sampling

Periodically, foliage samples were collected 9, 15 and 25 days after initiation of the soil propensity experiment. Whole plants were harvested after 67 days (first set of duplicate pairs) and 121 days (second set) and separated into tubers, roots and tops. Roots and tubers were washed to remove surface residues of non-absorbed ^{14}C-glyphosate. Tubers were diced into small pieces, then all parts were frozen and lyophilised until sample processing. Post-harvest soil samples were taken additionally.

For the foliar application experiment, replicate pairs of plants were harvested 1, 3, 14 and 34 DALT. Two untreated controls were harvested at 34 days. The plants were separated into the top part, tubers and roots. The top part was further subdivided into individual leaf and stem clusters for combustion and LSC analysis. Foliage samples were frozen, lyophilised and ground. Tubers and roots were rinsed in water and cut into small pieces prior freezing and lyophilisation.

3. Analytical procedures

Total radioactive residues (TRR) in foliage samples of the soil propensity experiments were determined by Liquid Scintillation Counting (LSC) following combustion.

In the soil propensity study, potato tubers were extracted in small scale and large scale experiments.

For the extraction in the small scale experiment, tuber samples were extracted four times with 0.1 N HCl, concentrated and stored refrigerated until analysed. The concentrated aqueous extract was taken through fractionation and clean-up steps to provide material for identification of radioactive compounds. Concentrated extracts were applied on AG 50W-X8 cation exchange resin and separated into non-retained and retained fractions by elution with water. Similarly, concentrated aqueous sample extracts were alkalified to pH 8 with ammonium hydroxide and purified by an anion exchange column with AG 1-X8 resin and separated into non-retained and retained fractions by elution with ammonia hydroxide.

A large scale experiment was performed in order to isolate and identify the AMPA-metabolite observed in the small scale experiment. Therefore, the remaining dry powdered tuber from four plants were combined, mixed and extracted four times with water. The extract was alkalified to pH 8.6 with ammonium hydroxide and applied to AG 1-X8 anion exchange resin and separated into non-retained and retained fractions. After collection of 160 fractions, the column was further eluted with water, 0.1 M ammonium hydrogen carbonate and 0.2 M ammonium hydrogen carbonate, respectively. Fractions containing ^{14}C-AMPA were pooled and aliquots were analysed by LSC. The pooled extract was concentrated and chromatographed on AG 50W-X8 and analysed by LSC, resulting in two major peaks. The peak fractions containing the major radioactivity was diluted in water, streaked across 6 cm of pH 5.9 buffered paper and HVE was performed at 3000 V for 30 min. Two major regions of radioactivity were detected. The region containing majority of radioactivity was used for derivatisation to produce trifluoroacetyl dimethyl ester derivatives. The sample was diluted in water and ammonium hydrogen carbonate, dried and dissolved in a mixture of trifluoroacetic acid and trifluoroanhydride at 25°C for 30 min. A stream of nitrogen gas was used to blow of the reagents, resulting in a dry crystalline material. The dry material was dissolved with benzene and diazomethane. After removal of diazomethane by a stream of nitrogen, ethyl acetate and benzene were added to the solution. Aliquots of this mixture were analysed by LSC and GLC to determine the yields of ^{14}C-AMPA-TAM. ^{14}C-AMPA-TAM was purified by silica gel purification and subsequently filtered through a sodium sulfate filter prior to quantitation by GC/MS/COM.

In the soil propensity study, post-harvest soil samples were analysed by LSC following combustion and aliquots were extracted with ammonium hydroxide for AG-1-X8 chromatography.
For the foliar application experiment, dry tuber samples were extracted three times with 0.1 N HCl and aliquots were analysed by LSC. After concentration of the remainders, the dried extracts were dissolved in a mixture of 1 N NaOH/pyridine HVE buffer, pH 5.9 (1:9, v/v) and analysed by HVE at 1500 V for 90 min or 3000 V for 30 min. Dry leaf samples were directly extracted with a mixture of 1 N NaOH/pyridine HVE buffer, pH 5.9 (1:9, v/v) for 4 hours. Aliquots were analysed by HVE as described for the soil propensity experiment and the remaining pellets were additionally extracted twice with water and analysed by LSC.

The quantification and identification following 0.1 N HCl or 1 N NaOH/pyridine buffer (1:9, v/v) extraction and concentration was performed by GC/MS following derivatisation to N-trifluoroacetyltrimethyl-esters, Ion exchange chromatography or high voltage electrophoresis (HVE) against reference substances.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) determined by LSC following combustion in soil treatment experiments for and soil are summarised in Table 6.2.1-48. Glyphosate treated soil loses about 78 % of its ^{14}C activity over the course of about 3 months, while AMPA treated soil, loses only about 49 % of its ^{14}C activity during the same period. The application of glyphosate to weeds showed a negligible effect on the uptake and growth of the potato plants. By the end of the growth period, soil treated with ^{14}C-HCO_3 is completely void of radioactivity

The TRRs in potato foliage, tubers, tops, roots were measured, but the results were presented only in dpm/µg dry weight. As the wet weight of the corresponding matrices was not reported, the recalculation in mg/kg was performed only for soil samples.

Table 6.2.1-48: Total radioactive residues in soil following soil application of glyphosate, AMPA and $NaHCO_3$

	TRR, mg/kg		% Lost
DALT	**0**	**67/121[1]**	
Control	*<0.001*	*<0.001*	---
^{14}C-$NaHCO_3$	*4.196*	*<0.001*	100
^{14}C-Glyphosate	*2.925*	*0.660*	78
^{14}C-Glyphosate (weed[2])	*2.925*	*0.680*	77
^{14}C-AMPA	*2.931*	*1.506*	49

DALT: days after last treatment
[1] Values are average of two replications, one harvested at 67 days and the other at 121 days
[2] Application was done to the weeds
Values calculated upon dossier compilation are presented in italics
TRRs were calculated based on dry weight

B. Extraction and characterisation of residues

Soil propensity experiments

Small scale extractions

The ^{14}C-levels found in fractions of potato tubers following soil application are shown in Table 6.2.1-49 to Table 6.2.1-54. Radioactivity of tuber samples of the soil propensity experiments following soil application, was approx. 50 % extractable for glyphosate and AMPA (see Table 6.2.1-49). Tuber that had grown in soil containing no ^{14}C was spiked with ^{14}C standards gave high recovery rates (≥ 80 %).

Table 6.2.1-49: Small Scale: Extractability of radioactive residues in potato tubers from plants harvested at 67 days following soil application

Tuber sample	Extract % TRR	Pellet % TRR
Control	44	n.d.
Control + A (spiked)	92	n.d.
Control + B (spiked)	80	n.d.
^{14}C-glyphosate	56	49
^{14}C-glyphosate (weed[1])	47	37
^{14}C-AMPA	56	31

Control: refers to tuber samples grown in non-radioactive soil
n.d. not determined
[1]Application was done to the weeds
A was spiked with a mixture of approx. 12 % ^{14}C-N-methyl-AMPA 66 % ^{14}C-AMPA and 22 % ^{14}C-glyphosate
B was spiked with a mixture of approx. 50 % ^{14}C-AMPA and 50 % ^{14}C-glyphosate
Values calculated upon dossier compilation are presented in italics

Aliquots of tuber extracts grown in soil containing glyphosate or AMPA or grown in control conditions were used for column chromatographies. AG-50W-X8 chromatography shows trace radioactivity in the glyphosate elution volume (up to 1.1 % of the TRR), but main radioactivity in the AMPA elution volume (up to 38.1 % of the TRR) and in the void elution volumes.

The results of the AG-1-X8 chromatography (parent compound: 7.5 % of the TRR) are in good agreement with the results obtained in the AG-50W-X8 chromatography experiment. With regard to extracts from tubers produced in soil, which had been treated with either ^{14}C-glyphosate or ^{14}C-AMPA, both methods indicated the presence of AMPA (up to 44.8 % of the TRR) and the presence of non-retarded, unidentified ^{14}C. Additionally, HVE at pH 5.9 of tuber extracts grown in soil containing glyphosate or AMPA or grown in control conditions was performed. HVE results were in good agreement with the results from the ion exchange chromatographies (mentioned above) on the same extracts.

Comparison of the results of the ion exchange chromatographies and HVE show that AMPA was the major fraction in each method. However, ^{14}C-N-methyl-AMPA, which exists as trace impurity in certain samples of ^{14}C-glyphosate, was not separated from the ^{14}C-AMPA in samples obtained by AG-50W-X8 chromatography and in samples obtained by HVE. The significant radioactive fractions in the non-retarded elution volumes of the ion exchange chromatograms and comparable radioactive levels which behaved neutrally by HVE suggest the presence of neutral ^{14}C-compounds in the extracts.

Comparisons of the levels of glyphosate fractions point to the absence of glyphosate and rather suggest the presence of low levels of acidic material with AG-1-X8 retention volume similar to glyphosate.

Table 6.2.1-50: Soil propensity experiment: AG-50W-X8 chromatography of extracted radioactive residues in potato tubers from plants harvested at 67 days following foliar application of ^{14}C-glyphosate and ^{14}C-AMPA

Residues in tubers, % TRR						
Fraction/ Soils	**Control**	**Control + A**	**Control + B**	**^{14}C-glyphosate treated**	**^{14}C-glyphosate treated (weed[1])**	**^{14}C-AMPA treated**
DALT	67					
TRR	100	100	100	100	100	100
Aqueous extract[2]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aqueous concentrate	44	92	80	56	47	56
AG-50W-X8 (water/ 1 N HCl eluate)						
Glyphosate[3]	---	16.6	32.0	1.1	0.9	0.6
AMPA/ N-methyl-AMPA	---	75.4	48.0	32.5	32.4	38.1
non-retarded (neutral compounds)	---	<1	<1	22.4	13.6	17.4
Identified	---	---	---	---	---	---
Characterised	44	92.0	80.0	56.0	47.0	56.0
ERR	44.0	92.0	80.0	56.0	47.0	56.0
RRR	n.d.	n.d.	n.d.	49.0	37.0	31.0
Total recovery	44.0	92.0	80.0	105.0	84.0	87.0

Table 6.2.1-50: Soil propensity experiment: AG-50W-X8 chromatography of extracted radioactive residues in potato tubers from plants harvested at 67 days following foliar application of ^{14}C-glyphosate and ^{14}C-AMPA

DALT days after last treatment
[1]Application was done to the weeds
[2] Samples were only LSC measured after concentration
[3] Not enough material could be isolated to permit positive identification. Comparisons of the results obtained in the tables below, point to the absence of glyphosate and suggest the presence of low levels of acidic material with AG-1-X8 (HCO_3^-) retention volume similar to glyphosate. Hence, the amounts are accounted as characterised.
n.d. not determined
Identified = AMPA was identified, but it occurs in a mixture of AMPA/N-methyl-AMPA.
Characterised = sum of non-retarded, non-specific identified (AMPA/N-methyl-AMPA) and not identified (glyphosate)
Radioactivity
TRR = total radioactive residues determined by combustion followed by LSC analysis
ERR = extracted radioactive residues
RRR= residual radioactive residues (radioactivity found in pellet)
Total recovery represents sum of concentrated extracts and RRR
A was spiked with a mixture of approx. 12 % ^{14}C-N-methyl-AMPA, 66 % ^{14}C-AMPA and 22 % ^{14}C-glyphosate
B was spiked with a mixture of approx. 50 % ^{14}C-AMPA and 50 % ^{14}C-glyphosate
Values calculated upon dossier compilation.

Table 6.2.1-51: Soil propensity experiment: AG-1-X8 chromatography of extracted radioactive residues in potato tubers from plants harvested at 67 days following foliar application of ^{14}C-glyphosate and ^{14}C-AMPA

Residues in tubers, % TRR						
Fraction/ Soils	**Control**	**Control + A**	**Control + B**	**^{14}C-glyphosate treated**	**^{14}C-glyphosate treated (weed[1])**	**^{14}C-AMPA treated**
DALT	67					
TRR	100	100	100	100	100	100
Aqueous extract[2]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aqueous concentrate	44	92	80	56	47	56
AG-1-X8 (water/ 1 N NH_4OH eluate)						
Glyphosate[3]	---	16.6	29.6	5.6	7.5	5.0
AMPA	---	66.2	48.8	35.3	31.0	44.8
non-retarded (neutral compounds)[4]	---	9.2	1.6	15.1	8.5	6.2
Identified	---	66.2	48.8	35.3	31.0	44.8
Characterised	44	25.8	31.2	20.7	16.0	11.2
ERR	44.0	92.0	80.0	56.0	47.0	56.0
RRR	n.d.	n.d.	n.d.	49.0	37.0	31.0
Total recovery	44.0	92.0	80.0	105.0	84.0	87.0

Table 6.2.1-51: Soil propensity experiment: AG-1-X8 chromatography of extracted radioactive residues in potato tubers from plants harvested at 67 days following foliar application of ^{14}C-glyphosate and ^{14}C-AMPA

DALT days after last treatment
[1] Application was done to the weeds
[2] Samples were only LSC measured after concentration
[3] Not enough material could be isolated to permit positive identification. Comparisons of the results obtained in the tables below and above, point to the absence of glyphosate and suggest the presence of low levels of acidic material with AG-1-X8 (HCO_3^-) retention volume similar to glyphosate. Hence, the amounts are accounted as characterised.
[4] includes N-methyl-AMPA
n.d. not determined
Identified = glyphosate + AMPA
Characterised = sum of non-retarded and not identified (glyphosate) radioactivity
TRR = total radioactive residues determined by combustion followed by LSC analysis
ERR = extracted radioactive residues
RRR= residual radioactive residues (radioactivity found in pellet)
Total recovery represents sum of concentrated extracts and RRR
A was spiked with a mixture of approx. 12 % ^{14}C-N-methyl-AMPA, 66 % ^{14}C-AMPA and 22 % ^{14}C-glyphosate
B was spiked with a mixture of approx. 50 % ^{14}C-AMPA and 50 % ^{14}C-glyphosate
Values calculated upon dossier compilation.

Table 6.2.1-52: Soil propensity experiment: HVE of extracted radioactive residues in potato tubers from plants harvested at 67 days following foliar application of ^{14}C-glyphosate and ^{14}C-AMPA

Residues in tubers, % TRR						
Fraction/ Soils	Control	Control + A	Control + B	^{14}C- glyphosate treated	^{14}C- glyphosate treated (weed[1])	^{14}C- AMPA treated
DALT	67					
TRR	100	100	100	100	100	100
Aqueous extract[2]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aqueous concentrate	44	92	80	56	47	56
HVE[3]						
Zone of glyphosate[4]	---	12.0	33.6	0.0	3.3	10.1
AMPA/ N-methyl-AMPA	---	80.0	46.4	28.0	28.7	36.4
Neutral compounds	---	<1	<1	28.0	15.0	9.5
Identified	---	---	---	---	---	---
Characterised	44	92.0	80.0	56.0	47.0	56.0
ERR	44.0	92.0	80.0	56.0	47.0	56.0
RRR	n.d.	n.d.	n.d.	49.0	37.0	31.0
Total recovery	44.0	92.0	80.0	105.0	84.0	87.0

Table 6.2.1-52: Soil propensity experiment: HVE of extracted radioactive residues in potato tubers from plants harvested at 67 days following foliar application of ^{14}C-glyphosate and ^{14}C-AMPA

DALT days after last treatment
[1] Application was done to the weeds
[2] Samples were only LSC measured after concentration
[3] HVE was performed using pyridine buffer with 1 N NaOH
[4] Comparisons of the results obtained in the tables above, point to the absence of glyphosate and suggest the presence of low levels of acidic material with AG-1-X8 (HCO_3^-) retention volume similar to glyphosate. Hence, the amounts are accounted as characterised.
n.d. not determined
Identified = AMPA was identified, but it occurs in a mixture of AMPA/N-methyl-AMPA.
Characterised = sum of non-retarded, non-specific identified (AMPA/N-methyl-AMPA) and not identified (glyphosate)
Radioactivity
TRR = total radioactive residues determined by combustion followed by LSC analysis
ERR = extracted radioactive residues
RRR= residual radioactive residues (radioactivity found in pellet)
Total recovery represents sum of concentrated extracts and RRR
A was spiked with a mixture of approx. 12 % ^{14}C-N-methyl-AMPA, 66 % ^{14}C-AMPA and 22 % ^{14}C-glyphosate
B was spiked with a mixture of approx. 50 % ^{14}C-AMPA and 50 % ^{14}C-glyphosate
Values calculated upon dossier compilation.

Large Scale Extraction

The ^{14}C-levels found in fractions of potato tubers following soil application are shown in in the table below. For potato tubers grown on non-radioactive soil spiked with ^{14}C-AMPA, extraction released 80 % of the TRR. Ion exchange chromatography of the extract using AG-1X8 followed by AG-50W-X8, resulted in one peak fraction, representing AMPA.
For potato tubers, extraction released 56 % of the TRR after soil application with glyphosate in the large scale experiment. Subsequent Ion exchange chromatography of the extract using AG-1X8 followed by AG-50W-X8, resulted in two peak fractions. The major peak fraction represents AMPA and the minor peak fraction in the void volume a possible AMPA-conjugate. HVE at pH 5.9 of non-spiked AG-50 fraction recovered 63 % of the fraction (6.6 % TRR) in the ^{14}C-AMPA zone. Further workup of the sample resulted in ^{14}C-AMPA-TAM. After purification of this compound it was compared to a ^{14}C-AMPA-TAM standard by GC/MS/COM, confirming its identity.

Table 6.2.1-53: Soil Propensity Experiment: Extractability of radioactive residues in potato tubers following soil application (large scale experiment)

Residues in tubers		
Fraction/ Soils	**Control (spiked with 0.141 mg/kg)**	**^{14}C-glyphosate treated**
	% TRR	**% TRR**
TRR	100.0	100.0
Extract	*80.0[1]*	56.0
AG-1-X8	*78.4*	*22.4*
^{14}C-AMPA + unknown	*78.4*	*22.4*
AG-50W-X8	*78.4*	*10.5*
^{14}C-AMPA	*78.4*	*6.6[1]*
^{14}C-AMPA-conjugate	---	*3.9*
Pellet	8.0	45.0

Table 6.2.1-53: Soil Propensity Experiment: Extractability of radioactive residues in potato tubers following soil application (large scale experiment)

Total	*88.0*	*101.0*

[1] identified as ^{14}C-AMPA-TAM by GC-MS
DALT days after last treatment
Values calculated upon dossier compilation are presented in italics.% recovered was recalculated in %TRR.
TRR = total radioactive residues determined by combustion followed by LSC analysis

Extraction of soil samples that were made up by combining each two replicates per treatment were extracted with NH_4OH, centrifuged and analysed by combustion followed by LSC.

Table 6.2.1-54: Extraction of post-harvest soil samples following soil application of glyphosate and AMPA

Soil	**^{14}C-glyphosate treated**	**^{14}C-AMPA treated**
DALT	67/121[1]	
	% TRR	**% TRR**
TRR	100.0[2]	100.0
Extract	*55.2*[3]	*73.0*
AG-1-X8	*63.0*	*69.4*
^{14}C AMPA + unknown	*63.0*	*69.4*
RRR	*44.8*	*27.0*
Total	*100.0*	*100.0*

DALT days after last treatment
[1] Values are averages of two replications, one harvested at 67 days and the other at 121 days.
[2] Average value of bare soil treated with ^{14}C-glyphosate (0.660 mg/kg) and weed-grown soil treated with ^{14}C glyphosate (0.680 mg/kg)
[3] Average value of bare soil treated with ^{14}C-glyphosate (0.407 mg/kg) and weed-grown soil treated with ^{14}C glyphosate (0.337 mg/kg)
Values calculated upon dossier compilation are presented in italics.

AG-1-X8 chromatography with either soil extracts which had been treated either with ^{14}C-AMPA or with ^{14}C-glyphosate was performed, resulting in very little radioactivity in the void volumes of the chromatographies. Hence, uptake of large quantities of neutral activity of the soil was not observed. Both chromatographies revealed major radioactivity in the ^{14}C-AMPA elution volume.

Foliar treatment experiments:

For potato leaves and tubers, extraction released 74 - 86 % of the TRR and 86 - 102 % of the TRR, respectively, after foliar application with ^{14}C-glyphosate.
HVE at pH 5.9 of aliquots of the treated-leaf and treated-tuber extracts indicate that most of the radioactivity, which still resided in the treated leaves at harvest time was unchanged ^{14}C-glyphosate. ^{14}C-AMPA was present to amounts ≤ 5 % and possibly ≤ 2 % for treated leaf extracts and ≤ 3 % for treated tuber extracts; but even these low amounts might also originate from streaking effects of ^{14}C-glyphosate zones during the HVE.

Table 6.2.1-55: Foliar application experiment: Extraction of ^{14}C-glyphosate treated potato leaves and β-camera zone-analysis of electropherograms from HVE of treated-leaf extracts

Residues in leaves, %[1]								
DALT	**1**		**3**		**14**		**34**	
Replicate	1	2	1	2	1	2	1	2
TRR	100	100	100	100	100	100	100	100
Aqueous extract	86	86	81	76	82	74	76	75
AG-50W-X8 (water elutions / 1 N HCL)								
Glyphosate zone	98	96	97	98	100	98	95	97
AMPA zone	2	4	3	2	---	2	5	3
Identified	---	---	---	---	---	---	---	---
Characterised	86	86	81	76	82	74	76	75
ERR	86	86	81	76	82	74	76	75
RRR	*14*	*14*	*19*	*24*	*18*	*26*	*24*	*25*
Total	100	100	100	100	100	100	100	100

[1] % extracted
Control samples did not have any radioactivity
DALT days after last treatment
Values calculated upon dossier compilation are presented in italics.
Identified = AMPA was identified, but it occurs in a mixture of AMPA/N-methyl-AMPA.
Characterised: Sum of not identified compounds (glyphosate and AMPA zone)

Table 6.2.1-56: Foliar application experiment: Extraction of ^{14}C-glyphosate treated potato tubers

Residues in tubers		
DALT	Replicate number	Extract %
1	1	86
1	2	92
3	1	98
3	2	102
14	1	97
14	2	95
34	1	87
34	2	87
34 (Control)	1	---
34 (Control)	2	---

DALT days after last treatment

C. Storage stability

No data on storage stability are available for this study. However, all experiments were completed within 11 months (from February 1974 to December 1974). All of the plants in the soil propensity experiment

were started on April 23, 1974 and harvested on June 29, 1974 (set 1) and August 23, 1974 (set 2). Hence, all samples of the soil propensity experiments were stored for a maximum of up to 6 months and no storage stability analysis was required.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in root and tubers category at the end of this chapter.

III. Conclusions

The nature and magnitude of glyphosate-derived residues after different treatments with glyphosate of potato plants was studied. The nature of residues resulting from soil uptake was investigated after a pre-emergence application of equivalent to 4.48 kg glyphosate acid equivalents /ha to bare soil immediately before planting of potato plants. Foliage samples were collected 9, 15 and 25 days after initiation of the soil propensity experiment. Whole plants were harvested after 67 days (first set of duplicate pairs) and 121 days (second set) and separated into tubers, roots and tops.
The translocation and metabolism of ^{14}C-glyphosate resulting from foliar uptake was investigated by single applications of 100 µL application solution containing ^{14}C-glyphosate (108 µg, 1.28×10^7 dpm) per plant to 40 day old- pre-bloom stage potato plants. Potato parts (tubers, roots, tops) were collected 1, 3, 14 and 34 days after treatment, respectively.

Uptake of radioactivity from the soil did occur as shown by the fact that control plants grown side by side with the treated-soil plants contained less than 1/10th of the radioactivity of the treated soil plants. However, in the treated plants parent compound was only found in trace amounts using ion exchange chromatographies (AG-50W-X8 chromatography: 1.1 % of the TRR and AG-1-X8 chromatography: 7.5 % of the TRR). Furthermore, the significant radioactive fractions in the non-retarded elution volumes of the ion exchange chromatograms and comparable radioactive levels which behaved neutrally by HVE suggest the presence of neutral ^{14}C-compounds in the extracts. Comparisons of the levels of potential ^{14}C-glyphosate fractions of ion exchange chromatographies and HVE analysis point to the absence of ^{14}C-glyphosate and rather suggest the presence of low levels of acidic material with AG-1-X8 retention volume similar to ^{14}C-glyphosate.

The only metabolite identified in tubers from the soil propensity experiment was ^{14}C-AMPA accounting for up to 38.1 % of the TRR. However, ^{14}C-N-methyl-AMPA, which exist as trace impurity in certain samples of ^{14}C-glyphosate, was not separated from the ^{14}C-AMPA in this sample. Additionally, the finding of ^{14}C-AMPA was in contrast to the finding of only parent ^{14}C-glyphosate in tubers when the herbicide was applied foliarly. Thus, it seems likely that the ^{14}C-AMPA found in tubers of the soil propensity experiment was not really a plant-produced metabolite, but rather one produced by soil microorganisms.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behavior of glyphosate in potato has been previously evaluated at EU level. It was not performed under GLP, as GLP was not established at the study facility at the time of the study conduction (1974). However, the study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with major deficits:
The radiochemical purity of the test item(s) is not specified.
No data on storage stability are available for this study. However, all experiments were completed within 11 months (up to 333 days) (from February 1974 to December 1974). During the course of the study samples of potato foliage, tops and roots (water rich matrix group) and potato tubers (starch rich matrix group) were analysed. A high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies. No degradation of glyphosate and its metabolites was found in matrices with high water content comparable to the present study (like corn

forage, fodder, cotton forage, soybean forage) over an investigated storage duration of 215-393 days ([redacted] 1995, CA 6.2.1-24; [redacted] 1997, CA 6.2.1-21 and [redacted] *et al.*, 1994, CA 6.2.1-20). In commodities with high starch content represented by corn grain, soybean hay and barley straw no degradation of glyphosate related residues was determined over a period of 264 days to 15 months ([redacted] 1995, CA 6.2.1-24 [redacted] *et al.*, 1994, CA 6.2.1-20, McMullan *et al.*, 1990, CA 6.6.2-02). Moreover, all of the plants in the soil propensity experiment were started on April 23, 1974 and harvested on June 29, 1974 (set 1) and August 23, 1974 (set 2). Extraction and analyses dates are not specified in the report, but the first page of the report states, that work was finished in December 1974, so samples were stored for a maximum of 185 days. Hence, all samples of the soil propensity experiments were stored for a maximum of up to 6 months and no storage stability analysis was required.
Developmental stages of the crop at harvesting are not reported, however, mature and immature stages at sampling were defined. Moreover, days after sampling were specified.
Only water has been used for extraction. Not extracted residues after solvent extractions were high (31.0 – 49.0 %) and no release and characterisation and/or identification was attempted.
Data to account for or track the loss of radioactivity in each subsequent step of the fractionation and isolation procedure are not provided. Total recovery (ERR + RRR) was partly < 90 % (The radioactivity balances are partly below 90 %).
For some matrices less than 90 % has been identified and characterised due to high level of non-extractable radioactivity in potato tubers of the pre-emergence experiment (up to approx. 49 %). Extractability of tubers in the foliar post-emergence experiments were higher accounting for 86 - 102 % (averaging 93 %). Moreover, radioactivity isolated from treated leaves or tubers from the foliar experiment was determined to be practically exclusively parent compound glyphosate (≥ 95 %). The only metabolite identified in tubers from the soil propensity experiment was AMPA.
Radioactive residues in RACs are expressed in dpm/µg dry weight values, recalculation in mg/kg dry weight was performed only for soil samples.
Limit of detection (LOD) for LSC analysis is not provided.
Physical facility and environmental conditions are poorly described.
For foliar application experiment the application rate is given as amount of glyphosate in mg per plant. Recalculation to g a.s./ha could be done if needed.
No flow sheet or diagram depicting the overall extraction and fractionation strategies available.

Therefore, the study is considered as not reliable for the assessment of the metabolic behavior of glyphosate in potato plants and in the whole group of root vegetables.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/009
Report author	[redacted]
Report year	1976
Report title	CP 67573 residue and metabolism Part 29: The metabolism of CP 67573 in sugar beets
Report No	394
Document No	M-649164-01-1
Guidelines followed in study	Not specified

Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • The radiochemical purity of the test substances is not clearly specified • Soil characteristics are not reported • Developmental stages of the crop at application and harvesting are not reported • Radioactive residues in samples determined by combustion are expressed in % of applied radioactivity (as %AR) rather than as TRR values in terms of mg eq./kg. Recalculation is not possible with the data reported • No quantitative description of the extraction and fractionation of the radioactive residues in the various crop matrices • No full accountability reported. Considerable portions of the radioactive residues were characterised as "neutral material" only by ion exchange chromatography • No information of the storage stability for all major components of the total radioactive residues • No description of conditions and length of storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

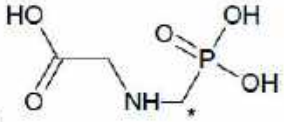
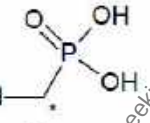
Executive summary

In this study the uptake of radioactivity into sugar beets grown in soil treated either with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) or ^{14}C-aminomethylphosphonic acid (^{14}C-AMPA) was investigated. Both radiolabelled analytes were applied to the soil of planting pots at rates of 8 mg per pot (corresponding to 4.48 kg a.s./ha). The observed uptake of radioactivity into roots or leaves of sugar beets was minimal. Less than 0.2 % of the applied radioactivity following soil treatment with ^{14}C-glyphosate or ^{14}C-AMPA were recovered in the plant samples. After soil treatment with ^{14}C-glyphosate, ion exchange chromatography and high voltage electrophoresis of the aqueous extracts of roots and leaves indicated ^{14}C-glyphosate (30 % of the extracted radioactivity), ^{14}C-AMPA (10 % of the extracted radioactivity), and neutral material (60 % of the extracted radioactivity) for roots, and ^{14}C- glyphosate (70 % of the extracted radioactivity) and neutral material (30 % of the extracted radioactivity) in the leaves, respectively. The aqueous extracts of the roots and the leaves from the ^{14}C-AMPA treatments contained ^{14}C-AMPA (90 % of the extracted radioactivity) and neutral material (10 % of the extracted radioactivity). The only identifiable metabolite was ^{14}C-AMPA in the roots of the plants grown in ^{14}C-glyphosate soil.

After foliar treatment with ^{13}C-glyphosate and ^{14}C-glyphosate, the major ^{13}C-, ^{14}C-labeled material detected in the water extracts was ^{13}C-, ^{14}C-glyphosate (85 - 90 % of extracted) as indicated by ion exchange chromatography and high voltage electrophoresis. The presence of ^{13}C-, ^{14}C-AMPA was not detectable; similarly, no other ^{13}C-, ^{14}C-labeled metabolites were observed. The mass spectral data taken with the ^{13}C-NMR characterisations confirmed the chromatographic characterisations and the presence of glyphosate as the only phosphonate containing residue in foliar treated sugar beets.

I. Materials and Methods

A. Materials

Test material:	a) N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) b) ^{14}C-aminomethylphosphonic acid (^{14}C-AMPA) c) N-(phosphono-^{13}C-methyl)glycine (^{13}C-glyphosate)
Chemical structure:	 a, c) * Position of ^{14}C or ^{13}C radiolabel  b) * Position of ^{14}C radiolabel
Radiochemical purity*:	Not specified; test substances were purified to 99 % by chromatography on AG 50W-X8 ion exchange resin
Specific activity:	a) 0.41 MBq/mg (1.87 mCi/mmol) 1.98 MBq/mg (9.07 mCi/mmol) b) 1.28 mBq/mg (3.84 mCi/mM)
CAS No:	a), c) 1071-83-6 b) 1066-51-9
Log $P_{o/w}$:	a), c) -3.2 b) -2.47

Test system:

Soil:	Norfolk sandy loam
Crop:	Sugar beet (Variety: Great Western Mono hy D2)
Botanical name:	*Beta vulgaris subsp. vulgaris*
Crop part(s):	Leaves, roots

*It is not clear from the report if purity stated refers to chemical or radiochemical purity.

B. Study design

1. In-life phase

Soil treatment

Sugar beets were grown in planting pots. Three pots of sugar beets were each treated on the soil surface with 8.0 mg (1.967×10^8 dpm, 0.41 MBq/mg) of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate). Three further pots were each treated with 8.0 mg (3.07×10^8 dpm, 0.64 MBq/mg) of ^{14}C-aminomethylphosphonic acid (^{14}C-AMPA) and unlabelled AMPA (1:1), prepared by adding equal parts of labelled (1.28 MBq/mg) and unlabelled material. This treatment rates corresponded to 4.48 kg a.s./ha.

Directly afterwards the pots were covered with plastic bags and kept in greenhouse for 4, 6 or 8 weeks until sampling. In order to remove any $^{14}CO_2$ arising from soil metabolism of the labelled compounds, the air inside the plastic bags was continuously pumped out of the greenhouse by means of plastic tubing connected to an air pump.
In parallel, untreated control samples were grown.

Foliar treatment
Thirteen sugar beet plants were each treated on the lower surface of four leaves with 25 µL of a test formulation containing 1300 µg of ^{13}C-glyphosate, 100 µg of ^{14}C-glyphosate, 700 µg of isopropylamine, 700 µg of Atlas G3780A adjuvant, and H_2O ad 700 µL. The composition of this solution was similar to the commercial formulation (Roundup®). This corresponded to 3.57 µg ^{14}C-glyphosate per plant and 0.89 µg ^{14}C-glyphosate per leaf, respectively.
All of the treated leaves were covered with small plastic bags each of which had a hole cut in the bottom for ventilation.

2. Sampling

Soil treatment
One treated and one control plant for each treatment was harvested at 4, 6 and 8 weeks.

Foliar treatment
Five weeks after treatment the plants were dissected into treated leaves, untreated leaves, and roots.

3. Analytical procedures

The total radioactive residues were determined in the lyophilised plant samples by liquid scintillation counting (LSC) after combustion.

For roots and leaves samples from the soil treatment experiments with ^{14}C-glyphosate or ^{14}C-AMPA, dried plant samples were extracted with water. The plant residue after extraction was lyophilised, and non-extractable radioactivity was assayed by combustion.
The combined extracts were frozen, lyophilised and re-dissolved in water. After cation exchange chromatography (AG 50W-X4 (H^+ form) (Bio-Rad)) and subsequent anion exchange chromatography (AG 1-X8 (HCO_3^- form) (Bio-Rad)), the combined and concentrated radioactive fractions were analysed by high voltage electrophoresis (HVE) with pH 5.9 pyridine-acetic acid-water buffer. The distribution of radioactivity on the electrophoretogram was determined by β-camera analysis.

Labelled material for spectral identification was isolated from treated leaves, untreated leaves and roots from the foliar treatment experiment with ^{13}C/^{14}C-glyphosate by extracting dried plant material with water. The extracts were frozen, lyophilised and applied to cation exchange columns (AG 50W-X4 (H^+ form; 5 x 60 cm) (Bio-Rad)), resulting in two radioactive elution peaks. For the first radioactive elution peaks, elution volumes from the cation exchange columns were 500 – 700 mL, 725 – 875 mL and 775 – 1250 ml for treated leaves, untreated leaves and roots extracts, respectively. This fraction was designated "neutral". For the second radioactive elution peaks, elution volumes from the cation exchange columns were 875 – 1250 mL, 1075 – 1500 mL and 1375 – 2150 mL for treated leaves, untreated leaves and roots extracts, respectively. This fraction was designated "1" (corresponding to glyphosate).
The fractions containing the radioactivity were combined separately for the two peaks and applied to anion exchange chromatography columns (AG 1-X8 (HCO_3^- form) (Bio-Rad)), resulting in a single elution peak from each column. The fractions containing the radioactivity were examined by HVE with pH 5.9 pyridine-acetic acid-water buffer.
The material contained in the second fraction from cation exchange chromatography was further purified by an additional cation exchange chromatography on AG 50W-X8 (H^+ form) (Bio-Rad) and subsequent clean-up by chromatography on Bio-Gel P-2. After evaporation to dryness, the samples from the treated leaves and the roots which contained about 75 µg of labelled material were dissolved in water and ^{13}C-NMR spectra were obtained at 22.6 MHz and 35 °C by standard pulsed techniques.

Purified samples from treated leaves, untreated leaves, and roots from the foliar treatment experiment with $^{13}C/^{14}C$-glyphosate together with a standard sample of ^{13}C-glyphosate were derivatised for GC-MS analysis by treating with trifluoroacetic acid and trifluoroacetic anhydride at room temperature.
The derivatised samples were separated by gas chromatography on a glass column packed with 1.5 % OV-17 on Chromosorb W-HP and the analytes detected and confirmed by mass spectrometry.

II. Results and Discussion

A. Total radioactive residues (TRRs)

Soil uptake
The uptake of radioactivity from the treated soils is expressed as percent uptake of the radioactivity applied to the soil in the report. As the weight of the samples analysed is not reported, no calculation of the total radioactive residue in mg/kg is possible. Therefore, data are expressed as percent radioactivity applied in the table below.

The level of soil uptake was very low. During the in-life phase, $^{14}CO_2$ evolved from soil metabolism of the labelled compounds was removed by pumping the air inside the plastic bags containing the treated plants out of the greenhouse to lower the amount of $^{14}CO_2$ photofixation. In the experiments with ^{14}C-glyphosate 0.033 – 0.040 % and 0.023 – 0.037 % of the applied radioactivity was recovered in leaves and roots respectively. In the experiments with ^{14}C-AMPA 0.033 – 0.151 % and 0.015 – 0.149 % of the applied radioactivity was recovered in leaves and roots. In the tests with ^{14}C-glyphosate the uptake of radioactivity remained nearly constant between 4 and 8 weeks after treatment while in the tests with ^{14}C-AMPA the uptake of radioactivity was found to increase steadily during this period of time.

Table 6.2.1-57: Uptake of radioactivity into sugar beet roots and leaves following soil treatment with ^{14}C-glyphosate or ^{14}C-AMPA at rates equivalent to 4.48 kg a.s./ha

Analyte applied	Interval	Sample	% AR recovered
^{14}C-glyphosate	4 weeks	Leaves, treated	0.033
		Leaves, control	0.003
		Roots, treated	0.037
		Roots, control	0.003
	6 weeks	Leaves, treated	0.038
		Leaves, control	0.004
		Roots, treated	0.023
		Roots, control	0.005
	8 weeks	Leaves, treated	0.040
		Leaves, control	0.003
		Roots, treated	0.030
		Roots, control	0.003
^{14}C-AMPA	4 weeks	Leaves, treated	0.033
		Leaves, control	0.002
		Roots, treated	0.015
		Roots, control	0.002
^{14}C-AMPA	6 weeks	Leaves, treated	0.124
		Leaves, control	0.003
		Roots, treated	0.042
		Roots, control	0.002
	8 weeks	Leaves, treated	0.151
		Leaves, control	0.002
		Roots, treated	0.149
		Roots, control	0.003

Results are not corrected for recovery in controls

Foliar treatment
After foliar treatment with ^{13}C-glyphosate and ^{14}C-glyphosate, the untreated leaves were found to contain 11.9 % of the applied radioactivity while 31.2 % of the applied radioactivity had translocated to the roots and 30.2 % remained on the treated leaves. The total accountability was 73.3 %.

B. Extraction and characterisation of residues

Soil treatment
After soil treatment with ^{14}C-glyphosate or ^{14}C-AMPA, respectively, 70 - 85 % of the radioactivity in dried plant material (70 – 85 % TRR) from the 4 and 6 week soil treatments was extractable with water. From 15 to 25 % of ^{14}C-activity (15 – 25 % TRR) was unextractable.
These aqueous extracts were each examined by ion exchange chromatography and HVE. The aqueous extracts of the roots and the leaves from the ^{14}C-AMPA treatments contained ^{14}C-AMPA (90 % of the extracted radioactivity) and neutral material (10 % of the extracted radioactivity).
The extracts of roots and leaves from the ^{14}C-glyphosate treatments indicated ^{14}C-glyphosate (30 % of the extracted radioactivity), ^{14}C-AMPA (10 % of the extracted radioactivity), and neutral material (60 % of the extracted radioactivity) for roots and ^{14}C- glyphosate (70 % of the extracted radioactivity) and neutral material (30 % of the extracted radioactivity) in the leaves, respectively.
It is stated in the report that "*The neutral material observed is probably a result of photofixation of $^{14}CO_2$ from soil metabolism of the labelled compounds. The concentration of the neutral material in the roots of the plants grown in the ^{14}C-1 treated soil indicates that this material is probably sugars*." (^{14}C-1 = ^{14}C-glyphosate)

The only identifiable metabolite was ^{14}C-AMPA in the roots of the plants grown in ^{14}C-glyphosate soil.

Foliar treatment
After foliar treatment with ^{13}C-glyphosate and ^{14}C-glyphosate, 70 - 75 % of the ^{14}C-activity in the various plant parts (70 – 75 % TRR) was extractable with water. The water extracts from each of the plant parts were examined by ion exchange chromatography on cation and anion exchange columns and found to contain 85 - 90 % ^{13}C-, ^{14}C-glyphosate (85 – 90 % of extracted) and 10 - 15 % neutral material (10 – 15 % of extracted). The presence of ^{13}C-, ^{14}C-AMPA was not detectable; similarly, no other ^{13}C-, ^{14}C-labeled metabolites were observed. The neutral material eluted in the void volume when chromatographed on both anion and cation exchange columns.

The material which eluted from both columns with a retention time characteristic of parent glyphosate was further shown to be ^{14}C-glyphosate by HVE.

Spectral characterisation of ^{13}C, ^{14}C-glyphosate from foliar treatment
In addition to ion exchange chromatography and HVE, the extracts of foliar treated sugar beets were further analysed by ^{13}C-NMR and GC-MS. The sample from the roots showed the characteristic doublet with a chemical shift of 46.1 ppm from TMS and ^{13}C-^{31}P coupling constant of 138.0 Hz. The spectrum obtained for the treated leaves sample showed a broad doublet at the right chemical shift and with the correct coupling constant for ^{13}C-glyphosate. The broadening was apparently caused by binding of glyphosate to impurities in the sample. A ^{13}C-NMR spectrum of ^{13}C-glyphosate from the untreated leaves could not be obtained due to lack of a sufficient quantity of material.

GC-MS analysis of the derivatised samples confirmed that the major ^{13}C-, ^{14}C-labelled material in the treated leaves, untreated leaves, and the roots is ^{13}C-, ^{14}C- glyphosate.

These mass spectra were essentially identical to the mass spectrum of a standard sample of the trimethyl N-trifluoroacetyl derivative of ^{13}C-, ^{14}C- glyphosate. The mass spectral data taken with the ^{13}C-NMR characterisations confirmed the chromatographic characterisations and the presence of glyphosate as the only phosphonate containing residue in foliar treated sugar beets.

C. Storage stability

No information on storage intervals of samples is given in the report. Storage stability was not investigated.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in root and tuber crops at the end of this chapter.

III. Conclusions

The observed uptake of radioactivity into roots or leaves of sugar beets after soil application of ^{14}C-glyphosate or ^{14}C-AMPA was minimal. In the experiments with ^{14}C-glyphosate 0.033 – 0.040 % and 0.023 – 0.037 % of the applied radioactivity was recovered in leaves and roots respectively while in the experiments with ^{14}C-AMPA 0.033 – 0.151 % and 0.015 – 0.149 % of the applied radioactivity was recovered in leaves and roots.

After foliar treatment with ^{13}C-glyphosate and ^{14}C-glyphosate, the untreated leaves were found to contain 11.9 % of the applied radioactivity while 31.2 % of the applied radioactivity had translocated to the roots and 30.2 % remained on the treated leaves.

The extracts from the ^{14}C-glyphosate soil treatments indicated ^{14}C-glyphosate (30 % of the extracted radioactivity), ^{14}C-AMPA (10 % of the extracted radioactivity), neutral material (60 % of the extracted radioactivity) for roots and ^{14}C-glyphosate (70 % of the extracted radioactivity) and neutral material (30 % of the extracted radioactivity) in the leaves, respectively. The only identifiable metabolite was ^{14}C-AMPA in the roots of the plants grown in ^{14}C-glyphosate treated soil. The aqueous extracts of the roots and the leaves from the ^{14}C-AMPA soil treatments contained ^{14}C-AMPA (90 % of the extracted radioactivity) and neutral material (10 % of the extracted radioactivity).
The major ^{13}C-, ^{14}C-labeled material detected in the water extracts after foliar treatment was ^{13}C-, ^{14}C-glyphosate (85 - 90 % of extracted). The presence of ^{13}C-, ^{14}C-AMPA was not detectable; similarly, no other ^{13}C-, ^{14}C-labeled metabolites were observed.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in sugar beets has been previously evaluated at EU level. The study is deemed to partly comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with major deficits (radiochemical purity of the test substances not clearly specified; soil characteristics not reported; developmental stages of the crop at application and harvesting not reported; radioactive residues in samples determined by combustion are expressed in % of applied radioactivity (as %AR) rather than as TRR values in terms of mg eq./kg, recalculation is not possible with the data reported; no quantitative description of the extraction and fractionation of radioactive residues in the various crop matrices; no full accountability reported, considerable portions of the radioactive residues were characterised as "neutral material" only by ion exchange chromatography; no information of the storage stability for all major components of the total radioactive residues; no description of conditions and length of storage of samples).
No information on storage duration of frozen plant samples and extracts is given in the study report.
However, the study duration is given on the front page with October 1973 to December 1974 (~15 months). No degradation of glyphosate and its metabolites was found in matrices with high water content comparable to the present study, like corn forage, fodder, cotton forage, soybean forage. Over an investigated storage duration of 215-393 days no degradation was observed ([redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/022). The storage duration is also well covered by storage stability studies of glyphosate and its metabolites available under CA 6.1.
Quantitative information in terms of absolute amounts of radioactive residues in mg/kg is not available

and cannot be derived by recalculation with the data reported. However, relative amounts in terms of percentage of applied radioactivity, as reported in the study, allow for an assessment of the relative uptake and distribution of ^{14}C-glyphosate or ^{14}C-AMPA after soil treatment and of ^{14}C-glyphosate after foliar treatment.

Identification of glyphosate by high voltage electrophoresis, ^{13}C-NMR and GC-MS was achieved for extracts of sugar beet treated leaves, untreated leaves and roots treated foliar with $^{13}C/^{14}C$-glyphosate. Residues in sugar beet roots and leaves after soil treatment with ^{14}C-glyphosate or ^{14}C-AMPA, respectively, were characterised by ion exchange chromatography and HVE. In roots and leaves extracts from the ^{14}C-AMPA treatments, 90 % ^{14}C-AMPA and 10 % neutral material were indicated, while in the extracts from the ^{14}C-glyphosate treatments 30 % ^{14}C-glyphosate, 10 % ^{14}C-AMPA, and 60 % neutral material for roots, and 70 % ^{14}C- glyphosate and 30 % neutral material for leaves were indicated, respectively (percentages as % in extract). The neutral material eluted in the void volume when chromatographed on both anion and cation exchange columns. The chromatographic behaviour of this fraction was interpreted that it consisted of uncharged natural plant constituents probably formed by incorporation of $^{14}CO_2$

Therefore, the study data allow for a qualitative assessment of the nature of the residue in sugar beet leaves and roots after soil and foliar treatment.

Total residues in sugar beet leaves and roots were determined by LSC as total ^{14}C-derived radioactivity which is expected to be stable during the course of the study.

Thus, although the study does not comply with current guideline requirements in major aspects, it still gives relevant and consistent qualitative information on the uptake and distribution of glyphosate-derived residues in sugar beets after soil and foliar application and on the nature of the residues in sugar beet leaves and roots.

Therefore, this study is considered to be supportive for the assessment of the metabolic behaviour of glyphosate in root and tuber crops.

Assessment and conclusion by RMS:

Leafy crops

Please refer to CA 6.6.1 for investigation of leafy crops (as part of confined rotational crop studies)

Cereals

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/010
Report author	[redacted] *et al.*
Report year	1989
Report title	ICIA0224: Metabolism on wheat following a pre-harvest foliar spray
Report No	RJ0778B
Document No	Not available
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501 • No information of the storage stability for all major components of the total radioactive residues • No description of length of storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The nature of the residues in plants following the use of glyphosate-trimesium was studied in cereals. In this study wheat was treated with N-(phosphono-methyl)glycine trimesium salt, the trimethylsulfonium salt of glyphosate, labelled either in the glyphosate- or the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively). The test item was applied at a rate equivalent to 5.64 kg a.s./ha for the PMG-label (3.89 kg a.s./ha expressed as glyphosate equivalents) and 7.20 kg a.s./ha for the TMS-label (4.96 kg a.s./ha expressed as glyphosate equivalents) to wheat close to harvest when the moisture content in grain was <20 %. After 7 days samples of wheat were collected.

For the ^{14}C-PMG-label experiment the calculated total radioactive residues in grain accounted for 2.68 mg/kg, 327.5 mg/kg in chaff and 124.2 mg/kg in straw. For the ^{14}C-TMS-label experiment the calculated total radioactive residues in grain accounted for 8.22 mg/kg, 363.9 mg/kg in chaff and 151.2 mg/kg in straw.

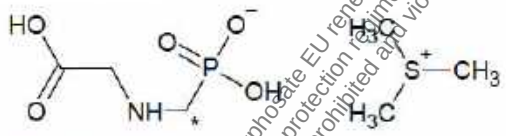
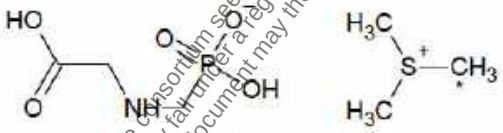
The extraction of samples was performed with water for the ^{14}C-PMG-label and with methanol followed by water for the ^{14}C-TMS-label.

The unaltered anion, phosphonomethyl glycine (PMG) was the major residue detected in ^{14}C-PMG labelled treated wheat accounting for 90.8, 85.0 and 82.6 % of the TRR in grain, chaff and straw. AMPA was detected in grain, chaff and straw accounting for 2.8, 3.9 and 3.3 % of the TRR. One very minor unknown (0.5 % of the TRR) was detected in grain and two minor unknowns (<2.0 % of the TRR) were detected in chaff and straw.

The unaltered cation, trimethylsulfonium ion (TMS) was the only major residue detected in the ^{14}C-TMS-labelled treated wheat. The total residues accounted for 95.3, 76.2 and 77.0 % of the TRR in grain, chaff and straw respectively. One minor unknown was detected in chaff (0.7 % of the TRR and straw (0.2 % of the TRR).

I. Materials and Methods

A. Materials

Test Material:	N-(phosphono-methyl)glycine trimesium salt, (ICIA0224; glyphosate-trimesium), radiolabelled (^{14}C) in either the N-phosphonomethylglycine (PMG) anion or the trimethylsulfonium (TMS) cation
Chemical structure:	a) ^{14}C-PMG label  * Position of the radio label b) ^{14}C-TMS label  * Position of the radio label
Radiochemical purity:	a) ^{14}C-PMG: 98.5 % b) ^{14}C-TMS: 98.0 %
Specific activity (in radiodiluted treatment solution):	a) ^{14}C-PMG-labelled glyphosate-trimesium: 0.836 MBq/mg b) ^{14}C-TMS- labelled glyphosate-trimesium, 1.350 MBq/mg
CAS No:	81591-81-3
Log $P_{o/w}$ for glyphosate-trimesium:	-2.9

Test system:

Soil:	Sandy clay loam
Crop:	Wheat, variety Broom
Botanical name:	*Triticum*
Crop part(s):	Grain, chaff and straw

B. Study design

1. In-life phase

The study was performed to determine the uptake and metabolism of N-(phosphono-^{14}C-methyl)glycine trimesium salt, the trimethylsulfonium salt of glyphosate in wheat grown in the UK.
The active substance was ^{14}C-radiolabelled either in the glyphosate- (PMG-label) or the trimethylsulfonium-moiety (TMS-label). The objective was to apply 150 mg of glyphosate to two separate areas of wheat with a size of 0.25 m^2 (target rate 6.0 kg a.s./ha). The actual rates were equivalent to 5.64 kg a.s./ha for the ^{14}C-PMG-labelled glyphosate-trimesium (PMG-label) (3.89 kg a.s./ha expressed as glyphosate equivalents) and 7.20 kg a.s./ha for ^{14}C-TMS labelled glyphosate-trimesium (TMS-label) (4.96 kg a.s./ha expressed as glyphosate equivalents).
The applications were performed close to harvest of wheat when the moisture in grain was <20 %.
For both labels the radiolabelled test compound was diluted with an aqueous concentrate of the non-radiolabelled test compound, a surfactant (AL-2042 (a blend of glucosides (67 %) amine ethoxylate (5 %)) at 240 g/L)) and water. The specific activity of the treatment solution was 0.836 MBq/mg for the PMG-label and 1.350 MBq/mg for the TMS-label. The amount of radioactivity applied was 120 MBq (^{14}C-PMG) or 240 MBq (^{14}C-TMS).

2. Sampling

After 7 days samples of wheat were collected. The wheat was harvested by cutting it off ~ 5 cm above the ground and was not allowed to come in contact with the soil. Prior to analysis wheat samples were separated into grain, chaff and straw. The straw was further processed by cutting the stems into ~ 1 cm lengths. After processing each of the individual crop samples were stored as a bulk homogeneous sample. All crop samples and extracts were stored frozen at ≤ -18°C during storage until analysis.

3. Analytical procedures

The extraction of samples was performed with water for the ^{14}C-glyphosate-label and with methanol followed by water for the ^{14}C-trimethylsulfonium-label. In general, samples were repeatedly extracted until the level of activity recovered in the last extract fell below 5 % of the total amount of activity extracted to that point. The remaining residues after extraction were combusted followed by liquid scintillation counting (LSC). The extracts were combined, and the total residue was determined by LSC.
For the identification of metabolites six different thin layer chromatography (TLC) systems were used (silica gel and cellulose plates using different solvent systems) for investigation of PMG-labelled compounds and three different thin layer chromatography (TLC) systems for metabolites related to the TMS-label.
The reference compounds were visualised by reaction with specific spray reagents (molybdenum blue/ninhydrin/potassium iodoplatinate, potassium iodide solution and Dragendorff´s reagent).

The study also compared the relative efficiencies and effects of extracting straw sub-samples of the PMG-label with water, 1 M ammonium chloride, 1 M hydrochloric acid and 1 M sodium hydroxide.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in wheat grain, chaff and straw was determined by summation of the extracted radioactivity in the water extracts (PMG-label) and methanol and water extracts (TMS-label) plus the radioactivity remaining in the solids. For the PMG-label experiment the calculated total radioactive residues in grain accounted for 2.68 mg/kg, 327.5 mg/kg in chaff and 124.2 mg/kg in straw. For the TMS-label experiment the calculated total radioactive residues in grain accounted for 8.22 mg/kg, 363.9 mg/kg in chaff and 151.2 mg/kg in straw.
The total radioactive residues (TRR) in wheat samples are summarised in the table below.

Table 6.2.1-58: Total radioactive residues in wheat

Sample description	Days after last treatment (DALT)	Experiment	TRR_{calc} (calculated as sum of ERR + RRR) (mg/kg)[1]
Grain	7	^{14}C-glyphosate-label (^{14}C-PMG-glyphosate) Extraction with water (3-4x)	2.68
Chaff			327.5
Straw			124.2
Grain	7	^{14}C-trimethylsulfonium-label (^{14}C-TMS-glyphosate) Extraction with methanol (3-4x) followed by water (2-4x)	8.22
Chaff			363.9
Straw			151.2

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] all residue data are expressed as mg/kg glyphosate equivalents

B. Extraction and characterisation of residues

The ^{14}C-levels found in extracts of wheat grain, chaff and straw are shown in the tables below.
For the PMG-label experiment 94.2 % of the TRR of grain, 94.9 % of the TRR of chaff and 92.4 % of the TRR of straw was extractable after repeated extraction (3-4 times) with water (values refer to the measured combined extracts).
For the TMS-label experiment 97.8 and 80.0 % of the TRR of grain and chaff and 80.1 % of the TRR of straw were extractable after repeated extraction (3-4 times) with methanol followed by extraction with water (2-4 times) (values refer to the measured combined extracts).

Table 6.2.1-59: Extraction of the radioactive residues of glyphosate in wheat following application of glyphosate at a dose rate of 6 kg a.s./ha - ^{14}C-glyphosate-label (PMG-label)

Experiment	^{14}C-PMG-label					
	Grain		Chaff		Straw	
DALT	7 days		7 days		7 days	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
TRR	**2.68**	**100.0**	**327.5**	**100.0**	**124.2**	**100.0**
Aqueous extract 1	1.40	52.3	243.3	74.3	64.7	52.1
Aqueous extract 2	0.85	31.8	56.3	17.2	26.0	20.9
Aqueous extract 3	0.17	6.5	13.1	4.0	15.6	12.6
Aqueous extract 4	0.13	4.9	NA	NA	5.5	4.4
Combined aqueous extracts (measured)	2.52	94.2	310.8	94.9	114.8	92.4
Combined aqueous extract after clean up	2.56	95.7	NA	NA	NA	NA
Combined aqueous extract concentrated	2.54	94.7	NA	NA	NA	NA
ERR	**2.52**	**94.2**	**310.8**	**94.9**	**114.8**	**92.4**
RRR	**0.12**	**4.5**	**14.7**	**4.5**	**12.4**	**10.0**
Accountability	***2.64***	***98.7***	***325.5***	***99.4***	***127.2***	***102.4***

Table 6.2.1-59: Extraction of the radioactive residues of glyphosate in wheat following application of glyphosate at a dose rate of 6 kg a.s./ha - ^{14}C-glyphosate-label (PMG-label)

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
Accountability Sum of extractable radioactive residue and residual radioactive residue
NA not applicable
[1] all residue data are expressed as mg/kg glyphosate equivalents
Values given in *italics* were recalculated during dossier compilation.

Table 6.2.1-60: Extraction of the radioactive residues of glyphosate in wheat following application of glyphosate at a dose rate of 6 kg a.s./ha - ^{14}C-trimethylsulfonium-label (TMS-label)

Experiment	^{14}C-TMS-label					
	Grain		**Chaff**		**Straw**	
DALT	7 days		7 days		7 days	
	mg/kg[1]	**% TRR**	**mg/kg[1]**	**% TRR**	**mg/kg[1]**	**% TRR**
TRR	**8.22**	**100.0**	**363.9**	**100.0**	**151.2**	**100.0**
Methanol extract 1	5.20	63.3	118.6	32.6	48.4	32.0
Methanol extract 2	0.83	10.1	27.7	7.6	17.1	11.3
Methanol extract 3	0.54	6.6	17.1	4.7	15.1	10.0
Methanol extract 4	0.25	3.1	7.6	2.1	NA	NA
Combined methanol extracts (measured)	7.01	85.2	174.4	47.9	80.4	53.2
Combined methanol extract concentrated	6.90	83.9	NA	NA	NA	NA
Aqueous extract 1	0.79	9.6	48.4	13.3	21.5	14.2
Aqueous extract 2	0.25	3.0	38.9	10.7	9.8	6.5
Aqueous extract 3	NA	NA	20.0	5.5	5.3	3.5
Aqueous extract 4	NA	NA	9.50	2.6	3.0	2.0
Combined aqueous extracts (calc.)[2]	1.04	12.6	116.8	32.1	39.6	26.2
Combined aqueous extracts (measured)	1.04	12.6	116.8	32.1	40.7	26.9
Combined aqueous extract after clean up	1.04	12.7	NA	NA	NA	NA
Combined aqueous extract concentrated	1.08	13.2	NA	NA	NA	NA
ERR	***8.1***	***97.8***	***291.2***	***80.0***	***121.1***	***80.1***
RRR	***0.34***	**4.2**	***76.1***	**20.9**	***30.8***	**20.4**
Accountability	***8.39***	***102.0***	***367.3***	***100.90***	***151.9***	***100.5***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
Accountability Sum of extractable radioactive residue and residual radioactive residue
NA not applicable
[1] all residue data are expressed as mg/kg glyphosate equivalents
[2] calculated by summing each of the individual extracts prior to combination
Values given in *italics* were recalculated during dossier compilation.

The distribution of glyphosate and its metabolites found in wheat grain, chaff and straw is shown in the the tables below.

For the PMG-label the main constituent of the TRR in grain, chaff and straw was glyphosate accounting for 90.8, 85.0 and 82.6 % of the TRR respectively (corresponding to 2.43, 278 and 103 mg/kg respectively). In addition to the parent compound, one metabolite was identified in grain, chaff and straw: Aminomethylphosphonic acid (AMPA) which accounted for 2.8, 3.9 and 3.3 % of the TRR in grain, chaff and straw respectively (corresponding to 0.08, 12.8 and 4.1 mg/kg respectively). One unknown with 0.5 % of the TRR (0.01 mg/kg) was found in grain, while two unknowns were determined in chaff and straw accounting for 2.0 and 1.8 % of the TRR respectively (corresponding to 6.6 and 2.2 mg/kg respectively).

For the TMS-label the main constituent of the TRR in grain, chaff and straw was the trimethylsulfonium ion accounting for 95.3, 76.2 and 77.0 % of the TRR respectively (corresponding to 7.83, 277.3 and 116.4 mg/kg respectively). One unknown compound was determined in chaff and straw accounting for 0.72 and 0.2 % of the TRR respectively (corresponding to 2.6 and 0.3 mg/kg respectively). Radioactivity at the TLC origin accounted for 0.4 to 0.9 % of the TRR (0.03 to 2.1 mg/kg).

Table 6.2.1-61: Distribution of the radioactive residues of glyphosate in wheat following application of glyphosate at a dose rate of 6 kg a.s./ha - ^{14}C-glyphosate-label (PMG-label)

	^{14}C-PMG-label					
	Grain		Chaff		Straw	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
TRR	**2.68**	**100.0**	**327.5**	**100.0**	**124.20**	**100.0**
ERR (calc.)	**2.56**	**95.5**	**312.8**	**95.5**	**111.78**	**90.0**
ERR (measured)	**2.52**	**94.2**	**310.8**	**94.9**	**114.8**	**92.4**
Glyphosate	2.43	90.8	278.4	85.0	102.60	82.6
AMPA	0.08	2.8	12.8	3.9	4.10	3.3
Total identified	***2.51***	***93.6***	***291.2***	***88.9***	***106.70***	***85.9***
Unknown	0.01[2]	0.5[2]	6.6[3]	2.0[3]	2.20[4]	1.8[4]
TLC-origin	0.03	1.3	5.6	1.7	3.70	3.0
Total characterised	***0.04***	***1.8***	***12.2***	***3.7***	***5.90***	***4.8***
Total identified and characterised	***2.55***	***95.4***	***303.4***	***92.6***	***112.60***	***90.7***
RRR	**0.12**	**4.5**	**14.7**	**4.5**	**12.40[c]**	**10.0[c]**

PHI pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] all residue data are expressed as mg/kg glyphosate equivalents
[2] consists of one unknown
[3] consists of two unknowns
[4] the results of the extraction experiment showed that extraction with 1 M sodium hydroxide would reduce the residual radioactive residue. The additional extracted activity was entirely identified as glyphosate.
Values given in *italics* were recalculated during dossier compilation.

Table 6.2.1-62: Distribution of the radioactive residues of glyphosate in wheat following application of glyphosate at a dose rate of 6 kg a.s./ha - ^{14}C-glyphosate-label (TMS-label)

	^{14}C-TMS-label					
	Grain		Chaff		Straw	
	mg/kg[1]	% TRR	mg/kg[1]	% TRR	mg/kg[1]	% TRR
TRR	**8.22**	**100.0**	**363.9**	**100.0**	**151.20**	**100.0**
ERR	**7.87**	**95.7**	**324.23**	**89.1**	**105.08**	**69.5**
ERR (measured)	**7.01**	**85.2**	**174.40**	**47.9**	**121.10**	**80.1**
Trimethylsulfonium-ion	7.83	95.3	277.3	76.2	116.4	77.0
Total identified	***7.83***	***95.3***	***277.30***	***76.2***	***116.40***	***77.0***
Unknown	-	-	2.6[2]	0.72[2]	0.30[2]	0.2[2]
TLC-origin	0.03	0.4	2.1	0.58	1.30	0.9
Total characterised	***0.03***	***0.4***	***4.70***	***1.3***	***1.60***	***1.1***
Total identified and characterised	***7.86***	***95.7***	***282.00***	***77.5***	***118.00***	***78.1***
RRR	**0.34**	**4.2**	**76.1**	**20.90**	**30.8**	**20.4**

PHI pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] all residue data are expressed as mg/kg glyphosate equivalents
[2] consists of one unknown
Values given in *italics* were recalculated during dossier compilation.

C. Storage stability

Storage intervals for samples and extracts are not reported. No information on storage stability is reported. It is stated that all crop samples and extracts were stored frozen at <-18 °C.

The field phase was conducted between 16th September 1988 and 23rd September 1988, which was immediately followed by the analytical section of the study between September 1988 and August 1989 (assumed maximum duration of sample storage: 342 days).

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusions

The nature of the residues in plants following the use of glyphosate trimesium was studied in wheat. N-(phosphono-methyl)glycine trimesium salt, labelled either in the glyphosate- or the trimethylsulfonium-ion (^{14}C-PMG-label and ^{14}C-TMS-label, respectively) was applied at a rate equivalent to 5.64 kg a.s./ha for the PMG-label (3.89 kg a.s./ha expressed as glyphosate equivalents) and 7.20 kg a.s./ha for the TMS-label (4.96 kg a.s./ha expressed as glyphosate equivalents) to wheat close to harvest (<20 % moisture in grain). After 7 days samples of wheat were collected.

The extraction of samples was performed with water for the ^{14}C-PMG-label and with methanol followed by water for the ^{14}C-TMS-label.

For the ^{14}C-PMG-label experiment the calculated total radioactive residues in grain accounted for 2.68 mg/kg, 327.5 mg/kg in chaff and 124.2 mg/kg in straw. For the ^{14}C-TMS-label experiment the

calculated total radioactive residues in grain accounted for 8.22 mg/kg, 363.9 mg/kg in chaff and 151.2 mg/kg in straw.

Extractable activity was characterised by thin layer chromatography. A high degree of characterisation was achieved for all samples. The unaltered anion, phosphonomethyl glycine (PMG) was the major residue detected in ^{14}C-PMG labelled treated wheat accounting for 90.8, 85.0 and 82.6 % of the TRR in grain, chaff and straw. AMPA was detected in grain, chaff and straw accounting for 2.8, 3.9 and 3.3 % of the TRR. One very minor unknown (0.5 % of the TRR) was detected in grain and two minor unknowns (<2.0 % of the TRR) were detected in chaff and straw.

The unaltered cation, trimethylsulfonium ion (TMS) was the only major residue detected in the ^{14}C-TMS-labelled treated wheat. The total residues accounted for 95.3, 76.2 and 77.0 % of the TRR in grain, chaff and straw respectively. One minor unknown was detected in chaff (0.7 % of the TRR and straw (0.2 % of the TRR).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate wheat has been previously evaluated at EU level. It was performed under GLP and is considered to be scientifically valid. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with some deficits (no information of the storage stability for all major components of the total radioactive residues, no description of length of storage of samples).

No information on storage duration of frozen plant samples and plant extracts is given in the study report. As there are no detailed information on storage duration, whole information given in the report may be considered: Based on the dates of the field phase and end of analytical phase stated the calculated maximum storage period of stored samples is 342 days.

A high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies.

No degradation of glyphosate and its metabolites was found in matrices with high water content comparable to the present study (like corn forage, fodder, cotton forage, soybean forage and in commodities with high starch content) over an investigated storage duration of 215-393 days ([redacted] 1995, CA 6.2.1/020; [redacted], 1997, CA 6.2.1/023 and [redacted] *et al.*, 1994, CA 6.2.1/022).

In commodities with high starch content represented by corn grain, soybean hay and barley straw no degradation of glyphosate related residues was determined over a period of 264 days to 15 months ([redacted] 1995, CA 6.2.1/020, [redacted] *et al.*, 1994, CA 6.2.1/022, McMullan *et al.*, 1990, CA 6.6.2/003).

Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1).

It is considered that the study was performed in a reasonable timeframe (~342 days) and therefore the present study is considered reliable to support the uses in the crop category cereal/grass crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/011
Report author	[redacted]
Report year	1974
Report title	CP 67573 residue and metabolism Part 22: The metabolism of N-phosphonomethylglycine in barley, oats, rice and sorghum
Report No	341
Document No	Not available
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • No information of the storage stability for all major components of the total radioactive residues. • No description of conditions and length of storage of samples. • Developmental stages of the crop at application and harvesting are not reported. • The sampled RACs (raw agricultural commodities) were not appropriate (4, 6 and 8 weeks old plants taken after soil treatment and plants taken at from hydroponic experiment at 7, 14, 20 (for rice only) and 28 days followed by separation into roots and aerial parts (tops)). • No flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed. • Relevant amounts of non-extractable residues were not characterised / not investigated, especially for root matrices (residual radioactive residue > 30 %). No exhaustive extraction procedures were applied.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The uptake and nature of the residues in plants following the use of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) was studied in cereal grains (barley, oats, rice and sorghum).

The uptake of radioactivity from treated soil at a rate of 4.5 kg a.s./ha was very limited. A range of 0.03 to 0.13 % of the applied radioactivity was found in the plants.

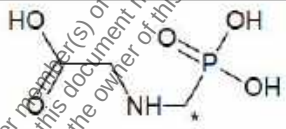
However, following application of ^{14}C-glyphosate via hydroponic solution, glyphosate gave a better uptake into the plants (2.70 to 4.68 % of the applied radioactivity into aerial parts and 6.53 to 23.05 % of the applied radioactivity into roots) and it was possible to investigate the nature of the residues in tops and roots of barley, oats, rice and sorghum.

The extractabilities of the terminal samples of barley, rice, oats and sorghum forage ranged between 85.36 and 107.9 % of the TRR. The extractability of terminal samples of roots ranged between 33.23 and

61.23 % of the TRR for barley, rice, oats and sorghum. Rice was the most difficult crop to extract, especially the roots from the 7 day harvest showing only 44.8 % extractability and the 28 day root with only 33.23 % of the TRR.
The aerial portion of all crops showed that 73.25 to 76.63 % of the TRR (0.165 to 2.076 mg/kg) was glyphosate. 6.51 to 13.97 % of the TRR (0.027 to 0.243 mg/kg) was identified as the metabolite AMPA and 1.41 to 5.43 % of the TRR (0.012 to 0.040 mg/kg) was N-methyl-AMPA.
In the roots 19.10 to 52.60 % of the TRR (0.703 to 3.221 mg/kg) were identified as glyphosate, 2.18 to 7.42 % of the TRR (0.037 to 0.273 mg/kg) as AMPA and 0.43 to 1.41 % of the TRR (0.008 to 0.071 mg/kg) as N-methyl-AMPA.
Barley and sorghum tops, however, contained the highest percentage of AMPA, 13.97 and 12.67 % of the TRR (0.080 and 0.027 mg/kg) respectively; as well as the highest percentage of N-methyl-AMPA accounting for 3.50 and 5.43 % of the TRR (0.020 and 0.012 mg/kg) respectively.
Unknown radioactivity (activity remaining at the TLC-origin or indeterminate) accounted for 1.88 to 9.28 % of the TRR (0.012 to 0.053 mg/kg) in the aerial parts and 1.49 to 5.15 % of the TRR (0.036 to 0.271 mg/kg) in the roots.
The origin of N-methyl-AMPA remained unclear. The occurrence of N-methyl-AMPA is discussed not be due to plant metabolism but due to hydroponic media metabolism.

I. Materials and Methods

A. Materials

Test Material:	N-(phosphono-^{14}C-methyl)glycine
Chemical structure:	 * Position of the radio label
Radiochemical purity:	A: 95.3 % (TLC) used for hydroponic plant uptake; 99.9 % after purification B: 96.4 % (TLC) used for soil experiment, 99.5 % after purification
Specific activity:	A: 1.98 MBq/mg (9.07 mCi/mmol) B: 0.41 MBq/mg (1.87 mCi/mmol)
CAS No:	1071-83-6
Log $P_{o/w}$:	- 3.2

Test system:

Crop:	Rice (Variety: Blue Bell) Oats (Rodney type) Sorghum (Surgro grain) Barley (Variety: Larker)
Botanical name:	*Oryza sativa* *Avena sativa* L. *Sorghum sp.* *Hordeum vulgare*
Soil:	Drummer: Silty clay loam (6 % OM, pH 7.0, 36.8 % clay, 55.4 % silt, 2.0 % sand) Ray: Silt loam (1.0 % OM, pH 6.5, 0.6 % clay, 82.3 % silt, 6.0 % sand)

Crop part(s):	Tops and roots

B. Study design

1. In-life phase

In this study the uptake and metabolism of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) in cereal grain (barley, oats, rice and sorghum) following soil treatment or application via hydroponic solution was investigated.

Soil uptake experiment:
N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) with a specific activity of 1.87 mCi/mmol (0.41 MBq/mg) was applied to soil surface of three pots per crop (containing 8 plants of barley, 9 plants of oats, 5 plants of sorghum or 6-8 plants of rice). The plants used were twelve days old.
The application rate was 4.5 kg a.s./ha in the soil uptake experiment (2.59 mg ^{14}C-glyphosate, corresponding to 6.37 x 10^7 dpm; 1.06 MBq). Three other pots per plant were used as controls.
The pots were placed on a cart filled with sand. The pots were buried about ~2.5 cm in the sand and watered from the bottom twice daily for the duration of the experiment.

Hydroponic uptake experiment:

Rice:
Soil used for growing rice was a 1:1-mixture of peat moss and Ray silt loam soil. Ten rice seeds were planted per container.

Sorghum, oats and barley:
Planters were filled with sand. Ten seeds of sorghum, oats and barley were planted per container. The pots were watered twice daily from the top and periodically inorganic nutritients (modified Hoagland´s solution) was applied after germination.
Sorghum (grown twelve days in sand), oats and barley (eighteen days in sand) and rice (nineteen days in mixed peat moss and Ray silt loam soil) were cleaned from sand or soil with distilled water and placed in tanks with nutrient solution.
After four days in the hydroponic solution (three days for rice) N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) (0.183 mg/mL) with a specific activity of 9.07 mCi/mM (1.98 MBq/mg) was used in the hydroponic uptake experiment. The amount used for rice and sorghum was 3 mg ^{14}C-glyphosate (corresponding to 35.72 x 10^7 dpm; 5.95 MBq) and for oats and barley 6 mg ^{14}C-glyphosate (corresponding to 71.45 x 10^7 dpm; 11.91 MBq) per tank.
Control plants were maintained for each crop in order to determine the level of incorporation via $^{14}CO_2$ fixation derived from both the plant and hydroponic nutrient media.
The duration of all the hydroponic experiments was 4 weeks starting from the day ^{14}C-glyphosate was added to the hydroponic solution.

2. Sampling

Soil uptake experiment:
Following soil treatment plant samples were taken from one pot after 4, 6 and 8 weeks and control plant from each crop was harvested. The plants were cut off about ~ 2.5 cm above the soil level. Each sample was frozen, lyophilised and ground to 40-mesh size in a Wiley mill. Aliquots were taken for combustion.

Hydroponic uptake experiment:
At 7, 14, 20 (for rice only) and 28 days plants in holes from all treated tanks were harvested by carefully separating their roots from the rest of the plant roots. The treated roots and roots from control tanks were washed by soaking them sequentially with distilled water followed by drying with paper towels. The roots were cut from the aerial part of the plant. The washings were analysed by LSC. Samples were frozen, lyophilised and ground to 40 mesh in a Wiley mill. Hydroponic solutions from all treated tanks were analysed by LSC and chromatographically by TLC at 4, 7, 11, 14, 18, 21, 24 and 28 days.

3. Analytical procedures

All treated plant samples were extracted with distilled water using a magnetic stirrer at room temperature. The remaining solids were removed by centrifugation. The extracts were assayed by liquid scintillation counting (LSC).

To separate and characterise glyphosate and its metabolites, thin layer chromatography (TLC), column chromatography (Dowex 50 cation and Dowex 1 anion exchange resins, Bio-Gel P-2 size exclusion resin) and high voltage electrophoresis (HVE) were applied.
Both aerial and root portions of all four crops were analysed by cation and anion exchange column chromatography and high voltage electrophoresis (HVE) at pH 5.9. In addition, the aerial and root parts of rice as well as oat roots were analysed by HVE at pH 10.1 to verify the high amount of N-methyl-AMPA obtained from anion exchange column chromatography.
Two dimensional thin layer chromatography on cellulose plates was used for analysis of standard compounds, hydroponic solutions and chromatographic fractions.
Different ion exchange and size exclusion chromatographic resins were used (AG-50W-X4, AG-50W-X8, AG-1-X8, Bio-Gel P-2) for metabolite purification, purification of ^{14}C-glyphosate, metabolite purification of fortified extracts or identification of parent compound and metabolites.

For the identification and quantification of metabolites thin layer chromatography (TLC) in combination with radio-detection, GC-FPD and GC-MS were used, and the results compared with reference substances (glyphosate, AMPA and N-methyl-AMPA).

II. Results and discussion

A. Total radioactive residues (TRRs)

Soil uptake experiment

The results of the soil uptake experiment are summarised in the table below. The resulting uptake showed a range of 0.03 to 0.13 % of the applied radioactivity for treated plants. Control plants showed an uptake of 0.01 to 0.07 %. Therefore, the treated plants showed only an uptake of 0.02 to 0.09 % above controls after 8 weeks.
Uptake by both treated and control plants did not increase significantly from the 4th week to the 8th week. Actually, in oats and barley (both treated and control) uptake was less at 8 weeks. Control sorghum and rice plants similarly had less uptake at 8 weeks. Rice showed the highest concentration while oats and barley had the lowest.

Table 6.2.1-63: Radioactivity found in sorghum, rice, oats and barley grown in soil treated with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) at rates equivalent to 4.5 kg a.s./ha

Sample description	Weeks after treatment	^{14}C in plants	
		% applied	TRR (mg eq/kg)
Sorghum, whole plant	4 (control)	0.06	0.048
	4 (treated)	0.08	0.073
	6 (control)	0.05	0.030
	6 (treated)	0.06	0.085
	8 (control)	0.05	0.024
	8 (treated)	0.08	0.085
Rice, whole plant	4 (control)	0.04	0.23
	4 (treated)	0.03	0.11
	6 (control)	0.01	0.038
	6 (treated)	0.04	0.077
	8 (control)	0.01	0.061

Table 6.2.1-63: Radioactivity found in sorghum, rice, oats and barley grown in soil treated with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) at rates equivalent to 4.5 kg a.s./ha

Sample description	Weeks after treatment	^{14}C in plants	
		% applied	TRR (mg eq/kg)
	8 (treated)	0.04	0.157
Oats, whole plant	4 (control)	0.06	0.050
	4 (treated)	0.09	0.062
	6 (control)	0.05	0.017
	6 (treated)	0.07	0.029
	8 (control)	0.04	0.018
	8 (treated)	0.13	0.050
Barley, whole plant	4 (control)	0.07	0.084
	4 (treated)	0.08	0.093
	6 (control)	0.03	0.026
	6 (treated)	0.07	0.078
	8 (control)	0.03	0.024
	8 (treated)	0.05	0.059

Hydroponic uptake experiment

The following table shows the plant uptake for 7, 14, 20 (only for rice) and 28 day samplings. The highest uptake at the termination of the experiment was by barley roots which was 23.05 % of the applied activity (normalised to the total number of plants) (corresponding to 6.12 mg/kg) and the lowest was by sorghum tops which was 2.70 % of the applied radioactivity (corresponding to 0.216 mg/kg).

Uptake into the aerial portion of terminal plants ranged from 2.70 to 4.68 % and into the roots from 6.53 to 23.05 % of the applied radioactivity. Despite the high percentage of activity in barley roots, barley tops showed one of the lowest percentages of uptake (2.87 %). Uptake by plant parts progressively increased as a function of time, excluding the results from the 20-day rice harvest.

The 20-day rice harvest might not give realistic results since the rice plants were in poor condition. This group of rice plants were growing poorly with half of their leaves turning to straw.

The results of the control and treated crops clearly indicate that $^{14}CO_2$ was being evolved from the ^{14}C-experiments and photosynthetically fixed by both the treated and untreated plants. However, the percent activity accountable via this route into the plant of the control group is small compared to the uptake by treated plants. The highest uptake by control plants (excluding rice) was by oat tops which accounted to only 0.1 % of applied activity at day 28. Uptake by treated oat tops by this time was 3.49 % of the applied activity.

Table 6.2.1-64: Radioactivity found in sorghum, rice, oats and barley treated hydroponically with 0.183 mg N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) per mL

Sample description	Days after treatment	Roots, ^{14}C in plants		Tops, ^{14}C in plants	
		% applied[1]	TRR (mg eq/kg)	% applied[1]	TRR (mg eq/kg)
Sorghum	7 (control)	0.012	0.003	0.016	0.005
	7 (treated)	1.94	1.095	0.08	0.037
	14 (control)	0.11	0.020	0.042	0.004
	14 (treated)	5.64	1.596	0.48	0.073
	28 (control)	0.112	0.004	0.119	0.004
	28 (treated)	**13.40**	**1.710**	**2.70**	**0.216**

Table 6.2.1-64: Radioactivity found in sorghum, rice, oats and barley treated hydroponically with 0.183 mg N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) per mL

Sample description	Days after treatment	Roots, ^{14}C in plants		Tops, ^{14}C in plants	
		% applied[1]	TRR (mg eq/kg)	% applied[1]	TRR (mg eq/kg)
Rice	7 (control)	0.004	0.003	0.002	0.003
	7 (treated)	2.88	2.504	0.77	0.534
	14 (control)	0.02	0.010	0.008	0.008
	14 (treated)	5.59	3.302	1.98	1.545
	20 (control)	0.08	0.078	0.19	0.266
	20 (treated)	12.36	10.743	5.58	8.782
	28 (control)	NP	NP	NP	NP
	28 (treated)	**6.53**	**3.682**	**4.68**	**2.815**
Oats	7 (control)	0.006	0.003	0.012	0.004
	7 (treated)	2.10	1.796	0.59	0.250
	14 (control)	0.03	0.014	0.04	0.013
	14 (treated)	4.96	2.978	3.21	0.999
	28 (control)	0.10	0.007	0.10	0.008
	28 (treated)	**13.76**	**6.478**	**3.49**	**0.706**
Barley	7 (control)	0.004	0.009	0.003	0.004
	7 (treated)	3.36	4.539	0.51	0.270
	14 (control)	0.01	0.005	0.02	0.009
	14 (treated)	6.31	7.976	1.62	1.178
	28 (control)	0.026	0.004	0.04	0.007
	28 (treated)	**23.05**	**6.124**	**2.87**	**0.570**

NP: Not performed
[1] The amount of radioactivity was normalised to the total number of plants.
Remark: As several values were not readable in the corresponding table of the pdf of the study, all values in mg/kg were recalculated based on dpm-value of the sample, the wet weight and the specific activity (barley and oats: 71.45*10^{7} dpm; 6 mg test substance per pot; rice and sorghum: 35.72*10^{7} dpm; 3 mg test substance per pot). Minor deviations may occur due to roundings.

B. Extraction and characterisation of residues

The aerial plant parts (sampled from the hydroponic nutrient uptake experiment) were extracted with water. In general, the radioactivity in the aerial portion of the plants was 85 to 100 % extractable (except for rice at day 7). The extractabilities of the terminal samples (28 days) of barley, rice, oats and sorghum forage were 102.50, 85.36, 89.0 and 107.9 % of the TRR, respectively.

The extractabilities of the roots were in the range of 42.69 to 75.6 % of the TRR. The terminal samples had extractabilities of 61.23, 33.23, 40.82 and 49.57 % of the TRR for barley, rice, oats and sorghum, respectively.

Rice was the most difficult crop to extract, especially the roots from the 7-day harvest showing only 44.8 % extractability and the 28-day root with only 33.23 % of the TRR.

The ^{14}C-activity levels found in aqueous extracts of barley, rice, oats and sorghum tops and roots are shown in the table below.

Table 6.2.1-65: Extraction of the radioactive residues of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) in barley, oats, rice and sorghum (tops and roots) following hydroponic treatment

	Barley						Oats					
	Tops			Roots			Tops			Roots		
DALT (days)	**7**	**14**	**28**	**7**	**14**	**28**	**7**	**14**	**28**	**7**	**14**	**28**
	% TRR			% TRR			% TRR			% TRR		
Aqueous extract	99.0	103.23	102.50[1]	44.2	64.4	61.23[1]	110.8	88.7	89.0[1]	75.6	57.35	40.82
	Rice						Sorghum					
	Tops			Roots			Tops			Roots		
DALT (days)	**7**	**14**	**28**	**7**	**14**	**28**	**7**	**14**	**28**	**7**	**14**	**28**
	% TRR			% TRR			% TRR			% TRR		
Aqueous extract	66.2	95.32	85.36[2]	44.8	42.69	33.23[2]	99.4	88.36	107.9[1]	72.6	74.11	49.57[1]

DALT Days after last treatment
TRR Total radioactive residue
[1] Mean of two extractions
[2] Mean of three extractions

The aqueous extracts of samples collected after 28 days were analysed by TLC, column chromatography and high voltage electrophoresis to identify the radioactive residues.
Excellent agreement was found between the methods identifying glyphosate and its metabolites. In addition, electrophoresis revealed a substantial percentage of an unknown material at the origin, suggesting the existence of an electrically neutral metabolite/natural product at pH 5.9.
Finally, averaged data of the different techniques (cation and anion exchange, HVE (□-Camera and PACA) were used to calculate the total radioactive residue of metabolites (TRR) based on uptake by the plants by multiplying the averaged data with the percent water extractable. The distribution of glyphosate and its metabolites found in barley, oats, rice and sorghum (tops and roots) is shown in below.

The aerial portion of all crops showed that 73.25 to 76.63 % of the TRR was glyphosate (corresponding to 0.165 to 2.076 mg/kg). 6.51-13.97 % of the TRR (corresponding to 0.027 to 0.243 mg/kg) was identified as the metabolite AMPA and 1.41 to 5.43 % of the TRR (corresponding to 0.012 to 0.040 mg/kg) was N-methyl-AMPA.
In the roots 19.10 to 52.60 % of the TRR (corresponding to 0.703 to 3.221 mg/kg) were identified as glyphosate, 2.18 to 7.42 % of the TRR (corresponding to 0.037 to 0.273 mg/kg) as AMPA and 0.43 to 1.56 % of the TRR (corresponding to 0.008 to 0.071 mg/kg) as N-methyl-AMPA. While barley and sorghum did not show any activity at the origin in the high voltage electrophoresis runs, oats and rice did. Barley and sorghum tops, however, contained the highest percentage of AMPA, 13.97 and 12.67 % of the TRR, respectively; as well as the highest percentage of N-methyl-AMPA accounting for 3.50 and 5.43 % of the TRR respectively.
Unknown radioactivity (activity remaining at the TLC-origin or indeterminate) accounted for 1.88 to 9.28 % of the TRR in the aerial parts and 1.49 to 4.95 % of the TRR in the roots.

Results of aerial plant parts were compared to the results of the hydroponic solution. It is apparent that based on percentage there is more AMPA in the aerial portions of all crops at 28 days than in the hydroponic solution. Even if there was uptake of AMPA from the hydroponic solution a good portion of AMPA in the aerial portion is likely due to plant metabolism of the compound. On the other hand, except for the sorghum, N-methyl-AMPA is less in the aerial portions.

The production of N-methyl-AMPA is traceable in the hydroponic solutions with the presence of the plant system. The analysis of a control hydroponic nutrient media containing only ^{14}C-glyphosate did not show any N-methyl-AMPA over the 28 day time period. In contrast, the hydroponic solutions with the plants contained 3.4 to 5.2 % over the same time span.

Table 6.2.1-66: Distribution of radioactive residues of glyphosate and its metabolites in barley, oats, rice and sorghum roots and tops following hydroponic treatment

	Barley, 28 days				**Oats, 28 days**			
	Tops		**Roots**		**Tops**		**Roots**	
	% TRR	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
	100.00	**0.570**	**100.00**	**6.124**	**100.00**	**0.706**	**100.00**	**6.478**
Glyphosate	73.25	*0.418*	52.60	*3.221*	76.63	*0.541*	35.70	*2.312*
AMPA	13.97	*0.080*	3.77	*0.231*	6.51	*0.046*	2.54	*0.165*
N-methyl-AMPA	3.50	*0.020*	0.43	*0.027*	1.69	*0.012*	1.09	*0.071*
Total identified	***90.72***	***0.517***	***56.80***	***3.479***	***84.83***	***0.599***	***39.33***	***2.548***
TLC-origin	0.00	*0.000*	0.00	*0.000*	4.36	*0.031*	1.02	*0.066*
In-determinate	9.28	*0.053*	4.43	*0.271*	0.00	*0.000*	0.47	*0.030*
Total characte-rised	**9.28**	***0.053***	**4.43**	***0.271***	**4.36**	***0.031***	**1.49**	***0.096***
Total identified and characte-rised	**100.00**	***0.570***	**61.23**	***3.750***	**89.19**	***0.630***	**40.82**	***2.644***
ERR	**100.00**	***0.570***	**61.23**	***3.750***	**89.00**	***0.628***	**40.82**	***2.644***
RRR	**0.00**	***0.000***	**38.77**	***2.374***	**11.00**	***0.078***	**59.18**	***3.834***
	Rice, 28 days[1]				**Sorghum, 28 days**			
	Tops		**Roots**		**Tops**		**Roots**	
	% TRR	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
	100.00	**2.815**	**100.00**	**3.68**	**100.00**	**0.216**	**100.00**	**1.710**
Glyphosate	73.75	*2.076*	19.10	*0.703*	76.23	*0.165*	44.80	*0.766*
AMPA	8.62	*0.243*	7.42	*0.273*	12.67	*0.027*	2.18	*0.037*
N-methyl-AMPA	1.41	*0.040*	1.56	*0.058*	5.43	*0.012*	0.50	*0.008*
Total identified	**83.78**	***2.358***	**28.08**	***1.03***	**94.33**	***0.204***	**47.47**	***0.812***
TLC-origin	1.88	*0.053*	4.95	*0.182*	0.00	*0.000*	0.00	*0.000*
In-determinate	0.00	*0.000*	0.20	*0.007*	5.67	*0.012*	2.10	*0.036*
Total characte-rised	**1.88**	***0.053***	**5.15**	***0.190***	**5.67**	***0.012***	**2.10**	***0.036***

Table 6.2.1-66: Distribution of radioactive residues of glyphosate and its metabolites in barley, oats, rice and sorghum roots and tops following hydroponic treatment

	Barley, 28 days				Oats, 28 days			
	Tops		Roots		Tops		Roots	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Total identified and characte-rised	**85.66**	***2.411***	**33.23**	***1.22***	**100.00**	***0.216***	**49.57**	***0.848***
ERR	**85.36**	***2.403***	**33.23**	***1.22***	**100.00**	***0.216***	**49.57**	***0.848***
RRR	**14.64**	***0.412***	**66.77**	***2.46***	**0.00**	***0.000***	**50.43**	***0.862***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
AR Applied radioactivity
[1] Rice plants were not healthy
Remark: Values in %TRR and mg/kg were recalculated during dossier compilation. Input values in % of radioactivity in the extract were taken from table 27 of the report and used for the recalculation of % TRR and mg/kg values. Total radioactive residues in mg/kg were taken from table above. Minor deviations to values in % TRR given in table 2 of the report may occur due to rounding.

C. Storage stability

No dates are reported for the experimental work from sampling to extraction and analysis of extracts. Thus, it is not possible to conclude on storage stability.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusions

In cereal grains (barley, oats, rice and sorghum) the uptake of radioactivity of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) from treated soil at a rate of 4.5 kg a.s./ha was very limited. A range of 0.03 to 0.13 % of the applied radioactivity was found in the plants. Following application of ^{14}C-glyphosate via hydroponic solution, glyphosate gave a better uptake into the plants (2.70 to 4.68 % of the applied radioactivity into aerial parts and 6.53 to 23.05 % of the applied radioactivity into roots) and it was possible to investigate the nature of the residues in tops and roots of barley, oats, rice and sorghum.
Radioactivity in the aerial portion of the plants was 85 to 100 % extractable with water (except for rice at day 7). The extractabilities of the terminal samples of barley, rice, oats and sorghum forage were 102.5, 85.36, 89.0 and 107.9 % respectively. The extractabilities of the roots were in the range of 42.69 to 75.6 % of the TRR. The terminal samples had extractabilities of 61.23, 33.23, 40.82 and 49.57 % for barley, rice, oats and sorghum, respectively. Rice was the most difficult crop to extract, especially the roots from the 7 day harvest showing only 44.8 % extractability and the 28 day root with only 33.23 %.

The aerial portion of all crops showed that 73.25 to 76.63 % of the TRR (0.165 to 2.076 mg/kg) was glyphosate. 6.51 to 13.97 % of the TRR (0.027 to 0.243 mg/kg) was identified as the metabolite AMPA and 1.41 to 5.43 % of the TRR (0.012 to 0.040 mg/kg) was N-methyl-AMPA.
In the roots 19.10 to 52.60 % of the TRR (0.703 to 3.221 mg/kg) were identified as glyphosate, 2.18 to 7.42 % of the TRR (0.037 to 0.273 mg/kg) as AMPA and 0.43 to 1.41 % of the TRR (0.008 to 0.071 mg/kg) as N-methyl-AMPA.

Barley and sorghum tops, however, contained the highest percentage of AMPA, 13.97 and 12.67 % of the TRR (0.080 and 0.027 mg/kg) respectively; as well as the highest percentage of N-methyl-AMPA accounting for 3.50 and 5.43 % of the TRR (0.020 and 0.012 mg/kg) respectively.
Unknown radioactivity (activity remaining at the TLC-origin or indeterminate) accounted for 1.88 to 9.28 % of the TRR (0.012 to 0.053 mg/kg) in the aerial parts and 1.49 to 5.15 % of the TRR (0.036 to 0.271 mg/kg) in the roots.
The origin of N-methyl-AMPA remained unclear. The occurrence of N-methyl-AMPA is discussed not be due to plant metabolism but due to hydroponic media metabolism.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in cereals (barley, rice, sorghum, oats) has been previously evaluated at EU level. It was not performed under GLP (as in 1974 GLP was not yet established at the test facility). The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with some deficits (no information of the storage stability for all major components of the total radioactive residues; no description of conditions and length of storage of samples; sampled RACs (raw agricultural commodities) (tops and roots) were not appropriate; no flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed; relevant amounts of non-extractable residues were not characterised / not investigated, especially for root matrices (residual radioactive residue > 30 %). No exhaustive extraction procedures were applied).
No information on storage duration of frozen plant samples and aqueous plant extracts is given in the study report. Analysis of top and root extracts showed that glyphosate was the major residue followed by AMPA. However, a high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies.
No degradation of glyphosate and its metabolites was found in matrices with high water content comparable to the present study, like corn forage, fodder, cotton forage, soybean forage. Over an investigated storage duration of 215-393 days no degradation was observed ([redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/022). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1).

Thus, although the study does not comply with current guideline requirements in some aspects, it still gives relevant and consistent qualitative information on the uptake of glyphosate-derived residues after soil application and growing in hydroponic solution as well as information on the nature of the residues in barley, oats, rice and sorghum (tops and roots) after hydroponical treatment. Therefore, this study is considered to be reliable to support the metabolic behaviour of glyphosate in cereal/grass crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/012 and CA 6.2.1/015
Report author	[redacted]
Report year	1973
Report title	CP 67573 residue and metabolism, part 10: The metabolism of CP 67573 in soybeans, cotton, wheat and corn
Report No	304

Document No	M-648850-02-1
Guidelines followed in study	Not specified
Deviations from current test guideline	Guideline for the Testing of Chemicals, 501: • Developmental stages (e.g. BBCH codes) of the crop at application and harvesting are not reported. • No relevant RAC (raw agricultural commodities) samples taken (maize/corn, soybean, wheat and cotton, edible commodity), only roots and forage and developmental stage of forage was not defined properly (i.e. not evident if maybe relevant as feed item) • In some cases the radioactive residues in RAC are expressed in % of applied activity rather than in terms of TRR. Fresh sample weights were not available therefore no calculation of mg/kg values was possible. • In some cases the radioactive residues in RAC are expressed in % TRR only. Fresh sample weights were not available therefore no calculation of mg/kg values was possible. • In some cases % TRR values of fractions/non-extractable radioactivity exceeded the trigger value of 10 % but the sample was not further analysed/extracted. • Unextracted radioactive residues not precisely quantified • No flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed. • No photographs/images/figures of TLC plates critical to the identification. • No description of conditions and length of storage of samples and extracts, therefore it can´t be decided if storage stability investigation of samples would be necessary for this study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this study the uptake and metabolism of ^{14}C-glyphosate (three different labels: N-(phosphono-^{14}C-methyl)glycine (Methane-^{14}C-glyphosate), N-(phosphonomethyl)-^{14}C-carboxy-glycine (Glycine-1-^{14}C-glyphosate) and N-(phosphonomethyl)-^{14}C-methyl-glycine (Glycine-2-^{14}C-glyphosate)) and Amino-^{14}C-methylphosphonic acid (^{14}C-AMPA) in soybeans, cotton, wheat and maize were investigated. Several routes of uptake were examined: soil uptake, sand culture uptake and hydroponic uptake.

Two soil uptake experiments per crop using N-(phosphono-^{14}C-methyl)glycine (4.5 kg a.s./ha) or Amino-^{14}C-methylphosphonic acid (1.7 kg/ha corresponding to 2.6 kg glyphosate equiv./ha) resulted in a very low uptake. The maximum uptake for any of the four crops after eight weeks was only 0.28 % of the applied dose on cotton using N-(phosphono-^{14}C-methyl)glycine, and in this case the untreated control showed 0.20 % of the applied dose uptake based on total ^{14}C-content. Amino-^{14}C-methylphosphonic acid (^{14}C-AMPA), the major soil metabolite, showed considerably lower uptake than N-(phosphono-^{14}C-

methyl)glycine. The maximum uptake for any of the four crops after eight weeks was 0.03 % of the applied dose on soybean.

Uptake of N-(phosphono-^{14}C-methyl)glycine into plants growing in sand culture after application of an aqueous solution of N-(phosphono-^{14}C-methyl)glycine to the sand has also been examined. Only maize gave an uptake of 11.3 % of the applied dose into the aerial portion after 18 days. Cotton, soybean and wheat had aerial uptakes of only 0.03, 0.07, and 0.03 %, respectively, of the applied ^{14}C-activity after 18 days. The extraction data (aqueous extraction followed by 1 N NH_4OH) for the treated sand show that N-(phosphono-^{14}C-methyl)glycine was not available for uptake into the plants.

After hydroponic treatment, uptakes of radioactivity in the aerial portions in all crops at the comparable time period of 26-28 days ranged from 1.71 % to a maximum of 7.70 % (both soybean). Uptake of ^{14}C-activity into the root portions at the comparable time period of 25-28 days ranged from 5.48 % (soybean) to a maximum of 19.34 % (cotton). In the ^{14}C-pulse experiment, the data show a decrease of radioactivity in roots from 2.40 at day 6 to 0.66 % applied radioactivity at day 28, and from 0.28 to 0.25 % applied radioactivity in aerial parts.

Plants (aerial parts and roots) after hydroponic uptake were extracted and the residues were analysed further. With the exception of maize forage, the major ^{14}C-containing component in the aqueous extracts in all cases was parent glyphosate in aerial parts and in roots; in maize forage, comparable amounts of glyphosate and AMPA were observed.

The major ^{14}C-containing degradate in all four crops was AMPA accounting for up to 38.0 % of the TRR in aerial parts and up to 21.6 % of the TRR in roots.

Several minor metabolites were also detected and were identified as N-methyl-aminomethyl phosphonic acid (N-methyl AMPA), methyl-phosphonic acid, and N-methyl-glyphosate.

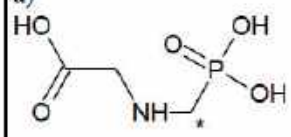
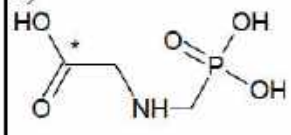
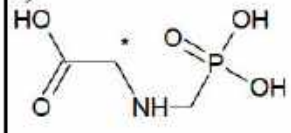
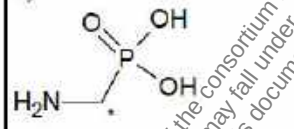
Some of the minor detectable metabolites are discussed as artefacts resulting from the starting glyphosate-^{14}C-methane and/or the hydroponic solution. In addition, their identification on the basis of TLC alone is inherently tenuous particularly in lieu of natural product formation from both $^{14}CO_2$ and/or metabolic fragments of glyphosate-^{14}C.

Separate extractions to investigate the radioactivity in natural products indicated the incorporation of fragments or $^{14}CO_2$ into natural products (e.g. amino acids and peptides or citric acid cycle intermediates).

I. Materials and methods

A. Materials

Test Material:	a) N-(phosphono-^{14}C-methyl)glycine (Methane-^{14}C-glyphosate) b) N-(phosphonomethyl)-^{14}C-carboxy-glycine (Glycine-1-^{14}C-glyphosate) c) N-(phosphonomethyl)-^{14}C-methyl-glycine (Glycine-2-^{14}C-glyphosate) d) Amino-^{14}C-methylphosphonic acid (^{14}C AMPA)

Chemical structure:	a)  b)  c)  d)  * Position of radiolabel
Radiochemical purity:	a) 97 % (reported)96.0 % (with the presence of 3.3 % AMPA and 0.6 % N-methyl-AMPA) b) 96 % c) 99 % d) 97 % (determined for all standard solutions by TLC/β-camera analysis)
Specific activity:	a) 8.03 mCi/mmol (corresponding to 1.76 MBq/mg) (reported) 8.06 mCi/mmol (corresponding to 1.76 MBq/mg) (LSC) b) 10.02 mCi/mmol (corresponding to 2.19 MBq/mg) c) 9.40 mCi/mmol (corresponding to 2.06 MBq/mg) d) 9.15 mCi/mmol (corresponding to 3.05 MBq/mg) (reported) 9.23 mCi/mmol (corresponding to 3.08 MBq/mg) Calculations based on molecular weights of 169.1 g/mol for glyphosate and 111.1 g/mol for AMPA
CAS No:	Glyphosate: 1071-83-6 AMPA: 1066-51-9
Log $P_{o/w}$:	Glyphosate: -3.2 Aminomethylphosphonic acid: -2.47

Test system:

Crop:	Winter varieties Soybean (Stoneville) Maize (DeKalb KL-45) Wheat (Clark) Cotton (Thacher)
Botanical name:	*Glycine max* *Zea mays* *Triticum aestivum* *Gossypium hirsutum*
Soil: Nutrient media:	Silty clay loam 36.8 % clay, 55.4 % silt, 2.0 % sand; pH 7.0; 6 % OM Modification of Hoagland and Arnon solution
Crop part(s):	Forage (tops) and roots

B. Study design

1. In-life phase

In this study the uptake and metabolism of ^{14}C- glyphosate (three different labels: N-(phosphono-^{14}C-methyl)glycine (^{14}C-methane-glyphosate), N-(phosphonomethyl)-^{14}C-carboxy-glycine (Glycine-1-^{14}C-glyphosate) and N-(phosphonomethyl)-^{14}C-methyl-glycine (Glycine-2-^{14}C-glyphosate) and Amino-^{14}C-methylphosphonic acid (^{14}C-AMPA) in soybeans, cotton, wheat and maize were investigated following soil or hydroponic treatment. Several routes of uptake were examined: soil uptake, sand culture uptake and hydroponic uptake.

In the first part of the study the crops were planted in pots containing one of the four crops (maize, cotton, wheat or soybean) (12 seeds per pot). After germination, the corn, cotton, and soybeans were thinned to give four plants per pot while eight wheat plants were retained per pot. The soil surface was treated with application rates equivalent to either 4.5 kg ^{14}C-methane-glyphosate or 1.7 kg ^{14}C-AMPA per ha (corresponding to 2.6 kg glyphosate equiv./ha). The activity applied was 8.61×10^8 dpm for ^{14}C-methane-glyphosate or 7.50 × 108 dpm for ^{14}C-AMPA, respectively (soil uptake experiment).

In a second set of experiments plants of maize, cotton, wheat or soybean growing in sand culture (six seeds each of corn, cotton, and soybean and twelve seeds of wheat). The pots were hydroponically treated with an aqueous solution containing ^{14}C-methane-glyphosate equivalent to 2.24 kg a.s./ha (applied activity 3.6×10^7 dpm).

The third set of experiments included various hydroponic studies to investigate the metabolism of ^{14}C-glyphosate. First, a preliminary study was conducted to investigate the uptake of ^{14}C-glyphosate from hydroponic solution. Plants were kept in hydroponic solution containing an activity of 1.11×10^8 dpm (maize, cotton, and wheat) or 5.50×10^7 (soyabean) for 3 days. After this interval the plants were separated into aerial parts and roots and analysed for the radioactivity taken up.

In the final set of experiments, the plants were kept in hydroponic solution to a maximum of 10 to 56 days with samples of plants and hydroponic solution collected in between.
The different experiments are summarised in the following table:

Table 6.2.1-67: Overview on soil and hydroponic uptake experiments in soybean, cotton, maize and wheat

	Experiment	Type of plant	Duration of the experiment (days)	Sampling (days)
Soil uptake experiments				
	Plants in soil, N-(phosphono-^{14}C-methyl)glycine 8.61 x 10^6 dpm equivalent to 4.5 kg glyphosate/ha	All crops	8 weeks	4, 6, 8 weeks
	Plants in soil, Amino-^{14}C-methylphosphonic acid 7.50 x 10^8 dpm, equivalent to 1.7 kg/ha (corresponding to 2.6 kg glyphosate equiv./ha)	All crops	8 weeks	4, 6, 8 weeks
Sand culture experiments				
	4 plants in sand, N-(phosphono-^{14}C-methyl)glycine 3.6 x 10^7 dpm, equivalent to 2.24 kg glyphosate/ha	All crops	18	4, 10, 18
Preliminary hydroponic experiment				
	Plants in hydroponic solution, 1 mg N-(phosphono-^{14}C-methyl)glycine (5.5 x 10^7 - 1.11 x 10^8 dpm)	All crops	3	3
Final hydroponic experiment				
1	99 plants in 20 L hydroponic solution, 50 mg N-(phosphono-^{14}C-methyl)glycine (5.15 x 10^9 dpm)	Soybean	28	Solution: 1, 6, 12, 20, 28 Plants: 28 (99 plants)
2	24 plants in 5 L hydroponic solution, 12 mg N-(phosphono-^{14}C-methyl)glycine (1.25 x 10^9 dpm)	Soybean	28	Solution: 1, 6, 12, 20, 28 Plants: 6, 12, 20 (2 each time), 28 (18 plants)
3	24 plants in 5 L hydroponic solution, 12 mg N-(phosphonomethyl)-^{14}C-carboxy-glycine (Glycine-1-^{14}C-glyphosate) (1.70 x 10^9 dpm)	Soybean	28	Solution: 1, 6, 12, 20, 28 Plants: 6, 12, 20 (2 each time), 28 (18 plants)
4	24 plants in 5 L hydroponic solution, 12 mg N-(phosphonomethyl)-^{14}C-methyl-glycine (Glycine-2-^{14}C-glyphosate) (1.63 x 10^9 dpm)	Soybean	25	Solution: 1, 6, 12, 20, 25 Plants: 6, 12, 20 (2 each time), 25 (18 plants)
5	24 plants in 5 L hydroponic solution, 3 mg N-(phosphono-^{14}C-methyl)glycine (3.13 x 10^8 dpm)	Maize	28	Solution: 1, 6, 12, 20, 28 Plants: 6, 12, 20 (2 each time), 28 (18 plants)
6	72 plants in 5 L hydroponic solution, 3 mg N-(phosphono-^{14}C-methyl)glycine (3.13 x 10^8 dpm)	Wheat	10	Solution: 1, 6, 10 6, 8 (2 each time), 10 (20 plants)
7	24 plants in 5 L hydroponic solution, 12 mg N-(phosphono-^{14}C-methyl)glycine (1.25 x 10^9 dpm)	Cotton	28	Solution: 1, 6, 12, 20, 28 Plants: 6,13, 20 (2 each time), 28 (16 plants)
8	Control, all crops; 6 plants each in 5 L hydroponic solution, except wheat with 18 plants	All crops	28 (wheat 10)	Solution: none Plants: 10 (18 wheat plants), 28 (6 of each crop)
9	198 plants in 20 L hydroponic solution, 2.96 mg N-(phosphono-^{14}C-methyl)glycine & 50.12 mg N-(phosphono-^{13}C-methyl)glycine (3.10 x 10^8 dpm)	Soybean	26	Solution: 1, 6, 12, 20, 26 Plants: 6 (8 plants), 12 (8 plants), 20 (4 plants), 26 (178 plants)

Table 6.2.1-67: Overview on soil and hydroponic uptake experiments in soybean, cotton, maize and wheat

	Experiment	Type of plant	Duration of the experiment (days)	Sampling (days)
10	24 plants in 5 L hydroponic solution, 11.85 mg N-(phosphono-^{14}C-methyl)glycine (1.24×10^9 dpm), pulse treatment for first 6 days only	Soybean	56	Solution: 4, 6 Plants: 6 (6 plants), 12 (6 plants), 20 (3 plants), 28 (3 plants), 42 (3 plants), 56 (3 plants)
11	Control, 48 plants in 5 L hydroponic solution, 12 mg N-(phosphono-^{12}C-methyl)glycine	Soybean	6	Solution: none Plants: 28 (4 plants), 42 (4 plants), 56 (36 plants)

2. Sampling

The sampling time schedule for each experiment is listed in the table above.
Samples of plants from the soil uptake experiment were harvested after 4, 6 and 8 weeks. In parallel control samples were kept in order to check for uptake of $^{14}CO_2$ from soil metabolism.
In the hydroponic sand culture experiments plant as well as sand samples were collected after 4, 10 and 18 days and analysed for radioactive residues.
In the hydroponic experiments, the roots of each plant from a given experiment were washed, the root and aerial portion were separated, weighed, frozen, lyophilised, the dry weight determined and ground to 40 mesh.
The ^{14}C-activity in hydroponic solution as well as the composition of the hydroponic solution was periodically monitored during the course of each experiment.

3. Analytical procedures

Total radioactive residues were determined using liquid scintillation counting (LSC). The analysis of standards, column fractions, nutrient solutions, and most plant extracts could be carried out rapidly using ß-camera quantitation with the validity of the TLC being subsequently established by spray detection of the co-chromatographed standard compounds.
Several column chromatographic systems were applied for purification and characterisation of glyphosate and its potential metabolites: DEAE cellulose, cation exchange resins (Dowex-50 (H^+)), anion exchange resins (Dowex-l (Cl^-, HCO_3^-, and $HCOO^-$)), and Bio-Gel P-2 for gel filtration.
For the identification of natural products aqueous plant extracts were fractionated into four components (basic, neutral, acidic fraction 1 and acidic fraction 2) followed by appropriate chromatographic methods. Initially, the dried plant sample was extracted with water and the ^{14}C-content was determined by LSC. The aqueous extract was then passed sequentially through a cation exchange column (Dowex-50/AG-50W) and an anion exchange column (Dowex-1/AG-1-X8). The effluent which passed through both columns was considered to be the neutral fraction and comprises mainly natural sugars. The basic fraction was obtained by washing the cation exchange column with ammonium hydroxide. The "acid-l" fraction was obtained from the anion exchange column with formic acid; further washing with hydrochloric acid gave the "acid-2" fraction. The acidic fraction 1 consists mainly of organic acids and monophosphate containing natural products while the acidic fraction 2 consists mainly of sugar diphosphates. Before concentration for further analysis, all four fractions were assayed for ^{14}C-activity by LSC.
The acidic fraction 1 was fractionated further on an anion exchange column (AG-1-X8, formate form) in order to separate the organic acid and monophosphate esters into several categories. The acidic fraction 2 was also fractionated on an AG-1-X8 column, chloride form to separate the natural mono- and diphosphate esters.

Standard compounds, hydroponic solutions, chromatographic fractions, and plant extracts were analysed routinely by two dimensional TLCs on cellulose plates and quantitated by β-camera. Derivatisation was done with ninhydrin (for the determination of amino acids and amino acid analogs) or Hanes reagent (for the determination of phosphorous containing compounds).
Aliquots of the evaporated basic fraction from the natural products screening study were spotted on silica gel TLC and quantitated by β-camera.

For the identification of metabolites in addition to TLC, GC-MS after derivatisation (n-butyl N-trifluoroacetyl derivative, trimethylsilyl N-trifluororacetyl derivative, n-butyl, N-formyl derivative, trimethylsilyl derivative), and NMR (^{1}H, ^{13}C and ^{31}P) were used and the results compared with reference substances.

II. Results and discussion

A. Total radioactive residues (TRRs)

Soil uptake experiment
The results of the soil uptake experiments are summarised in the tables below. Two experiments per crop using N-(phosphono-^{14}C-methyl)glycine (4.5 kg a.s./ha) or Amino-^{14}C-methylphosphonic acid (1.7 kg/ha corresponding to 2.6 kg glyphosate equiv./ha) resulted in a very low uptake.

The maximum uptake for any of the four crops after eight weeks was only 0.28 % of the applied dose on cotton using N-(phosphono-^{14}C-methyl)glycine, and in this case the untreated control showed 0.20 % of the applied dose uptake based on total ^{14}C-content.

Amino-^{14}C-methylphosphonic acid (^{14}C-AMPA), the major soil metabolite showed uptake considerably less than N-(phosphono-^{14}C-methyl)glycine. The maximum uptake for any of the four crops after eight weeks was 0.03 % of the applied dose in soybean.

Table 6.2.1-68: Radioactivity found in maize, cotton, soybean and wheat grown in soil treated with N-(phosphono-^{14}C-methyl)glycine at rates equivalent to 4.5 kg a.s./ha

Sample description	Weeks after treatment	Treatment	^{14}C in plants	
			% AR	mg equiv/kg
Maize	4	Control	0.0070	0.068
		Treated	0.0310	0.21
	6	Control	0.0361	0.0890
		Treated	0.0395	0.2434
	8	Control	0.0131	0.0218
		Treated	0.0472	0.0785
Cotton	4	Control	0.0015	0.04
		Treated	0.0180	0.26
	6	Control	0.0051	0.020
		Treated	0.0586	0.21
	8	Control	0.2001	0.27
		Treated	0.2760	0.42
Soybean	4	Control	0.0013	0.0294
		Treated	0.0237	0.202
	6	Control	0.0629	0.109
		Treated	0.138	0.293
	8	Control	0.0355	0.0453
		Treated	0.0726	0.0755

Table 6.2.1-68: Radioactivity found in maize, cotton, soybean and wheat grown in soil treated with N-(phosphono-^{14}C-methyl)glycine at rates equivalent to 4.5 kg a.s./ha

Sample description	Weeks after treatment	Treatment	^{14}C in plants	
			% AR	mg equiv/kg
Wheat	4	Control	0.00085	0.008
		Treated	0.0204	0.20
	6	Control	0.0030	0.013
		Treated	0.0335	0.18
	8	Control	0.0220	0.061
		Treated	0.1159	0.35

AR applied radioactivity

Table 6.2.1-69: Radioactivity found in maize, cotton, soybean and wheat grown in soil treated with Amino-^{14}C-methylphosphonic acid at rates equivalent to 1.7 kg AMPA/ha corresponding to 2.6 kg glyphosate equiv./kg

Sample description	Weeks after treatment	Treatment	^{14}C in plants		
			% AR	mg/kg[4]	mg glyphosate equiv/ kg
Maize	4	Control[1]	0.003	*0.011*	*0.017*
		Treated	0.007	0.021	*0.032*
	6	Control[1]	0.006	*0.014*	*0.021*
		Treated	0.017	0.032	*0.049*
	8	Control[1]	0.009	*0.011*	*0.017*
		Treated	0.044	0.031	*0.047*
Cotton	4	Control[1]	0.0033	*0.071*[2]	*0.108*
		Treated	0.0008	0.024	*0.037*
	6	Control[1]	0.0012	*0.010*	*0.015*
		Treated	0.0046	0.044	*0.067*
	8	Control[1]	0.0036	*0.011*	*0.017*
		Treated	0.0077	0.032	*0.049*
Soybean	4	Control[1]	0.003	*0.018*	*0.027*
		Treated	0.014	0.094	*0.143*
	6	Control[1]	0.006	*0.014*	*0.021*
		Treated	0.017	0.053	*0.081*
	8	Control[1]	0.01	*0.011*	*0.017*
		Treated	0.033	0.041	*0.062*
Wheat	4	Control[1]	0.0012	*0.019*	*0.029*
		Treated	0.0035	0.075	*0.114*
	6	Control[1]	0.0026	*0.028*	*0.043*
		Treated	0.0049	0.084	*0.128*
	8	Control[1]	0.0058	0.042[3]	*0.064*
		Treated	0.0076	*0.058*	*0.088*

[1] mean of two samples, recalculated during dossier compilation
[2] residues in one replicate of the control samples were higher than in treated sample: replicate 1: 0.126 mg/kg, replicate 2: 0.015 mg/kg
[3] residues in one replicate of the control samples were higher than in treated sample: replicate 1: 0.019 mg/kg, replicate 2: 0.065 mg/kg

AR applied radioactivity
[4] mg/kg = mg AMPA equivalents/kg, values expressed as glyphosate equivalents given in *italics* were recalculated

Hydroponic sand culture uptake experiment

Uptake of N-(phosphono-^{14}C-methyl)glycine into plants growing in sand culture after application of an aqueous solution of N-(phosphono-^{14}C-methyl)glycine to the sand has also been examined.

As can be seen in the table below, only maize gave an uptake of 11.3 % of the applied dose into the aerial portion after 18 days. Cotton, soybean and wheat had aerial uptakes of only 0.03, 0.07, and 0.03 %, respectively, of the applied ^{14}C-activity after 18 days.

The extraction data (aqueous extraction followed by 1 N NH_4OH) for the treated sand show that N-(phosphono-^{14}C-methyl)glycine was not available for uptake into the plants.

Table 6.2.1-70: Recovered radioactivity following hydroponic application of N-(phosphono-^{14}C-methyl)glycine to maize, cotton, soybean and wheat grown in sand at application rates equivalent to 2.24 kg a.s./ha

Treated crop	Sample	% AR		
		4 days	10 days	18 days
Maize	Plant, aerial part	0.87	1.45	11.29
	Plant, roots	2.73	0.49	2.24
	Root wash	0.06	0.18	-
	Sand wash[1]	72.83	62.8	52.0
	Total recovered	76.5	64.9	65.5
Cotton	Plant, total	0.09	0.06	-
	Plant, aerial part	-	-	0.03
	Plant, roots	-	-	0.16
	Sand wash[1]	86.2	83.4	62.4
	Total recovered	86.3	83.5	62.6
Soybean	Plant, total	0.03	0.02	-
	Plant, aerial part	-	-	0.07
	Plant, roots	-	-	0.22
	Sand wash[1]	84.9	82.0	82.9
	Total recovered	84.9	82.0	83.2
Wheat	Plant, total	0.09	0.16	-
	Plant, aerial part	-	-	0.03
	Plant, roots	-	-	0.37
	Sand wash[1]	93.9	92.2	85.8
	Total recovered	94.0	92.4	86.2

1 sum of extracts (aqueous followed by 1 N NH_4OH extraction)

AR applied radioactivity

Hydroponic uptake experiment (preliminary experiment)

Administration of N-(phosphono-^{14}C-methyl)glycine from the hydroponic nutrient media enabled to attain the balance between the potent phytotoxicity and the need of sufficient incorporation to permit rigorous quantitation and identification.

Incorporations into the aerial portions of these four crops ranged from 1.66-33.4 % of the applied dose after only three days; these incorporations are clearly greater than the uptakes observed from soil and sand culture after eight weeks or 18 days, respectively.

Table 6.2.1-71: Uptake of radioactivity after 3 days in hydroponic solution (1.1×10^7 dpm)

Treated crop	% AR			
	Maize	Cotton	Wheat	Soybean
Aerial part, dry	19.0	33.40	26.21	1.66
Roots, dry	7.31	10.09	7.24	1.75
Hydroponic solution	67.48	62.15	63.16	84.75
Root wash	11.90	8.21[1]	4.77	4.08
Total recovered	105.69	113.85	101.38	92.24

AR applied radioactivity
[1] calculated during dossier compilation

Hydroponic uptake experiments

The final set of experiments included various hydroponic studies to investigate the metabolism of ^{14}C-glyphosate. The plants were kept in hydroponic solution to a maximum of 10 to 56 days with samples of plants and hydroponic solution collected.

In order to maintain viable plants, to quantitate the ^{14}C-activity in solution, and to determine the composition of the solution ^{14}C-activity, the hydroponic nutrient solutions were periodically monitored during the course of each experiment. The ^{14}C-activity remaining in solution at a given time for each experiment is summarised in the table below.

The major findings were as follows: 37.44-73.80 % of the starting ^{14}C-activity remained in the hydroponic nutrient solutions after 26-28 days; as a consequence, these uptake studies represented continuous administration.

Uptakes of ^{14}C-activity in the aerial portions in all crops at the comparable time period of 26-28 days ranged from 1.71 % in experiment 2 to a high of 7.70 % in experiment 9 (both soybean). Uptake of ^{14}C-activity into the root portions at the comparable time period of 25-28 days ranged from 5.48 % in Experiment 9 (soybean) to a high of 19.34 % in experiment 7 (cotton). (The pulse-^{14}C uptake experiment (No. 10) in soybean has not been considered above since N-(phosphono-^{14}C-methyl)glycine was administered for only the first six days; similarly, the hydroponic wheat experiment 6 in which both root and aerial portions each had 2.5 % uptake was not considered since its duration was 10 days).

The uptake of ^{14}C-activity by the control crops was investigated (experiments 8 and 11) for all crops. The amount of ^{14}C-activity (dpm values given in the report) in corn, cotton, and soybeans indicates that ^{14}C-uptake is present also in control samples and is therefore discussed to occur due to the fixation of $^{14}CO_2$. These data clearly indicate that $^{14}CO_2$ evolved from the ^{14}C-experiments and was photosynthetically fixed by both the treated and untreated plants. The amount of ^{14}C-activity in corn, cotton, and soybeans indicates that approximately five percent of the ^{14}C-uptake in experiments 1-7 is due to the fixation of $^{14}CO_2$.

In the ^{14}C-pulse experiment, 24 soybean plants were hydroponically treated with 12 mg of N-(phosphono-^{14}C-methyl)glycine for 6 days and then removed to untreated, fresh nutrient media. At 6, 12, 20, 28, 42, and 56 days, plants were removed and analysed for ^{14}C-content. The data show a decrease of radioactivity in roots from 2.40 at day 6 to 0.66 % applied radioactivity at day 28, and from 0.28 to 0.25 % applied radioactivity in aerial parts, respectively.

Table 6.2.1-72: Uptake of ^{14}C-glyphosate in soybean plants grown in hydroponic solution

Time (days)	Matrix	% AR
Experiment 1: 99 plants in 20 L hydroponic solution, 50 mg N-(phosphono-^{14}C-methyl)glycine (5.15×10^9 dpm)[1]		
1	Hydroponic solution	119.0
6	Hydroponic solution	96.54
12	Hydroponic solution	82.25
20	Hydroponic solution	68.30
28	Hydroponic solution	66.39
	Aerial parts	4.19
	Roots	10.80
Experiment 2: 24 plants in 5 L hydroponic solution, 12 mg N-(phosphono-^{14}C-methyl)glycine (1.25×10^9 dpm)[1]		
1	Hydroponic solution	93.94
6	Hydroponic solution	80.22
	Aerial parts	0.93
	Roots	3.68
12	Hydroponic solution	67.49
	Aerial parts	1.12
	Roots	6.71
20	Hydroponic solution	55.96
	Aerial parts	1.42
	Roots	9.34
28	Hydroponic solution	43.88
	Aerial parts	1.71
	Roots	8.64
Experiment 3: 24 plants in 5 L hydroponic solution, 12 mg N-(phosphonomethyl)-^{14}C-carboxy-glycine (1.76×10^9 dpm)[1]		
1	Hydroponic solution	107.51
6	Hydroponic solution	77.47
	Aerial parts	1.46
	Roots	2.97
12	Hydroponic solution	58.45
	Aerial parts	1.10
	Roots	6.85
20	Hydroponic solution	43.95
	Aerial parts	1.56
	Roots	13.88
28	Hydroponic solution	37.44
	Aerial parts	1.79
	Roots	12.26
Experiment 4: 24 plants in 5 L hydroponic solution, 12 mg N-(phosphonomethyl)-^{14}C-methyl-glycine (1.63×10^9 dpm)[1]		
1	Hydroponic solution	109.89
6	Hydroponic solution	81.22
	Aerial parts	0.64
	Roots	4.25
12	Hydroponic solution	73.09
	Aerial parts	1.21
	Roots	5.30
20	Hydroponic solution	67.20
	Aerial parts	2.00
	Roots	8.56
25	Hydroponic solution	57.36
	Aerial parts	2.09

Table 6.2.1-72: Uptake of ^{14}C-glyphosate in soybean plants grown in hydroponic solution

Time (days)	Matrix	% AR
	Roots	10.30
28	Hydroponic solution	58.43
Experiment 9: 198 plants in 20 L hydroponic solution, 2.96 mg N-(phosphono-^{14}C-methyl)glycine & 50.12 mg N-(phosphono-^{13}C-methyl)glycine (3.10×10^8 dpm) [1]		
1	Hydroponic solution	98.23
6	Hydroponic solution	84.50
	Aerial parts	0.41
	Roots	1.71
12	Hydroponic solution	77.13
	Aerial parts	0.72
	Roots	3.49
20	Hydroponic solution	77.35
	Aerial parts	4.96
	Roots	3.47
26	Hydroponic solution	73.80
	Aerial parts	7.70
	Roots	5.48
Experiment 10: 24 plants in 5 L hydroponic solution, 11.85 mg N-(phosphono-^{14}C-methyl)glycine (1.24×10^9 dpm), pulse treatment for first 6 days only[2]		
1	Hydroponic solution	99.76
6	Hydroponic solution	93.02
	Aerial parts	0.29 (0.28)
	Roots	2.41 (2.40)
12	Aerial parts	0.29 (0.28)
	Roots	2.68 (2.67)
20	Aerial parts	0.39 (0.39)
	Roots	1.98 (1.96)
28	Aerial parts	0.30 (0.25)
	Roots	0.80 (0.66)
42	Aerial parts	0.45 (0.21)
	Roots	1.35 (1.26)
56	Aerial parts	0.78 (0.22)
	Roots	1.63 (1.38)

[1] Normalised to number of starting plants
[2] Values in brackets were corrected for ^{14}C-content in controls (experiment 11).
AR applied radioactivity

Table 6.2.1-73: Uptake of ^{14}C-glyphosate in maize, wheat and cotton plants grown in hydroponic solution

Time (days)	Matrix	% AR recovered
Experiment 5: Maize, 24 plants in 5 L hydroponic solution, 3 mg N-(phosphono-^{14}C-methyl)glycine (3.13×10^8 dpm) [1]		
1	Hydroponic solution	104.50
6	Hydroponic solution	79.48
	Aerial parts	0.96
	Roots	3.39
12	Hydroponic solution	66.69
	Aerial parts	3.80
	Roots	8.43
20	Hydroponic solution	56.93

Table 6.2.1-73: Uptake of ^{14}C-glyphosate in maize, wheat and cotton plants grown in hydroponic solution

Time (days)	Matrix	% AR recovered
	Aerial parts	7.01
	Roots	9.74
28	Hydroponic solution	44.76
	Aerial parts	4.73
	Roots	10.30
Experiment 6: Wheat, 72 plants in 5 L hydroponic solution, 3 mg N-(phosphono-^{14}C-methyl)glycine (3.13×10^8dpm) [1]		
1	Hydroponic solution	103.26
6	Hydroponic solution	81.81
	Aerial parts	1.280
	Roots	1.39
10	Hydroponic solution	58.31
	Aerial parts	2.46
	Roots	2.52
12	Hydroponic solution	63.92
20	Hydroponic solution	55.94
28	Hydroponic solution	50.00
Experiment 7: Cotton, 24 plants in 5 L hydroponic solution, 12 mg N-(phosphono-^{14}C-methyl)glycine (1.25×10^9 dpm) [1]		
1	Hydroponic solution	109.00
6	Hydroponic solution	87.81
	Aerial parts	0.40
	Roots	1.32
12	Hydroponic solution	83.12
	Aerial parts	1.28
	Roots	3.43
20	Hydroponic solution	72.32
	Aerial parts	2.98
	Roots	7.98
28	Hydroponic solution	58.17
	Aerial parts	2.15
	Roots	19.34

[1] normalised to number of starting plants
AR applied radioactivity

B. Extraction and characterisation of residues

Aqueous extraction

Plants (aerial parts and roots) from hydroponical uptake experiments were investigated further. The plant contained ^{14}C-activity has been analysed for extractability using water as the solvent. The extractabilities are summarised in below.

High extractability of the aerial (forage) portions was found, extractability for root portions of the plants was considerably lower.

In most forage samples, more than 80 % of the plant contained ^{14}C-activity was solubilised by a single water extraction at room temperature. The actual percent extractabilities for experiments 1-7 on the terminal sample in each case were 72.3, 80.3, 82.6, 91.8, 81.1, 77.3, and 89.2 %, respectively. In experiments 1-7, subsequent extraction with 0.5 N NH_4OH gave 4.3, 6.2, 4.2, 4.4, 15.1, 3.6 and 4.7 % respectively, of the plant contained ^{14}C-activity. A third extraction with 0.5 N HCl recovered 0.4-3.1 % of the starting activity.

In the root samples, the water extractability decreased significantly compared to the corresponding forage samples. In experiments 1-7, the extractabilities observed with the terminal samples were 42.3, 59.0, 70.0, 43.5, 55.5, 54.5, and 17.3 %, respectively. Significant ^{14}C-activity was released by extraction at room temperature with 0.5 N NH_4OH and HCl. The sum of three extractions for each terminal crop root sample ranged from 68.3 to 88.5 % of the root contained ^{14}C-activity.

Table 6.2.1-74: Extraction of the radioactive residues of glyphosate in soybean following application of glyphosate at a dose rate of 50 mg (experiment 1) or 12 mg (experiment 2) in hydroponic solution - N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean									
	Forage					Roots				
PHI (days)	6	12	20	28	28[1]	6	12	20	28	28[1]
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	69.2	70.8	82.3	80.3	72.3	37.6	59.7	54.3	59.0	42.3
0.5 N NH_4OH	NP	NP	NP	6.2	4.3	NP	NP	NP	13.0	19.5
0.5 N HCl	NP	NP	NP	2.2	2.3	NP	NP	NP	7.3	15.9
ERR	**69.2**	**70.8**	**82.3**	**88.7**	**78.9**	**37.6**	**59.7**	**54.3**	**79.3**	**77.7**
RRR[2]	***30.8***	***29.2***	***17.7***	***11.3***	***21.1***	***62.4***	***40.3***	***45.7***	***20.7***	***22.3***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] Data from experiment 1, all other data from experiment 2
[2] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-75: Extraction of the radioactive residues of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 3) in hydroponic solution – N-(phosphonomethyl)-^{14}C-carboxy-glycine

Experiment	Soybean							
	Forage				Roots			
PHI (days)	6	12	20	28	6	12	20	28
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	53.1	65.8	72.0	82.6	35.0	73.0	59.3	70.0
0.5 N NH_4OH	NP	NP	NP	4.2	NP	NP	NP	12.5
0.5 N HCl	NP	NP	NP	3.1	NP	NP	NP	5.4
ERR	**53.1**	**65.8**	**72.0**	**89.9**	**35.0**	**73.0**	**59.3**	**87.9**
RRR[1]	***46.9***	***34.2***	***28.0***	***10.1***	***65.0***	***27.0***	***40.7***	***12.1***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-76: Extraction of the radioactive residues of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 4) in hydroponic solution – N (phosphonomethyl)-^{14}C-methyl-glycine

Experiment	Soybean							
	Forage				Roots			
PHI (days)	6	12	20	25	6	12	20	25
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	66.8	78.6	73.9	91.8	38.40	62.2	41.5	43.5
0.5 N NH_4OH	NP	NP	NP	4.4	NP	NP	NP	18.7
0.5 N HCl	NP	NP	NP	1.7	NP	NP	NP	6.1
ERR	**66.8**	**78.6**	**73.9**	**97.9**	**38.40**	**62.2**	**41.5**	**68.3**
RRR[1]	***33.2***	***21.4***	***26.1***	***2.1***	***61.6***	***37.8***	***58.5***	***31.7***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-77: Extraction of the radioactive residues of glyphosate in maize following application of glyphosate at a dose rate of 3 mg (experiment 5) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Maize							
	Forage				Roots			
PHI (days)	6	12	20	28	6	12	20	28
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	70.6	73.0	71.4	81.1	68.6	66.7	99.9	55.5
0.5 N NH_4OH	NP	NP	NP	15.1	NP	NP	NP	21.4
0.5 N HCl	NP	NP	NP	0.4	NP	NP	NP	11.6
ERR	**70.6**	**73.0**	**71.4**	**96.6**	**68.6**	**66.7**	**99.9**	**88.5**
RRR[1]	***29.4***	***27.0***	***28.6***	***3.4***	***31.4***	***33.3***	***0.1***	***11.5***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-78: Extraction of the radioactive residues of glyphosate in wheat following application of glyphosate at a dose rate of 3 mg (experiment 6) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Wheat			
	Forage		Roots	
PHI (days)	6	10	6	10
	% TRR	% TRR	% TRR	% TRR
Aqueous extract	77.3	77.3	42.3	54.5
0.5 N NH_4OH	NP	3.6	NP	16.7
0.5 N HCl	NP	3.1	NP	15.0
ERR	**77.3**	**84.0**	**42.3**	**86.2**
RRR[1]	***22.7***	***16.0***	***57.7***	***13.8***
Total sum	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-79: Extraction of the radioactive residues of glyphosate in cotton following application of glyphosate at a dose rate of 12 mg (experiment 7) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Cotton							
	Forage				Roots			
PHI (days)	6	13	20	28	6	13	20	28
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	73.8	84.6	78.8	89.2	51.2	30.8	32.8	17.3
0.5 N NH_4OH	NP	NP	NP	4.7	NP	NP	NP	27.0
0.5 N HCl	NP	NP	NP	0.8	NP	NP	NP	34.6
ERR	**73.8**	**84.6**	**78.8**	**94.7**	**51.2**	**30.8**	**32.8**	**78.9**
RRR[1]	***26.2***	***15.4***	***21.2***	***5.3***	***48.8***	***69.2***	***67.2***	***21.1***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-80: Extraction of the radioactive residues of glyphosate in soybean following application of glyphosate at a dose rate of 2.96 mg and 50.12 mg respectively (experiment 9) in hydroponic solution – N-(phosphono-^{13}C-methyl)glycine and N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean			
	Forage			
PHI (days)	6	12	20	26
	% TRR	**% TRR**	**% TRR**	**% TRR**
Aqueous extract	85.3	63.1	66.2	66.6
0.5 N NH_4OH	NP	NP	NP	NP
0.5 N HCl	NP	NP	NP	NP
ERR	**85.3**	**63.1**	**66.2**	**66.6**
RRR[1]	***14.7***	***36.9***	***33.8***	***33.4***
Total sum	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Table 6.2.1-81: Extraction of the radioactive residues of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 10) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean			
	Forage			
PHI (days)	6	12	20	28
	% TRR	**% TRR**	**% TRR**	**% TRR**
Aqueous extract	67.4	55.0	50.8	43.2
0.5 N NH_4OH	NP	NP	NP	NP
0.5 N HCl	NP	NP	NP	NP
ERR	**67.4**	**55.0**	**50.8**	**43.2**
RRR[1]	***32.6***	***45.0***	***49.2***	***56.8***
Total sum	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed

Results of identification and characterisation in aqueous plant extracts of the different hydroponic experiments are summarised in the following tables.
The major ^{14}C-containing metabolite in all cases except maize forage was the parent glyphosate. In maize forage, comparable amounts of glyphosate and AMPA were observed at the 12, 20, and 28 day sampling periods.
In the terminal samples, ^{14}C-glyphosate constituted 55.1-85.5, 28.1, 70.7, and 70.5 % of the ^{14}C-content in soybean, maize, wheat, and cotton forage, respectively.

Considerably lower amounts of glyphosate parent compound were recovered in terminal samples from the pulse experiment 10 accounting for 29.4 % in soybean forage.
In the terminal root samples, glyphosate was the major ^{14}C-labelled compound detected in all cases in the water extracts. The major ^{14}C-containing metabolite based on analysis of these extracts was aminomethylphosphonic acid (AMPA). In the terminal samples, AMPA constituted 5.1-9.2, 27.0, 6.6, and 8.0 % of the ^{14}C content in soybean, maize, wheat, and cotton forages, respectively. AMPA was also the major ^{14}C-labelled metabolite in the aqueous root extracts in all cases (1.4-3.0, 8.0, 5.2, and 3.0 % of the ^{14}C content in soybeans, maize, wheat, and cotton forages, respectively). As expected, based on the metabolic pathways involved, AMPA-^{14}C was observed in neither the aerial nor the root extracts from soybeans (experiments 3 and 4) treated with the N-(phosphonomethyl)-^{14}C-carboxy-glycine and N-(phosphonomethyl)-^{14}C-methyl-glycine labels.

There were no detectable metabolites observed in the N-(phosphonomethyl)-^{14}C-carboxy-glycine and N-(phosphonomethyl)-^{14}C-methyl-glycine hydroponic uptake solutions.

A number of minor metabolites both natural and unnatural were observed in the plant extracts on the basis of TLC/β-camera analysis. Detected minor metabolites, which normally constitute less than one percent of the plant contained ^{14}C-activity, included N-methyl-AMPA, methyl phosphonic acid ($CH_3PO_3H_2$) and N-methyl-glyphosate as well as several unknowns.

These characterisations as well as the relative amounts detected should be viewed cautiously. Some of the minor detectable metabolites are discussed as artefacts from the starting glyphosate-^{14}C-methane and/or the hydroponic solution. In addition, their identification on the basis of TLC alone is inherently tenuous particularly in lieu of natural product formation from both $^{14}CO_2$ and/or metabolic fragments of glyphosate-^{14}C.

Table 6.2.1-82: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 50 mg (experiment 1) or 12 mg (experiment 2) in hydroponic solution - N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean									
	Forage					Roots				
PHI (days)	6	12	20	28	28[1]	6	12	20	28	28[1]
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	69.2	70.8	82.3	80.3	72.3	37.6	59.7	54.3	59.0	42.3
Parent	-	65.4	78.5	70.3	63.1	29.3	54.1	30.2	44.3	35.4
AMPA	-	5.4	3.8	5.1	9.2	2.3	5.6	3.5	1.4	3.0
N-methyl-AMPA	-	-	-	-	-	1.0	-	-	-	0.3
Methyl-phosphonic acid	-	-	-	-	-	-	-	-	-	0.3
Total	**-**	***70.8***	***82.3***	***75.4***	***72.3***	***32.6***	***59.7***	***33.7***	***45.7***	***39.0***

Table 6.2.1-82: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 50 mg (experiment 1) or 12 mg (experiment 2) in hydroponic solution - N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean									
	Forage					Roots				
identified										
Origin	-	-	-	-	-	4.1	-	-	13.3	2.8
Unknown	-	-	-	5.0	-	0.9	-	0.6	-	0.5[2]
Total characterised	**-**	**-**	**-**	**5.0**	**-**	**5.0**	**-**	**0.6**	**13.3**	**3.3**
0.5 N NH_4OH	NP	NP	NP	6.2	4.3	NP	NP	NP	13.0	19.5
0.5 N HCl	NP	NP	NP	2.2	2.3	NP	NP	NP	7.3	15.9
ERR	**69.2**	**70.8**	**82.3**	**88.7**	**78.9**	**37.6**	**59.7**	**54.3**	**79.3**	**77.7**
RRR[3]	***30.8***	***29.2***	***17.7***	***11.3***	***21.1***	***62.4***	***40.3***	***45.7***	***20.7***	***22.3***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] Data from experiment 1, all other data from experiment 2
[2] Consists of 2 unknown compounds
[3] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-83: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 3) in hydroponic solution – N-(phosphonomethyl)-^{14}C-carboxy-glycine

Experiment	Soybean							
	Forage				Roots			
PHI (days)	6	12	20	28	6	12	20	28
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
Aqueous extract	53.1	65.8	72.0	82.6	35.0	73.0	59.3	70.0
Parent	53.1	65.8	69.6	82.6	35.0	73.0	59.3	65.0
AMPA	-	-	-	-	-	-	-	-
N-methyl-AMPA	-	-	-	-	-	-	-	-
Methyl-phosphonic acid	-	-	-	-	-	-	-	-
Total identified	***53.1***	***65.8***	***69.6***	***82.6***	***35.0***	***73.0***	***59.3***	***65.0***
Origin	-	-	-	-	-	-	-	-
Unknown	-	-	2.4	-	-	-	-	5.0

Table 6.2.1-83: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 3) in hydroponic solution – N-(phosphonomethyl)-^{14}C-carboxy-glycine

Experiment	Soybean							
Total characterised	**-**	**-**	**2.4**	**-**	**-**	**-**	**-**	**5.0**
0.5 N NH_4OH	NP	NP	NP	4.2	NP	NP	NP	12.5
0.5 N HCl	NP	NP	NP	3.1	NP	NP	NP	5.4
ERR	**53.1**	**65.8**	**72.0**	**89.9**	**35.0**	**73.0**	**59.3**	**87.9**
RRR[1]	***46.9***	***34.2***	***28.0***	***10.1***	***65.0***	***27.0***	***40.7***	***12.1***
Total sum	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-84: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 4) in hydroponic solution – N-(phosphonomethyl)-^{14}C-methyl-glycine

Experiment	Soybean							
	Forage				Roots			
PHI (days)	6	12	20	25	6	12	20	25
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	66.8	78.6	73.9	91.8	38.4	62.2	41.5	43.5
Parent	66.8	78.6	71.6	85.5	30.3	53.7	28.3	21.5
AMPA	-	-	-	-	-	-	-	-
N-methyl-AMPA	-	-	-	-	2.3	-	-	-
Total identified	***66.8***	***78.6***	***71.6***	***85.5***	***32.6***	***53.7***	***28.3***	***21.5***
Origin	-	-	-	-	1.0	-	-	10.0
Unknown	-	-	2.2	6.4[5]	3.0[2]	8.6[3]	13.2[4]	11.9[6]
Total characterised	**-**	**-**	**2.2**	**6.4**	**4.0**	**8.6**	**13.2**	**21.9**
0.5 N NH_4OH	NP	NP	NP	4.4	NP	NP	NP	18.7
0.5 N HCl	NP	NP	NP	1.7	NP	NP	NP	6.1
ERR	**66.8**	**78.6**	**73.9**	**97.9**	**38.4**	**62.2**	**41.5**	**68.3**
RRR[1]	***33.2***	***21.4***	***26.1***	***2.1***	***61.6***	***37.8***	***58.5***	***31.7***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
[1] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
[2] Consists of two unknowns
[3] Consists of four unknowns
[4] Consists of more than eight unknowns
[5] Consists of three unknowns
[6] Consists of more than six unknowns
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-85: Distribution of the radioactive residue of glyphosate in maize following application of glyphosate at a dose rate of 3 mg (experiment 5) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Maize							
	Forage				Roots			
PHI (days)	6	12	20	28	6	12	20	28
	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
Aqueous extract	70.6	73.0	71.4	81.1	68.6	66.7	99.9	55.5
D-50 column extract	-	58.2	61.6	76.9	-	-	70.6	55.5
Parent	70.6	19.7	23.7	28.1	61.1	56.6	45.5	40.8
AMPA	-	16.2	22.2	27.0	7.4	10.1	8.4	8.0
N-methyl-AMPA	-	4.2	1.9	2.0	-	-	0.6	-
Total identified	***70.6***	***40.1***	***47.8***	***57.1***	***68.5***	***66.7***	***54.5***	***48.8***
Origin	-	-	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	-	-
Void volume[1]	-	-	-	-	-	-	-	5.6
Total characterised	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**5.6**
0.5 N NH_4OH	NP	NP	NP	15.1	NP	NP	NP	21.4
0.5 N HCl	NP	NP	NP	0.4	NP	NP	NP	11.6
Indeterminate[2]	-	18.0	13.8	19.8	-	-	16.2	
ERR	**70.6**	**73.0**	**71.4**	**96.6**	**68.6**	**66.7**	**99.9**	**88.5**
RRR[3]	***29.4***	***27.0***	***28.6***	***3.4***	***31.4***	***33.3***	***0.1***	***11.5***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction)
RRR Residual radioactive residue
[1] Expected to contain neutral and acidic natural products
[2] Indeterminates are defined as the extractable radioactivity which was lost during the chromatographic analyses of the extracts.
[3] The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-86: Distribution of the radioactive residue of glyphosate in wheat following application of glyphosate at a dose rate of 3 mg (experiment 6) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Wheat			
	Forage		Roots	
PHI (days)	6	10	6	10
	% TRR	% TRR	% TRR	% TRR
Aqueous extract	77.3	77.3	42.3	54.5
Parent	69.3	70.7	33.5	38.5
AMPA	8.0	6.6	8.8	5.2
N-methyl-AMPA	-	-	-	2.0
Total identified	***77.3***	***77.3***	***42.3***	***45.7***
Origin	-	-	-	3.1
Unknown	-	-	-	3.7
Total characterised	**-**	**-**	**-**	***6.8***
0.5 N NH_4OH	NP	3.6	NP	16.7
0.5 N HCl	NP	3.1	NP	15.0

Table 6.2.1-86: Distribution of the radioactive residue of glyphosate in wheat following application of glyphosate at a dose rate of 3 mg (experiment 6) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Wheat			
	Forage		Roots	
ERR	**77.3**	**84.0**	**42.3**	**86.2**
RRR*	***22.7***	***16.0***	***57.7***	***13.8***
Total sum	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
* The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-87: Distribution of the radioactive residue of glyphosate in cotton following application of glyphosate at a dose rate of 12 mg (experiment 7) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Cotton							
	Forage				Roots			
PHI (days)	6	13	20	28	6	13	20	28
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
Aqueous extract	73.8	84.6	78.8	89.2	51.2	30.8	32.8	17.3
Parent	73.8	80.0	70.8	70.5	38.8	21.3	24.5	11.1
AMPA	-	4.6	5.3	8.0	8.9	4.2	4.4	3.0
N-methyl-AMPA	-	-	-	-	-	0.8	0.2	0.4
N-methyl-glyphosate[1]	-	-	-	-	-	-	-	0.3
Methyl-phosphonic acid[2]	-	-	-	-	-	-	2.0	0.4
Total identified	***73.8***	***84.6***	***76.1***	***78.5***	***47.7***	***26.3***	***31.1***	***15.2***
Origin	-	-	-	-	3.5	2.8	-	1.7
Unknown	-	-	2.7	-	-	1.7	1.8	0.2
Void volume[3]	-	-	-	2.8	-	-	-	-
Total characterised	***-***	***-***	***2.7***	***2.8***	***3.5***	***4.5***	***1.8***	***1.9***
0.5 N NH_4OH	NP	NP	NP	4.7	NP	NP	NP	27.0
0.5 N HCl	NP	NP	NP	0.8	NP	NP	NP	34.6
ERR	**73.8**	**84.6**	**78.8**	**94.7**	**51.2**	**30.8**	**32.8**	**78.9**
RRR[4]	***26.2***	***15.4***	***21.2***	***5.3***	***48.8***	***69.2***	***67.2***	***21.1***
Total sum	***100***	***100***	***100***	***100***	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
NP Not performed
1 Named as CP67205 in the report ($HO_2CCH_2N(CH_3)CH_2PO_3H_2$)
2 Named as $CH_3PO_3H_2$ in the report
3 Expected to contain neutral and acidic natural products
4 The RRR was recalculated based on ERR and an assumed recovery of 100 %.
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-88: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 2.96 mg and 50.12 mg respectively (experiment 9) in hydroponic solution – N-(phosphono-^{13}C-methyl)glycine and N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean			
	Forage			
PHI (days)	6	12	20	26
	% TRR	% TRR	% TRR	% TRR
Aqueous extract	85.3	63.1	66.2	66.6
Parent	60.6	54.4	56.2	55.1
AMPA and/or N-methyl-AMPA	16.6	7.3	5.2	7.8
Methyl-phosphonic acid[1]	-	-	-	-
Total identified	***77.2***	***61.7***	***61.4***	***62.9***
Origin	-	-	-	-
Unknown	-	-	-	-
Total characterised	-	-	-	-
0.5 N NH_4OH	NP	NP	NP	NP
0.5 N HCl	NP	NP	NP	NP
ERR	**85.3**	**63.1**	**66.2**	**66.6**
RRR[2]	***14.7***	***36.9***	***33.8***	***33.4***
Total sum	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
1 Named as $CH_3PO_3H_2$ in the report
2 The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Table 6.2.1-89: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 10) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean			
	Forage			
PHI (days)	6	12	20	28
	% TRR	% TRR	% TRR	% TRR
Aqueous extract	67.4	55.0	50.8	43.2
Parent	55.3	46.5	39.3	29.4
AMPA and/or N-methyl-AMPA	8.8	4.7	5.5	5.8
Methyl-phosphonic acid[1]	-	-	-	-
Total identified	***64.1***	***51.2***	***44.8***	***35.2***
Origin	-	-	-	-
Unknown	-	-	-	-
Void volume[2]	3.2	3.7	6.0	7.2
Total characterised	**3.2**	**3.7**	**6.0**	**7.2**
0.5 N NH_4OH	NP	NP	NP	NP
0.5 N HCl	NP	NP	NP	NP

Table 6.2.1-89: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate at a dose rate of 12 mg (experiment 10) in hydroponic solution – N-(phosphono-^{14}C-methyl)glycine

Experiment	Soybean			
	Forage			
PHI (days)	6	12	20	28
ERR	**67.4**	**55.0**	**50.8**	**43.2**
RRR[3]	***32.6***	***45.0***	***49.2***	***56.8***
Total sum	***100***	***100***	***100***	***100***

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue (dependent on experiment equivalent to aqueous extract or aqueous extract + sum of extracts after exhaustive extraction).
RRR Residual radioactive residue
1 Named as $CH_3PO_3H_2$ in the report
2 Expected to contain neutral and acidic natural products
3 The RRR was recalculated based on ERR and an assumed recovery of 100 %.
NP Not performed
Values in *italics* were calculated during dossier compilation.
Note: Values in the report are described as "% starting activity", they are interpreted as % TRR.

Natural product analysis

Plants were separately extracted to investigate radioactivity incorporated into natural products. Results are comparable in efficiency to those obtained previously during the hydroponic uptake experiments despite a shorter extraction time and the extraction of larger samples for a given volume of water in the former experiments. In the N-(phosphono-^{14}C-methyl)glycine-treated forage samples collected at termination, after 28 days in all cases (except wheat, 10 days), 90.5, 73.4, 68.5, and 90.3 % of the ^{14}C-activity in soybeans, maize, wheat, and cotton, respectively, were solubilised with a single water extraction.
The distribution of ^{14}C-activity into the initial four fractions (neutral, basic, acid 1 and acid 2), showed that most (70 - 90 %) of the extractable ^{14}C-activity was found in the basic and acid-1 fractions, with essentially none in the neutral and a maximum of approximately 20 % in the acid-2 fraction. This finding is consistent with glyphosate and AMPA comprising the bulk of the extractable^{14}C-activity.
The basic fractions obtained were evaluated by TLC/ß-camera in order to determine the relative amounts of ^{14}C-labelled phosphonates and natural amino acids and peptides. ^{14}C-labelled phosphonates detected were glyphosate, AMPA and N-methyl-AMPA in the N-(phosphono-^{14}C-methyl)glycine treated samples. N-methyl-AMPA-^{14}C was observed in the N-(phosphonomethyl)-^{14}C-methyl-glycine treatments; AMPA-^{14}C was not detected. Neither AMPA nor N-methyl-AMPA were observed in the glycine-l-^{14}C-treatments.

A significant incorporation of ^{14}C-activity into the natural amino acids and peptides was determined in the case of soybeans and cotton. In the N-(phosphono-^{14}C-methyl)glycine treated cotton and soybeans, 3.3 % and 7.0 % of the extractable radioactivity of cotton and soybean forage consisted of natural basic materials (amino acids and peptides); no detectable activity of this type was observed in the corn and wheat samples. The most striking example of ^{14}C-activity in the natural basic materials is in the case of the N-(phosphonomethyl)-^{14}C-methyl-glycine-treated soybeans; 7.6 % and 25.8 % of the extractable radioactivity from the top and root portions, respectively, were coincident with natural basic products. Only traces of ^{14}C-labelled basic natural products were observed in the N-(phosphonomethyl)-^{14}C-carboxy-glycine treated soybeans.

Table 6.2.1-90: Extraction of the radioactive residue of glyphosate in soybean following application of N-(phosphono-^{14}C-methyl)glycine, N-(phosphonomethyl)-^{14}C-carboxy-glycine or N-(phosphonomethyl)-^{14}C-methyl-glycine in hydroponic solution

	Soybean	
	Forage	Roots

Label	N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine		N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine	
PHI (days)	28		28		25		28		28		25	
	% of extr.	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**
TRR		**100**		**100**		**100**		**100**		**100**		**100**
Aqueous extract		**90.5**		**75.5**		**82.6**		**43.7**		**58.7**		**37.0**
Neutral	**2.6**	***2.4***	**2.1**	***1.6***	**2.2**	***1.8***	**0.2**	***0.1***	**0.40**	***0.2***	**2.0**	***0.7***
Basic	**43.3**	***39.2***	**40.9**	***30.9***	**26.6**	***22.0***	**31.1**	***13.6***	**31.8**	***18.7***	**47.8**	***17.7***
Parent	28.9	*26.2*	40.9	*30.9*	19.0	*15.7*	21.0	*9.2*	30.8	*18.1*	19.8	*7.3*
AMPA	9.9	*9.0*	-	-	-	-	7.0	*3.1*	-	-	-	-
N-methyl-AMPA	1.2	*1.1*	-	-	-	-	0.8	*0.3*	-	-	1.2	*0.4*
Natural amino acids and peptides	3.3	*3.0*	-	-	7.6	*6.3*	2.3	*1.0*	1.3	*0.8*	25.8	*9.5*
Acid-1	**49.3**	***44.6***	**29.1**	***22.0***	**47.6**	***39.3***	**42.8**	***18.7***	**51.7**	***30.3***	**34.8**	***12.9***
Parent	42.4	*38.4*	24.9	*18.8*	39.0	*32.2*	40.6	*17.7*	49.8	*19.0*	32.4	*18.4*
Hydrolysed phosphate ester (sugars)	2.3	*2.1*	0.9	*0.7*	2.5	*2.1*	-	-	-	-	0.2	*0.1*
Glyceric region	-	-	0.3	*0.2*	-	-	0.1	*0.04*	0.1	0.1	-	-
Succinic region	0.5	*0.5*	0.5	*0.4*	1.0	0.8	0.1	*0.04*	-	-	0.2	0.1
Malic region	-	-	0.2	*0.2*	-	-	0.3	*0.1*	-	-	-	-
Phosphate sugars	0.1	*0.1*	1.0	*0.8*	0.5	0.4	-	-	-	-	-	-
Citric region	-	-	-	-	-	-	-	-	-	-	-	-
Fumaric region	-	-	-	-	-	-	-	-	-	-	-	-
Unknown	0.6	*0.5*	-	-	2.7	2.2	-	-	0.5	*0.3*	-	-
Acid-2	**6.7**	***6.1***	**12.4**	***9.4***	**2.0**	**1.7**	**26.1**	***11.4***	**17.7**	***10.4***	**8.2**	**3.0**
Parent	*5.1*	*4.6*	9.8	*7.4*	*1.13*	*0.9*	*23.8*	*10.4*	16.7	*9.8*	*6.7*	*2.5*
Sugars	0.1	*0.1*	-	-	0.0	0.0	0.2	*0.1*	-	-	0.0	0.0
Sugar mono-phosphate	-	-	-	-	-	-	-	-	-	-	-	-
3-PGA	0.1	*0.1*	0.2	*0.2*	0.3	*0.2*	-	-	-	-	0.1	0.04
Sugar diphosphate/ PEP	0.1	0.1	0.0	*0.0*	0.1	*0.1*	0.1	*0.04*	-	-	0.2	0.1
RUDP	0.1	0.1	0.1	*0.1*	0.1	*0.1*	-	-	-	-	0.1	0.04
Unknowns	0.2	0.2	0.1	*0.1*	0.1	*0.1*	-	-	-	-	0.0	0.0
Total identified	***87.5***	***79.2***	***75.6***	***57.2***	***59.1***	***48.8***	***93.2***	***40.7***	***79.9***	***46.9***	***77.5***	***28.7***
Total characterised	***10.0***	***9.05***	***5.4***	***4.08***	***17.1***	***14.1***	***3.3***	***1.4***	***2.3***	***1.35***	**28.60**	***10.58***
Indeterminate	2.5	2.3	18.9	14.3	23.7	19.6	3.6	1.6	0.6	0.4	11.3	4.2

Table 6.2.1-90: Extraction of the radioactive residue of glyphosate in soybean following application of N-(phosphono-^{14}C-methyl)glycine, N-(phosphonomethyl)-^{14}C-carboxy-glycine or N-(phosphonomethyl)-^{14}C-methyl-glycine in hydroponic solution

	Soybean											
	Forage						Roots					
Label	N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine		N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine	
PHI (days)	28		28		25		28		28		25	
[1]												
ERR		**90.5**		**75.5**		**82.6**		**43.7**		**58.7**		**37.0**
RRR		**9.5**		**24.5**		**17.4**		**56.3**		**41.3**		**63.0**
Total sum		**100**		**100**		**100**		**100**		**100**		**100**

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
Values in *italics* were calculated during dossier compilation.
[1] Indeterminates are defined as the extractable radioactivity which was lost during the chromatographic analyses of the extracts.
Note: recalculation of % TRR into mg/kg was not possible as sample weights available in the report were expressed as dry weights only.

Table 6.2.1-91: Extraction of the radioactive residue of glyphosate in corn, wheat and cotton following application of N-(phosphono-^{14}C-methyl)glycine in hydroponic solution

	Maize/Corn		Wheat		Cotton		Maize/Corn		Wheat		Cotton	
	Forage						Roots					
Label	N-(phosphono-^{14}C-methyl)glycine											
PHI (days)	28		10		28		28		10		28	
	% of extr.	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr**	**% TRR**	**% of extr**	**% TRR**	**% of extr**	**% TRR**
TRR		**100**		**100**		**100**		**100**		**100**		**100**
Aqueous extract		**73.4**		**68.5**		**90.0**		**64.3**		**45.7**		**13.0**
Neutral	**2.1**	***1.5***	**0.8**	***0.5***	**1.3**	***1.2***	**1.2**	***0.8***	**1.0**	***0.7***	**0.8**	***0.1***
Basic	**51.3**	***37.7***	**45.3**	***31.0***	**42.2**	***38.0***	**41.2**	***26.5***	**36.5**	***31.0***	**39.9**	***5.2***
Parent	13.3	*9.8*	36.6	*25.1*	25.2	*22.7*	34.4	*22.1*	20.4	*25.1*	10.2	*1.3*
AMPA	38.0	*27.9*	8.7	*6.0*	7.6	*6.8*	6.8	*4.4*	8.7	*6.0*	21.6	*2.8*
N-methyl-AMPA	-	-	-	-	2.2	*2.0*	-	-	-	-	3.3	0.4
Natural amino acids and peptides	-	-	-	-	7.0	*6.3*	-	-	-	-	4.8	0.6
Acid-1	**15.3**	***11.2***	**28.6**	***19.6***	**32.9**	***29.6***	**26.8**	***17.2***	**42.3**	***29.0***	**32.0**	***4.2***
Parent	11.0	*8.1*	26.5	*18.2*	38.5	*34.7*	24.1	*15.5*	40.6	*27.8*	32.8	*4.3*
Hydrolysed phosphate ester (sugars)	1.0	*0.7*	0.3	*0.2*	0.0	*0.0*	0.2	*0.1*	0.1	*0.1*	0.2	*0.0*
Glyceric region	0.6	*0.4*	0.1	*0.1*	0.1	*0.1*	0.2	*0.1*	0.1	*0.1*	-	-
Succinic region	0.5	*0.4*	0.1	*0.1*	0.3	*0.3*	-	-	0.0	*0.0*	-	-
Malic region	-	-	0.1	0.1	-	-	0.1	0.1	-	-	-	-
Phosphate sugars	0.6	0.4	-	-	-	-	-	-	-	-	0.6	0.1
Citric region	-	-	-	-	-	-	-	-	-	-	-	-
Fumaric region	-	-	-	-	-	-	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	-	-	-	-	-	-
Acid-2	**6.7**	***4.9***	**18.7**	***12.8***	**7.0**	***6.3***	**15.2**	***9.8***	**19.8**	***13.6***	**19.9**	***2.6***
Parent	*4.4*	*3.2*	*17.7*	*12.1*	*4.6*	*4.1*	0.8	*0.5*	*17.9*	*12.3*	*15.6*	*2.0*

Table 6.2.1-91: Extraction of the radioactive residue of glyphosate in corn, wheat and cotton following application of N-(phosphono-^{14}C-methyl)glycine in hydroponic solution

	Maize/Corn		Wheat		Cotton		Maize/Corn		Wheat		Cotton	
	Forage						Roots					
Label	N-(phosphono-^{14}C-methyl)glycine											
Sugars	0.0	*0.0*	-	-	0.7	*0.6*	1.3	*0.8*	-	-	0.9	*0.1*
Sugar monophosphate	-		-	-	-	-	-	-	-	-	-	-
3-PGA	0.4	*0.3*	0.1	0.1	0.3	*0.3*	-	-	0.1	*0.1*	0.3	*0.0*
Sugar diphosphate/ PEP	-	-	-	-	0.1	*0.1*	0.1	*0.1*	0.1	*0.1*	0.5	*0.1*
RUDP	-	-	0.0	0.0	-	-	12.4	*8.0*	0.1	*0.1*	0.2	*0.0*
Unknowns	0.3	0.2	0.1	0.1	0.1	0.1	-	-	0.2	*0.1*	0.3	*0.0*
Total identified	***66.7***	***49.0***	***89.5***	***61.3***	***78.1***	***70.3***	***66.1***	***42.3***	***103.8***	***71.1***	***83.5***	***10.9***
Total characterised	***5.5***	***1.6***	***1.6***	***1.1***	***9.9***	***8.9***	***15.5***	***10.0***	***1.7***	***1.2***	***8.6***	***1.1***
Indeterminate	27.8	20.4	11.7	8.0	12.7	11.4	18.4	11.8	2.8	1.9	8.3	1.1
ERR		**73.4**		**68.5**		**90.0**		**64.3**		**45.7**		**13.0**
RRR		**26.6**		**31.5**		**10.0**		**35.7**		**54.3**		**87.0**
Total sum		**100**		**100**		**100**		**100**		**100**		**100**

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
Note: recalculation of % TRR into mg/kg was not possible as sample weights available in the report were expressed as dry weights only.

Table 6.2.1-92: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate-^{14}C, ^{14}C-1-glyphosate or ^{14}C-2-glyphosate in hydroponic solution

	Soybean											
	Forage						Roots					
Label	N-(phosphono-^{14}C-methyl) glycine		N-(phosphonomethyl)-^{14}C-carboxy-glycine		N-(phosphonomethyl)-^{14}C-methyl-glycine		N-(phosphono-^{14}C-methyl) glycine		N-(phosphonomethyl)-^{14}C-carboxy-glycine		N-(phosphonomethyl)-^{14}C-methyl-glycine	
PHI (days)	28		28		25		28		28		25	
	% of extr.	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**
TRR		**100**		**100**		**100**		**100**		**100**		**100**
Parent	76.5	69.2	75.6	57.1	59.1	48.8	85.4	37.3	*79.9*[1]	*46.9*[1]	*76.3*[1]	*28.3*[1]
AMPA	9.9	9.0	-	-	-	-	7.0	3.1	-	-	-	-
N-methyl-AMPA	1.2	1.1	-	-	-	-	0.8	0.3	-	-	1.2	0.4
Total identified	***87.5***	***79.2***	***75.6***	***57.2***	***59.1***	***48.8***	***93.2***	***40.7***	***79.9***	***46.9***	***77.5***	***28.7***
Natural amino acids and peptides	3.3	3.0	-	-	7.6	*6.3*	2.3	1.0	1.3	0.8	25.8	9.5
Hydrolysed phosphate ester (sugars)	2.3	*2.1*	0.9	0.7	2.5	*2.1*	-	-	-	-	0.2	0.1
Glyceric region	-	-	0.3	0.2	-	-	0.1	*0.04*	0.1	0.1	-	-
Succinic region	0.5	*0.5*	0.5	0.4	1.0	*0.8*	0.1	*0.04*	-	-	0.2	0.1
Malic region	-	-	0.2	0.2	-	-	0.3	*0.1*	-	-	-	-

Table 6.2.1-92: Distribution of the radioactive residue of glyphosate in soybean following application of glyphosate-^{14}C, ^{14}C-1-glyphosate or ^{14}C-2-glyphosate in hydroponic solution

Phosphate sugars	0.1	*0.1*	1.0	0.8	0.5	*0.4*	-	-	-	-	-	-
Citric region	-	-	-	-	-	-	-	-	-	-	-	-
Fumaric region	-	-	-	-	-	-	-	-	-	-	-	-
Unknown 1	0.6	*0.5*	-	-	2.7	*2.2*	-	-	0.5	0.3	-	-
Sugars	0.1	*0.1*	-	-	0.0	0.0	0.2	*0.1*	-	-	0.0	0.0
Sugar mono-phosphate	-	-	-	-	-	-	-	-	-	-	-	
3-PGA	0.1	*0.1*	0.2	*0.2*	0.3	*0.2*	-	-	-		0.1	0.04
Sugar diphosphate/ PEP	0.1	*0.1*	0.0	*0.0*	0.1	*0.1*	0.1	*0.04*	-	-	0.2	0.1
RUDP	0.1	*0.1*	0.1	*0.1*	0.1	*0.1*	-	-	-	-	0.1	0.04
Unknowns 2	0.2	*0.2*	0.1	*0.1*	0.1	*0.1*	-	-	-	-	0.0	0.0
Total charac-terised	***10.0***	***9.05***	***5.4***	***4.08***	***17.1***	***14.1***	***3.3***	***1.4***	***2.3***	***1.35***	**28.60**	***10.58***
In-determinate[2]	2.5	2.3	18.9	14.3	23.7	19.6	3.6	1.6	0.6	0.4	11.3	4.2
ERR		**90.5**		**75.5**		**82.6**		**43.7**		**58.7**		**37.0**
RRR		**9.5**		**24.5**		**17.4**		**56.3**		**41.3**		**63.0**
Total sum		**100**		**100**		**100**		**100**		**100**		**100**

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
Values in *italics* were calculated during dossier compilation.
[1] Values differ from the values given in the report. Presented values are the sum of glyphosate within the different fractions.
[2] Indeterminates are defined as the extractable radioactivity which was lost during the chromatographic analyses of the extracts.
Note: recalculation of % TRR into mg/kg was not possible as sample weights available in the report were expressed as dry weights only.

Table 6.2.1-93: Distribution of the radioactive residue of glyphosate in corn, wheat and cotton following application of ^{14}C-methane-glyphosate in hydroponic solution

	Maize/Corn		**Wheat**		**Cotton**		**Maize/Corn**		**Wheat**		**Cotton**	
	Forage						**Roots**					
Label	N-(phosphono-^{14}C-methyl)glycine											
PHI (days)	28		10		28		28		10		28	
	% of extr.	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr**	**% TRR**	**% of extr**	**% TRR**	**% of extr**	**% TRR**
TRR		**100**		**100**		**100**		**100**		**100**		**100**
Parent	28.7	21.1	80.7	55.3	68.3	61.5	*59.3**	*38.1**	*95.1**	*65.1**	58.6	7.6
AMPA	38.0	*27.9*	8.7	*6.0*	7.6	*6.8*	6.8	*4.4*	8.7	*6.0*	21.6	*2.8*
N-methyl-AMPA	-	-	-	-	2.2	*2.0*	-	-	-	-	3.3	0.4
Total identified	***66.7***	***49.0***	***89.5***	***61.3***	***78.1***	***70.3***	***66.1***	***42.5***	***103.8***	***71.1***	***83.5***	***10.9***
Natural amino acids and peptides	-	-	-	-	7.0	*6.3*	-	-	-	-	4.8	0.6
Hydrolysed phosphate ester (sugars)	1.0	*0.7*	0.3	*0.2*	0.0	*0.0*	0.2	*0.1*	0.1	*0.1*	0.2	*0.0*
Glyceric region	0.6	*0.4*	0.1	*0.1*	0.1	*0.1*	0.2	*0.1*	0.1	*0.1*	-	-
Succinic region	0.5	*0.4*	0.1	*0.1*	0.3	*0.3*	-	-	0.0	*0.0*	-	-
Malic region	-	-	0.1	0.1	-	-	0.1	0.1	-	-	-	-

Table 6.2.1-93: Distribution of the radioactive residue of glyphosate in corn, wheat and cotton following application of ^{14}C-methane-glyphosate in hydroponic solution

	Maize/Corn		**Wheat**		**Cotton**		**Maize/Corn**		**Wheat**		**Cotton**	
	Forage						**Roots**					
Label	N-(phosphono-^{14}C-methyl)glycine											
PHI (days)	28		10		28		28		10		28	
	% of extr.	**% TRR**	**% of extr.**	**% TRR**	**% of extr.**	**% TRR**	**% of extr**	**% TRR**	**% of extr**	**% TRR**	**% of extr**	**% TRR**
Phosphate sugars	0.6	0.4	-	-	-	-	-	-	-	-	0.6	0.1
Citric region	-	-	-	-	-	-	-	-	-	-	-	-
Fumaric region	-	-	-	-	-	-	-	-	-	-	-	-
Unknown	-	-	-	-	-	-	-	-	-	-	-	-
Sugars	0.0	*0.0*	-	-	0.7	*0.6*	1.3	*0.8*	-	-	0.9	*0.1*
Sugar monophosphate	-		-	-	-	-	-	-	-	-	-	-
3-PGA	0.4	*0.3*	0.1	0.1	0.3	*0.3*	-	-	0.1	*0.1*	0.3	*0.0*
Sugar diphosphate/ PEP	-	-	-	-	0.1	*0.1*	0.1	*0.1*	0.1	*0.1*	0.5	*0.1*
RUDP	-	-	0.0	0.0	-	-	12.4	*8.0*	0.1	*0.1*	0.2	*0.0*
Unknowns	0.3	0.2	0.1	0.1	0.1	0.1	-	-	0.2	*0.1*	0.3	*0.0*
Total characterised	***5.5***	***1.6***	***1.6***	***1.1***	***9.9***	***8.9***	***15.5***	***10.0***	***1.7***	***1.2***	***8.6***	***1.1***
Indeterminate**	27.8	20.4	11.7	8.0	12.7	11.4	18.4	11.8	2.8	1.9	8.3	1.1
ERR		**73.4**		**68.5**		**90.0**		**64.3**		**45.7**		**13.0**
RRR		**26.6**		**31.5**		**10.0**		**35.7**		**54.3**		**87.0**
Total sum		**100**		**100**		**100**		**100**		**100**		**100**

PHI Pre-harvest interval
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
Values in *italics* were calculated during dossier compilation.
* Values differ from the values given in the report. Presented values are the sum of glyphosate within the different fractions.
** Indeterminates are defined as the extractable radioactivity which was lost during the chromatographic analyses of the extracts.
Note: recalculation of % TRR into mg/kg was not possible as sample weights available in the report were expressed as dry weights only.

C. Storage stability

No dates are reported for the experimental work from sampling to extraction and analysis of extracts. Thus, it is not possible to conclude on storage stability.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusions

In this study the uptake of ^{14}C-glyphosate and ^{14}C-AMPA via the roots in conventional soybeans, wheat, cotton, and maize grown in soil, sand culture and hydroponic nutrient solutions was investigated.
Less than 0.3 % of the applied radioactivity was taken up by the growing plants at 4, 6 and 8 weeks after soil application. The very low levels of ^{14}C-activity in plant samples did not allow to further study the distribution and metabolism of glyphosate in plants using this method of treatment.
Uptake of N-(phosphono-^{14}C-methyl)glycine into plants growing in sand culture after application of an aqueous solution of N-(phosphono-^{14}C-methyl)glycine to the sand has also been examined. Only maize gave an uptake of 11.3 % of the applied dose into the aerial portion after 18 days. Cotton, soybean and wheat had aerial uptakes of only 0.03, 0.07, and 0.03 %, respectively, of the applied ^{14}C-activity after 18

days. The extraction data (aqueous extraction followed by 1 N NH_4OH) for the treated sand show that N-(phosphono-^{14}C-methyl)glycine was not available for uptake into the plants.

Uptakes of ^{14}C-activity in the aerial portions in all crops at the comparable time period of 26-28 days ranged from 1.71 % to a maximum of 7.70 % (both soybean). Uptake of ^{14}C-activity into the root portions at the comparable time period of 25-28 days ranged from 5.48 % (soybean) to a maximum of 19.34 % (cotton). In the ^{14}C-pulse experiment, 24 soybean plants were hydroponically treated with 12 mg of N-(phosphono-^{14}C-methyl)glycine for 6 days and then removed to untreated, fresh nutrient media. At 6, 12, 20, 28, 42, and 56 days, plants were removed and analysed for ^{14}C-content. The data show a decrease of radioactivity in roots from 2.40 at day 6 to 0.66 % applied radioactivity at day 28, and from 0.28 to 0.25 % applied radioactivity in aerial parts, respectively.

Plants (aerial parts and roots) after hydroponic uptake were extracted and the residues were characterised. With the exception of maize forage, the major ^{14}C-containing component in the aqueous extracts in all cases was parent glyphosate in aerial parts and in roots; in maize forage, comparable amounts of glyphosate and AMPA were observed.

The major ^{14}C-containing degradate in all four crops was AMPA accounting for up to 38.0 % of the TRR in aerial parts and up to 21.6 % of the TRR in roots.

Several minor metabolites were also detected and were identified as N-methyl-aminomethyl phosphonic acid (N-methyl AMPA), methyl-phosphonic acid, and N-methyl-glyphosate, as well as some unknowns.

Some of the minor detectable metabolites are discussed as artefacts resulting from the starting glyphosate-^{14}C-methane and/or the hydroponic solution. In addition, their identification on the basis of TLC alone is inherently tenuous particularly in lieu of natural product formation from both $^{14}CO_2$ and/or metabolic fragments of glyphosate-^{14}C.

Separate extractions to investigate the radioactivity in natural products indicated the incorporation of fragments or $^{14}CO_2$ into natural products (e.g. amino acids and peptides or citric acid cycle intermediates).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in cereals (maize, cotton, soybean and wheat) has been previously evaluated at EU level. It was not performed under GLP but is still considered to be scientifically valid. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with some deficits (developmental stages of the crop at application and harvesting are not reported; no relevant RAC (raw agricultural commodities) samples taken (maize/corn, soybean, wheat and cotton, edible commodity), only roots and forage and developmental stage of forage was not defined properly (i.e. not evident if maybe relevant as feed item); in some cases the radioactive residues in RAC are expressed in % of applied activity rather than in terms of TRR; fresh sample weights were not available therefore no calculation of mg/kg values was possible; in some cases the radioactive residues in RAC are expressed in % TRR only. Fresh sample weights were not available therefore no calculation of mg/kg values was possible; in some cases % TRR values of fractions/non-extractable radioactivity exceeded the trigger value of 10 % but the sample was not further analysed/extracted; no flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed; no photographs/images/figures of TLC plates critical to the identification; no description of conditions and length of storage of samples and extracts, therefore it can´t be decided if storage stability investigation of samples would be necessary for this study.

Quantitative information in terms of absolute amounts of radioactive residues expressed in mg/kg fresh weight is available and no recalculation is possible based on missing fresh sample weights.
However, relative amounts in terms of percentage of applied radioactivity or percentage of TRR, as reported in the study, allow for an assessment of the relative uptake and distribution of ^{14}C-glyphosate or ^{14}C-AMPA after soil treatment, and of ^{14}C-glyphosate after sand culture or hydroponical treatment.

It is not stated of the storage duration of samples exceeded 6 months. However, it is considered that the study was performed in a reasonable timeframe of less than two years (on the front page of the report the timeframe of January 1972 to June 1973 is stated; the report date is given with July 1973) and therefore the qualitative and quantitative results of the present study are considered valid in context of storage stability.
In addition, a high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies. No degradation of glyphosate and its metabolites was found in matrices with high water content (corn forage, fodder, cotton forage, soybean forage). Over an investigated storage duration of 215-393 days no degradation was observed in the metabolic profile ([redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/022). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1).

Thus, although the study does not comply with current guideline requirements in some aspects, it still gives relevant and consistent qualitative information on the uptake and distribution of glyphosate-derived residues after soil application and growing in sand culture and hydroponic solution and information on the nature of the residues in forage and roots from maize, cotton, soybean and wheat after hydroponical treatment. Therefore, this study is considered to be reliable to support the metabolic behaviour of glyphosate in cereal/grass crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/013
Report author	[redacted]
Report year	1976
Report title	The metabolism of glyphosate in pasture crops
Report No	404
Document No	Not available
Guidelines followed in study	None
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • The radiochemical purity of the test substances is not unambiguously specified • Radioactive residues in RAC are expressed in % of applied activity (as %AR) rather than in terms of TRR in mg/kg. Recalculation is only possible for the soil application experiment I • No full description of the extraction and fractionation of radioactivity in the various crop matrices • Radioactive residues were characterised only in two out of 4 experiments. Results are given in % applied. No TRR is reported or can be calculated for the fractions characterised • Identification results by GC-MS are only described qualitatively • No full accountability reported • No information of the storage stability for all major components of the total radioactive residues • No description of length of storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

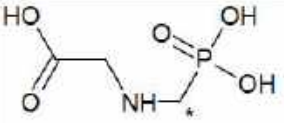
2. Full summary of the study according to OECD format

Executive summary

The uptake of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) in pasture crops was investigated after soil and foliar treatment. Application scenarios included pre-emergent treatment at 4.48 kg a.s./ha, incorporation of treated foliage into the seed bed and foliar treatment at 1.12 kg a.s./ha.
In all experiments with soil application the uptake was very limited, not exceeding 0.1 % of the AR. In directly treated foliage 41.8-69 % of the AR was recovered after one week. Regrowth after eradication of treated foliage showed residue levels at or below 0.2 % of the applied activity.

I. Materials and methods

A. Materials

Test Material:	a) N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) b) N-(phosphono-^{13}C-methyl)glycine (^{13}C-glyphosate)
Chemical structure:	 a), b) * Position of label
Radiochemical purity[1]:	Not specified (purification by D-50 [H$^+$] ion exchange chromatography prior to use)
Specific activity:	a) 0.41 MBq/mg (1.87 mCi/mmol) 1.98 MBq/mg (9.07 mCi/mmol)
CAS No:	1071-83-6
Log $P_{o/w}$:	- 3.2

[1] Purities are stated for a) N-(phosphono-^{14}C-methyl)glycine to be 96 % and 97 % for the two batches, respectively; it is not clear from the report if this refers to chemical or radiochemical purity.
For b) N-(phosphono-^{13}C-methyl)glycine stated purity is 97.7 %.

Test system:

Soil:	Silt loam (organic matter: 1.2 %; sand: 4.6 %; silt: 84.2 %; clay: 10.0 %; pH: 8.1; water holding capacity: 23.9 %; cation exchange capacity: 10.4 meq./mL; particle density 1.13 g/mL)
Crop:	Tall Fescue (Variety: Kentucky-31) Smooth Bromegrass (Origin: Kansas) Timothy (Origin: Missouri) Alfalfa (Variety: Vernal) White Clover (Variety: Ladino) Red Clover (Variety not known)
Botanical name:	*Festuca arundinacea* *Bromus inermis* *Phleum pratense* *Medicago sativa* *Trifolium repens* *Trifolium pratense*
Crop part(s):	Forage

B. Study design

1. In-life phase

In this study the behaviour of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) in pasture crops was investigated in four experiments.

In the first experiment (I) seed mixtures of fescue/alfalfa, bromegrass/red clover and timothy/white clover were planted in plastic pots. The experimental design included treated and untreated pots, replicated twice. Each treated pot received a 4.48 kg a.s./ha pre-emergent application of ^{14}C-glyphosate (11.54 mg, corresponding to 2.83×10^8 dpm).

The second experiment (II) involved treatment of established quackgrass with 1.68 kg glyphosate/ha. The treatment solution was formulated as Roundup®, containing 5.405 mg ^{14}C-glyphosate (corresponding to 1.32×10^8 dpm), 0.071 mL isopropylamine, 0.071 mL Atlas adjuvant G 3780A, and 4.0 mL water. One week after treatment the quackgrass foliage, roots and soil were thoroughly mixed and then used to form the top seed bed in two plastic pots. Each pot contained an activity of 6.64×10^7 dpm. One month after incorporation of the quackgrass a fescue/alfalfa mixture was sown.

In the third experiment (III) established fescue and alfalfa plants growing in separate plastic pots were foliar treated with 1.12 kg/ha N-(phosphono-^{13}C/^{14}C-methyl)glycine (90:10). The treatment solution for each pot was formulated as Roundup®, containing 0.278 mg ^{14}C-glyphosate (corresponding to 6.7×10^6 dpm), 2.505 mg N-(phosphono-^{13}C-methyl)glycine, 0.028 mL isopropylamine, 0.028 mL Atlas adjuvant G3780A, and 3.0 mL water. The treated foliage was removed after one week and the fescue and alfalfa regrowth was sampled.

The fourth experiment (IV) simulated pre-harvest use by treating established fescue and alfalfa plants growing in plastic pots with 1.12 kg ^{14}C-glyphosate/ha (2.045 mg ^{14}C-glyphosate, corresponding to 2.43×10^8 dpm) one week before harvest.
Frozen plant samples from study I to IV were lyophilised. The dried material was ground to a fine powder that would pass a 60 mesh screen.

2. Sampling

Experiment I: Samples of grass and legume forage were collected after 6, 12, 18, 24 and 32 weeks and frozen.
Experiment II: Samples of fescue and alfalfa forage were collected after 6, 12, 18 and 24 weeks and frozen.
Experiment III: After 9, 15 and 23 weeks the fescue and alfalfa regrowth (forage) was sampled and frozen.
Experiment IV: The fescue and alfalfa forage was harvested one week after treatment and allowed to air dry in the greenhouse for an additional week before freezing to simulate curing.

Frozen plant samples from experiments I through IV were lyophilised. The dried material was then ground to a fine powder that would pass a 60 mesh screen.

3. Analytical procedures

The total ^{14}C-activity present in samples was determined directly by combustion of homogenised and lyophilised plant samples. The total ^{14}C-activity present in aqueous extracts of plant samples from experiments III and IV were determined by liquid scintillation counting (LSC).

Radioactive components were extracted from dried forage three times with deionised water. The composited extracts contained >95 % of the initial radioactivity. The ^{14}C-activity in the extract was adsorbed onto anion exchange resin (A 101-D [HCO_3^-]) in a batch procedure. After separation of the resin by filtration, batch desorption of the ^{14}C-activity from the resin was accomplished by exposing to 1 M NH_4HCO_3, followed by filtration. Desorption with 1 M NH_4HCO_3 was repeated twice. The composited NH_4HCO_3 fractions contained 90 % of the initial ^{14}C-activity in the forage sample. The NH_4HCO_3 was removed from the extract by either repetitive evaporation under vacuum with a 50 °C water bath or by exposing the NH_4HCO_3 extract to an equivalent amount of cation exchange resin (D-50 [H^+] 20/50).
The plant extract of forage was diluted and pH adjusted to 9 with NH_4OH. The sample was added to a D-1 [HCO_3^-] column and eluted with a 0 to 1M NH_4HCO_3 concave gradient.

Radioactive residues were characterised and identified by comparison to standards using anion exchange column chromatography (A 101-D [HCO_3^-]), ^{13}C-NMR spectroscopy, or, following derivatisation to form the trimethyl-N-trifluoroacetamide of phosphonomethylglycine, gas chromatography with mass spectral characterisation and identification.

II. Results and discussion

A. Total radioactive residues (TRRs)

In the table below the uptake of radioactivity observed in experiment I, following soil treatment equivalent to 4.48 kg a.s./ha, is summarised. Radioactive residues in the analyzed fractions were reported in the original study as percentage of applied radioactivity. Total radioactive residues were calculated from the reported values upon dossier compilation; these values are shown in the table below in italics.

In all cases, the ^{14}C-content in grass or legume forage was less than 0.1 % of the applied radioactivity at 6 to 32 weeks harvest intervals. Total radioactive residues (TRR) in forage ranged between 3.045 – 5.449 mg/kg at the first sampling after 6 weeks and declined to 0.205 – 0.294 mg/kg after 32 weeks.
The ^{14}C-concentration in any given grass or legume forage from untreated controls was approximately 10 % of that found in the corresponding grass or legume forage harvested from ^{14}C-glyphosate-treated soil.

Table 6.2.1-94: Recovered radioactivity and total radioactive residue in pasture crops after pre-emergence treatment with ^{14}C-glyphosate at rates equivalent to 4.48 kg a.s./ha

Sample	TRR (mg equiv./kg)*					% AR					dpm/g measured				
	6 wks	12 wks	18 wks	24 wks	32 wks	6 wks	12 wks	18 wks	24 wks	32 wks	6 wks	12 wks	18 wks	24 wks	32 wks
Fescue, treated	*3.04*	*1.32*	*1.04*	*0.40*	*0.29*	0.08	0.03	0.05	0.03	0.03	74664	32440	25501	9884	7209
Fescue, control	*0.30*	*0.11*	*0.10*	*0.04*	*0.03*	-	-	-	-	-	7440	2780	2468	1071	704
Alfalfa, treated	*5.26*	*1.21*	*1.09*	*0.42*	*0.25*	0.03	0.02	0.02	<0.01	<0.01	129087	29636	26838	10194	6049
Alfalfa, control	*0.33*	*0.13*	*0.10*	*0.04*	*0.03*	-	-	-	-	-	8095	3180	2370	1104	732
Bromegrass treated	*2.06*	*1.52*	*1.10*	*0.36*	*0.22*	0.02	0.01	0.04	0.03	0.02	50562	37198	27061	8920	5428
Bromegrass control	*0.43*	*0.10*	*0.07*	*0.04*	*0.03*	-	-	-	-	-	10469	2525	1674	868	637
Red clover treated	*5.67*	*2.19*	*1.62*	*0.47*	*0.16*	0.07	0.04	0.02	0.01	<0.01	139148	53841	39693	11560	3993
Red clover control	*0.53*	*0.12*	*0.06*	*0.03*	*0.03*	-	-	-	-	-	12938	2892	1474	794	625
Timothy bromegrass, treated	*3.60*	*1.54*	*0.93*	*0.38*	*0.24*	0.06	0.04	0.06	0.04	0.03	88384	37912	22888	9238	5894
Timothy bromegrass, control	*0.46*	*0.15*	*0.07*	*0.30*	*0.02*	-	-	-	-	-	11337	3767	1694	7268	450
White clover, treated	*5.44*	*2.03*	*0.95*	*0.26*	*0.20*	<0.01	<0.01	<0.01	<0.01	<0.01	133621	49820	23257	6266	5024
White clover, control	*0.47*	*0.09*	*0.06*	*0.02*	*0.02*	-	-	-	-	-	11617	2176	1536	468	401

Table 6.2.1-94: Recovered radioactivity and total radioactive residue in pasture crops after pre-emergence treatment with ^{14}C-glyphosate at rates equivalent to 4.48 kg a.s./ha

TRR Total radioactive residue (*calculated based on given dpm/g values and specific activity of 1.87 mCi/mM)
Wks Weeks
% AR Percent of applied radioactivity (^{14}C-glyphosate (soil) initial: 2.83 x 10^8 dpm (11.54 mg N-(phosphono-^{14}C-methyl)glycine) per pot)
Values in *italics* were calculated from reported values upon dossier compilation

The radioactivity recovered in fescue and alfalfa forage regrowth, expressed in percent of applied radioactivity, after incorporation of treated quackgrass into the seed bed (experiment II) is shown in the table below.
In all cases, the ^{14}C content in fescue or alfalfa forage, respectively, was less than 0.1 % of the applied radioactivity at 6 to 24 weeks harvest intervals.

Table 6.2.1-95: Recovered radioactivity in fescue and alfalfa planted after treatment of quackgrass with ^{14}C-glyphosate at rates equivalent to 1.68 kg a.s./ha and incorporation into the soil

Sample	% AR after treatment			
	6 weeks	12 weeks	18 weeks	24 weeks
Fescue forage	0.09	0.05	0.02	0.09
Alfalfa forage	0.01	0.01	<0.01	0.04

% AR Percent of applied radioactivity (^{14}C-glyphosate (soil) initial: 6.64 x 10^7 dpm (2.7 mg ^{14}C-glyphosate) per pot)
Remark: Calculation of the total radioactive residues (TRR) was not possible from the data for experiment III provided in the report.

The radioactivity recovered in fescue and alfalfa forage regrowth, expressed in percent of applied radioactivity, following foliar treatment with 1.12 kg N-(phosphono-^{13}C/^{14}C-methyl)glycine (90:10) and removal of the treated foliage after one week (experiment III), is shown below.
Alfalfa regrowth occurred in only one out of 3 replications.
The fescue and alfalfa treated foliage contained 69 % and 55 %, respectively, of the applied radioactivity one week after treatment. The fescue and alfalfa regrowth harvested at 9 and 15 weeks after treatment contained 0.17 - 0.2 % and 0.18 – 0.19 %, respectively, of the applied radioactivity. By the 23rd week the ^{14}C concentration in fescue and alfalfa regrowth was less than 0.1 % of the applied radioactivity.

Table 6.2.1-96: Recovered radioactivity in fescue and alfalfa forage after foliar treatment with N-(phosphono-^{13}C/^{14}C-methyl)glycine at rates equivalent to 1.12 kg a.s./ha and the residues in regrowth

Sample	% AR after treatment[1]			
	1 week (treated foliage)	9 weeks (regrowth)	15 weeks (regrowth)	23 weeks (regrowth)
Fescue forage	69	0.17	0.18	0.05
Alfalfa forage	55	0.20[2]	0.19[2]	0.06[2]

% AR Percent of applied radioactivity (^{14}C-glyphosate (soil) initial: 6.7 x 10^6 dpm (0.278 mg ^{14}C-glyphosate per pot; 2.78 mg ^{13}C/^{14}C-glyphosate (90:10) per pot)
[1] Mean of 3 replications
[2] Regrowth only in one replication

Air-dried pasture that had been foliar treated with 1.12 kg ^{14}C-glyphosate/ha (2.045 mg ^{14}C-glyphosate, corresponding to 2.43 x 10^8 dpm; experiment IV) one week before harvest contained 41.8 % of the applied radioactivity for fescue and 63.7 % of the applied radioactivity for alfalfa.
Recalculation of reported values as TRR (mg equ./kg) was not possible based on the data available in the report for experiments II to IV.

B. Extraction and characterisation of residues

No characterisation and identification of the residue was attempted in experiments I and II.

The concentration of radioactivity in the regrowth of fescue and alfalfa forage in experiment III was insufficient to carry out chromatographic and spectroscopic evaluations. Anion exchange chromatography of water extracts from fescue and alfalfa treated forage harvested one week after treatment and containing 69 and 55 % of the applied radioactivity, respectively, revealed ^{14}C-histograms very similar to that given by anion exchange chromatography of the ^{13}C/^{14}C-glyphosate treatment solution. ^{13}C-NMR spectroscopy of the chromatographic fractions from fescue and alfalfa extracts containing >99 % of the radioactivity produced spectra that were identical to that obtained for ^{13}C/^{14}C-glyphosate. The presence of ^{13}C/^{14}C-glyphosate was shown by GC-MS; the mass spectral fragmentation patterns for fescue and alfalfa extracts (after derivatisation), respectively, were consistent with that for ^{13}C/^{14}C-glyphosate in the treatment solution after derivatisation.

The complete radioactivity recovered in water extracts of the treated alfalfa forage from experiment IV showed an elution profile similar to that of ^{14}C-glyphosate on an anion exchange column (D-1).
In the case of treated fescue forage, approximately 3 % of the radioactivity in the extract showed a chromatographic behaviour corresponding to the metabolite aminomethyl phosphonic acid (AMPA), while the majority of the radioactivity showed an elution profile similar to that of ^{14}C-glyphosate on an anion exchange column (D-1).
GC-MS analysis (after derivatisation) of the radioactivity extracted from alfalfa and fescue forage revealed mass fragmentation patterns consistent with ^{14}C-glyphosate.

C. Storage stability

Storage intervals for samples and extracts are not reported. No information on storage stability is reported.

D. Degradation pathway

Please refer to the pathway of glyphosate in miscellaneous crops presented further below.

III. Conclusions

After pre-emergent application of ^{14}C-glyphosate at a rate of 4.48 kg a.s./ha, the uptake of residues by grass or legume pasture did not exceed 0.1 % of the applied radioactivity.

Planting pasture crops in soil containing incorporated perennials previously treated with ^{14}C-glyphosate at a rate of 1.68 kg a.s./ha resulted in the same low uptake below 0.1 % of the applied radioactivity.

After foliar treatment of pasture crops with N-(phosphono-^{13}C/^{14}C-methyl)glycine (90:10) at a rate of 1.12 kg a.s./ha, 41.8 - 69 % of the applied radioactivity were recovered in treated foliage one week after application. Regrowth after eradication of treated foliage showed residue levels at or below 0.2 % of the applied activity.

The majority of the radioactive residues extracted from treated fescue and alfalfa forage was shown to be glyphosate by ion exchange chromatography, ^{13}C-NMR and GC-Mass spectroscopy. Approximately 3 % of the radioactivity recovered in extracts of dried fescue forage showed a chromatographic behaviour corresponding to the metabolite aminomethyl phosphonic acid (AMPA).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in pasture crops has been previously evaluated at EU level. It was not performed under GLP and partly meets current requirements (as laid down in Reg.

(EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501) with major deviations (radiochemical purity of the test substances not unambiguously specified, data largely insufficient to calculate TRRs, no quantitative extraction/fractionation reported, no full accountability reported, no quantitative identification results reported, no description of length of storage of samples).
Residues in pasture matrices were determined by LSC as total ^{14}C-derived radioactivity which is expected to be stable during the course of the study. Expression of the radioactive residues as percentage of the applied radioactivity allows to conclude on the relative amount of uptake of glyphosate-derived residues from soil.
The study is therefore considered to be supportive for the assessment of glyphosate metabolism in pasture crops.

Assessment and conclusion by RMS:

Pulses and oilseeds

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/014
Report author	[redacted]
Report year	1992
Report title	[^{14}C-Anion] ICIA0224: Nature of the residue: Soybeans
Report No	RR 91-092B
Document No	Not available
Guidelines followed in study	EPA §171-4(a): "Nature of Residues in Plants"
Deviations from current test guideline	A review of this study indicates no deviations from OECD Guideline for the Testing of Chemicals, 501.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary
The nature of the residues in plants following the use of glyphosate was studied in soybean. [^{14}C-PMG]glyphosate-trimesium was applied at a rate of 8.40 kg a.s./ha by soil drench method within two hours after planting the seeds.
The TRRs were 1.76 mg/kg in forage sampled 31 days after the application, 0.859 mg/kg in straw, 0.487 mg/kg in hulls, 0.772 mg/kg in green seeds and 1.31 mg/kg in yellow seeds, respectively, sampled 97 days after application.
Within extracts of forage, straw, hulls and yellow seeds PMG and its metabolite AMPA were identified accounting for 0.57 - 4.10 and 1.50 - 5.70 % of the TRR, respectively.

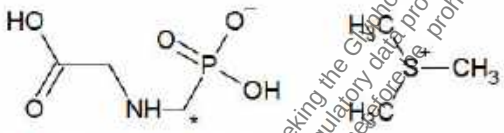
The remaining fractions of the extractable residue (34.13 - 48.9 % of the TRR), were shown to be radiolabelled natural products mainly consisting of mono- and disaccharides and amino acids and to a lower extent to smaller proteins.
The unextractable (bound) residues consisted of natural products, 16.9 - 25.3 % of the TRR carbohydrates, 1.43 - 2.93 % of the TRR lignin, 16.0 - 24.0 % of the TRR protein and 7-6 - 21.8 % of the TRR crude cellulose.

I. Materials and methods

A. Materials

Test Material: N-(phosphono-^{14}C-methyl)glycine trimesium salt ([^{14}C-PMG]glyphosate-trimesium)

Chemical structure:



* Position of Radiolabel

Radiochemical purity: > 95 % (stock solution radiopurity determined by three dissimilar TLC systems)

Specific activity: 1.71 MBq/mg (11.4 mCi/mmol)

CAS No: 81591-81-3

Log $P_{o/w}$ for glyphosate-trimesium: - 2.9

Test system

Soil: Loam (organic matter: 0.8 %; sand: 51.0 %; silt: 37.8 %; clay: 11.2 %; gravel: 0.3 %; pH: 7.8; half saturation: 14 %; ECe, 2.3)

Crop: Soybean (variety Corsoy)

Botanical name: *Glycine max*

Crop part(s): Whole immature plant, straw, hull and seeds (green and yellow)

B. Study design

1. In-life phase

The study was conducted under greenhouse conditions. Plastic pots were planted with 18-20 soybean seeds in rows approximately 10 cm apart, with seeds planted approximately 4 cm apart and 3 cm deep within the rows. After watering and fertilizing, the treatment solution was applied by soil drench method within two hours after planting. The treatment rate was calculated to be equivalent to 8.40 kg glyphosate-trimesium/ha. The radioactivity applied per pot was 208.6 MBq. Two soil core samples (1.2 cm diameter by 5 cm deep) were taken from each pot two hours after treatment, to verify the treatment rate.

The plants were checked daily, watered as needed, and fertilised once each month. Natural sunlight was supplemented with artificial light when needed to maintain 20 hours of sunlight each day. The ambient temperature (18-42 °C) was recorded on a chart recorder for the duration of the greenhouse portion of this study.

2. Sampling

Immature plants (9 from each of 2 pots) were harvested 31 days after treatment (31 DAT) by cutting every other plant just above the soil surface. The remaining plants (9 from each of 2 pots) were harvested at maturity, 97 DAT. One immature plant was reserved for autoradiography, to determine distribution of the radiolabel. The immature plants remained whole as forage, and the mature plants were separated into straw, hull and seeds. The seeds were further separated into yellow and green seeds.
Samples were stored frozen (-20 °C) prior to analysis. All tissue and soil samples were transported frozen to the analytical laboratory.

3. Analytical procedures

Pilot Characterisation for Storage Stability Determination
Forage, straw and hull samples were sequentially extracted four times with acetonitrile:water (1:1) in an ice bath. The extracts were separated by centrifugation and the first three extracts were pooled. Attempts were made to separate the acetonitrile from the water by freezing the water. The extracted pulp was further extracted with 1.0 N HCl with the same procedure.
Natural products were characterised from the bound residues by hydrolysis. Carbohydrates were liberated by an acid hydrolysis with 1.0 N HCl at 100 °C. The acid hydrolysate was separated from the pulp by centrifugation.
The pulp was then refluxed with 20 % aqueous NaOH at 100 °C. Base hydrolysis was used to separate protein and lignin from the cellulose. The base hydrolysate was separated from the cellulose by centrifugation. The lignin was precipitated from the protein (amino acids) in the base hydrolysate by adjusting the pH to ≤ 1.0. The precipitated lignin was removed by centrifugation.
Seed samples were extracted twice with hexane prior to extraction three times with acetonitrile:water (1:1). The hexane was used to extract soy oil which would form an emulsion with the acetonitrile:water (1:1) extract. Each liquid fraction was assayed by liquid scintillation counting (LSC) and each solid fraction by combustion/LSC.
The liquid fractions were further characterised with thin layer chromatography (TLC) spray reagents. Extracts from control soy plants were spotted on a silica TLC plate and allowed to dry. The plate was sprayed without developing in a solvent system. Ninhydrin was used to detect amino acids and Bial's Reagent was used to detect protein.

Analysis of Extractable Residues from Plant Tissue
Plant tissues were extracted in an ice bath. Soybean forage, straw and hulls, were extracted with 2-3 x with water followed by methanol. The extracts were assayed by liquid scintillation counting (LSC) and the aqueous extracts were typically pooled.
Soy seeds were extracted twice with hexane, to remove fat. The defatted soybean pulp was extracted with 1-2 x 30 mM Tris Buffer (pH 8.0). The extracts were separated from the pulp by centrifugation, the extracts were decanted and filtered when necessary. Extracted soy seed protein was precipitated by reducing the pH of the aqueous buffer extract.

The extracts were further characterised applying Chelex, C-18 and / or ion exchange chromatography:

Extracted ^{14}C-PMG and ^{14}C-AMPA in the extracts were separated from co-extracted ^{14}C natural products (carbohydrates) using a column made from Chelex 100 in iron form (Bio-Rad Laboratories, Richmond, CA), followed by a column made from AG 1-X8 anion exchange resin 200-400 mesh in chloride form (Bio-Rad Laboratories, Richmond, CA). Aqueous soy plant extracts were acidified to 0.1 N HCl with concentrated HCl, applied to the column and eluted sequentially with water, 0.2 N HCl and 6.0 N HCl. The PMG and AMPA were eluted from the column in the 6.0 N HCl fractions. Each eluent fraction was assayed by LSC.
Controls were performed with aqueous extracts spiked with [^{14}C-PMG]glyphosate-trimesium, to determine if parent material was retained on the Chelex column.
The 6.0 N HCl eluents from the Chelex purification were further purified by anion exchange. Each of the eluent fractions was assayed by LSC.

Alternatively, the soy plant extracts were purified on a column of Dowex 50W-X8 strong cation exchange resin, hydrogen form, 200-400 mesh. The isolated ^{14}C-PMG and ^{14}C-AMPA were identified and quantified by TLC co-chromatography and HPLC.

Aqueous samples were purified on C-18 silica preconditioned with methanol followed by water or 1.0 N HCl. The sample was eluted with several column volumes of water or 1-3 N HCl. The pigments were eluted with several column volumes of methanol. Each of the eluent fractions was assayed by LSC.
Controls were performed with aqueous extracts spiked with [^{14}C -PMG]glyphosate-trimesium, to determine if parent material was retained on the C-18 column.

After successive extraction with aqueous and organic solvents, nonextractable ^{14}C-residues in the pulp were further characterised by hydrolysis. The hydrolysis procedure was used to isolate natural products.
The non-extractable solid from soybean forage, straw and hulls was refluxed with 100 mL of 1.0 N HCl for 2 hours at 100 °C to release carbohydrates. Non-soluble solids were then separated from the soluble carbohydrates by centrifugation and filtration. The carbohydrate fraction was analysed by TLC and Chelex chromatography as described for the extractable fraction. The solids were refluxed with 20 % sodium hydroxide for 24 hours at 100 °C. The alkaline hydrolysate, which contained amino acids (hydrolysed proteins) and lignin, was separated from the crude cellulose fraction by centrifugation as above. The hydrolysate was acidified to pH 1.0 with concentrated HCl to precipitate lignin. The lignin precipitate was separated from the soluble protein amino acid) fraction by centrifugation and filtration as described above.
Each of the liquid samples was analysed by LSC. The solid fractions were analysed by combustion and LSC.

The solids remaining after hexane extraction of soybean seeds were extracted once with 30 mM Tris buffer (pH 8.0) in an ice bath. The mixture was then centrifuged, and the protein-containing extract decanted. In a second experiment the extraction step with of Tris buffer was performed twice. Extracted protein was precipitated by adjusting the supernatant to pH 5.0 with 1.0 N HCl. The suspension was centrifuged and the supernatant decanted.
In the first experiment the isolated protein was further purified by dialysis. The protein pellet was re-dissolved in 100 mL of 30 mM Tris buffer (pH 8.0) and dialysed overnight in a cold room (0-5 °C) in Spectrapore membrane tubing(MW cutoff 12,000-14,000). Protein was precipitated by adjusting the pH to 5.0 with 1.0 N HCl. The suspension was then centrifuged and the supernatant decanted. The purified protein pellet was air dried in a vacuum desiccator with anhydrous $CaSO_4$ and then analysed by combustion and LSC.

In the second experiment the dialysis step was omitted. The isolated protein was lyophilised before combustion and LSC analysis with no further purification.

Smaller whey proteins were not precipitated from the Tris buffer extract after pH was adjusted to 5.0. Therefore, remaining protein and other co-extracted macromolecules were precipitated from the decanted supernatant by saturation with ammonium sulfate in the first experiment. The precipitate was removed by centrifugation. The supernatant, which was expected to contain PMG and AMPA, was purified on cation exchange column and then analysed by LSC and TLC.

In the second experiment the pH 5.0 supernatant was purified on C-18 and cation exchange open column chromatography before HPLC analysis was performed.
Different HPLC systems with UV (190 or 200 nm) or radiodetection were used for the separation of PMG, AMPA and sugars.
Ten different TLC systems were used.The radiolabeled material was quantitated by scraping the silica gel from the TLC plate, counting the scrapings by liquid scintillation counting (LSC). Alternatively, the radioactivity was visualised and quantitated using an AMBIS Beta Scanning System TLC plate scanner or a Berthold Digital Autoradiograph.

The PMG and AMPA analytical standards as well as amino acids were detected by spraying the plates with Ninhydrin reagent. The monosaccharides were detected with Bials Reagent (0.9 % ferric chloride + 0.55 % orcinol in acidified ethanol).

All liquid samples and the silica scraped from TLC plates were radioassayed by LSC in a Packard Model 4430 Liquid Scintillation Counter equipped with an external standard (AES) for efficiency determination. Plant tissue and soil samples were combusted to CO_2 in a Packard Model 306A Sample Oxidizer. The CO_2 was trapped in Carbosorb and mixed with 12 mL of Permafluor scintillation cocktail. Counting and combustion efficiencies were determined by combusting and counting glycerol tri[1-^{14}C] palmitate standards
The minimum detection limit of ^{14}C-PMG in soil or plant tissue was calculated to be 0.0004 mg/kg, assuming average sample aliquot of 0.30 g.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in soybean forage, straw, hulls, green and yellow seeds was determined by combustion/LSC. The TRRs are summarised in Table 6.2.1-97. Plant tissue contained only 0.156 % of the applied treatment. In soybean forage, a TRR of 1.76 mg/kg was found, while in straw 0.859 mg/kg, in hull 0.487 mg/kg, in green seed 0.772 mg/kg and in yellow seed 1.31 mg/kg were detected. Hay (mature total aerial tissue) was not analysed directly, but residue values were calculated from measured values assuming a hay composition of 35.0 % straw, 12.8 % hull and 52.2 % seed, resulting in a calculated TRR of 0.8537 mg PMG equiv./kg.

Table 6.2.1-97: Total radioactive residues in soybean commodities

Sample description	Days after last treatment (DAT)	TRR (direct combustion) (mg eq./kg)
Forage	31	1.76
Straw	97	0.859
Hulls	97	0.487
Seeds, yellow	97	1.31
Seeds, green	97	0.772
Hay	97	0.8537[1]

DAT Days after treatment
TRR Total radioactive residue, expressed as glyphosate equivalents
[1] Calculated based on a hay composition of 35.0 % straw, 12.8 % hull and 52.2 % seed

B. Extraction and characterisation of residues

The results of the pilot extractions for storage stability are presented in the tables below No identification of glyphosate or its metabolites was done from these extractions.

Comprehensive characterisation and identification were done in the large scale extractions. The results for large-scale extraction of soybean forage, straw and hulls are summarised and the results of identification are presented in the table below. Large scale-extractions of soybean green and yellow seed are summarised in a separate table the results of identification of the radioactive residues are presented below.
From forage, the majority of residues were extracted with water alone, 42.22 % of the TRR (0.743 mg/kg) with water and 2.73 % of the TRR (0.048 mg/kg) with methanol.
Analysis of the extractable residues, after purification by Chelex chromatography and anion exchange, detected PMG at 3.30 % of the TRR (0.058 mg/kg) and AMPA at 5.70 % of the TRR (0.100 mg/kg) by HPLC.

The HPLC eluent fractions were quantified by LSC. This result was confirmed with TLC analysis, which showed ^{14}C-AMPA standard co-migrating with the radioactivity in a cation exchange column purified aqueous forage extract.
The remaining extractable residue, 36.0 % of the TRR (0.634 mg/kg), were radiolabelled natural products consisting of mono and disaccharides and amino acids. Evidence of this came from HPLC analysis of an aqueous extract after purification on a C-18 column.
Additional evidence for monosaccharides was obtained from TLC separation and Bial's Reagent, selective for the detection of sugars, of an aqueous extract after purification on a Chelex column which contained 14 % TRR (0.246 mg/kg).
The unextractable (bound) residues were separated into fractions consistent with the expected classes of natural products. Acid hydrolysis liberated carbohydrates, 25.3 % of the TRR (0.445 mg/kg). Monosaccharides were also separated in the acid hydrolysate by TLC and detected with Bial's Reagent.
The base hydrolysate contained 1.43 % of the TRR (0.025 mg/kg) in lignin and 17.2 % of the TRR (0.303 mg/kg) in protein. The amino acids from the hydrolysed protein were separated by TLC and detected with ninhydrin reagent. The remaining solids contained 15.8 % of the TRR (0.278 mg/kg) in crude cellulose.

From straw, the majority of unbound residues were extracted with water alone, 35.3 % of the TRR (0.303 mg/kg) with water and 2.12 % of the TRR (0.018 mg/kg) with methanol. Analysis of the extractable residues, after purification by Chelex chromatography and anion exchange, detected 0.57 % of the TRR PMG (0.005 mg/kg) and 2.70 % of the TRR AMPA (0.023 mg/kg) by HPLC. The HPLC eluent fractions were quantified by LSC. This result was confirmed with TLC analysis, which showed ^{14}C-AMPA standard co-migrating with the radioactivity in a cation exchange column purified aqueous straw extract.
The remaining extractable residue, 34.13 % of the TRR (0.293 mg/kg), were radiolabelled natural products consisting of mono and disaccharides and amino acids. Evidence of this came from HPLC analysis of an aqueous extract after purification on a C-18 column. Additional evidence for monosaccharides was obtained from TLC separation and Bial's Reagent, selective for the detection of sugars, of an aqueous extract after purification on a Chelex column which contained 14.0 % of the TRR (0.120 mg/kg). 2.2 % of the TRR (0.019 mg/kg) migrated with the same R_f as glucose and fructose.
The unextractable (bound) residues were separated into fractions consistent with the expected classes of natural products. Acid hydrolysis liberated carbohydrates, 17.2 % of the TRR (0.148 mg/kg). Only 0.34 % of the TRR (0.0029 mg/kg) was retained on the Chelex column. This indicates that little or no parent (PMG) or metabolite (AMPA) is present in the aqueous extracted pulp, because ^{14}C-PMG standards were retained on Chelex in spiked acid hydrolysis fractions.
Monosaccharides were also separated in acid hydrolysates by TLC and detected with Bial's Reagent. 5.3 % of the TRR (0.046 mg/kg) migrated with the same R_f as glucose and fructose.
The base hydrolysate contained 2.93 % of the TRR (0.025 mg/kg) in lignin and 16.0 % of the TRR (0.137 mg/kg) in protein. The amino acids from the hydrolysed protein were separated by TLC and detected with ninhydrin reagent. The remaining solids contained 21.8 % of the TRR (0.187 mg/kg) in crude cellulose.

From hulls, the majority of unbound residues were extracted with water alone, 43.2 % of the TRR (2.10 mg/kg) with water and 4.7 % of the TRR (0.023 mg/kg) with methanol. Analysis of the extractable residues, after purification by Chelex chromatography and anion exchange, detected 4.10 % of the TRR PMG (0.020 mg/kg) and 1.50 % of the TRR AMPA (0.007 mg/kg) by HPLC. The HPLC eluent fractions were quantified by LSC. This result was confirmed with TLC analysis, which showed ^{14}C-AMPA standard co-migrating with the radioactivity in a Chelex column and anion exchange purified aqueous hull extract.
The remaining extractable residue, 42.3 % of the TRR (0.206 mg/kg), were natural products consisting of mono and disaccharides and amino acids. Evidence of this came from HPLC analysis of an aqueous extract after purification on a C-18 column. Additional evidence for monosaccharides was obtained from TLC separation and Bial's Reagent, selective for the detection of sugars, of an aqueous extract after purification on a Chelex column which contained 14 % of the TRR (0.068 mg/kg).

The unextractable (bound) residues were separated into fractions consistent with the classes of natural products. Acid hydrolysis liberated carbohydrates, 16.9 % of the TRR (0.082 mg/kg). Only 0.34 % of the TRR (0.0016 mg/kg) was retained on the Chelex column. This indicates that little or no parent (PMG) or metabolite (AMPA) is present in the aqueous extracted pulp, because ^{14}C-PMG standards were retained on Chelex in spiked acid hydrolysis fractions. Monosaccharides were also separated in acid hydrolysates by TLC and detected with Bial's Reagent.
The base hydrolysate from the pilot extraction contained 14.5 % of the TRR (0.071 mg/kg) protein and lignin. The lignin was not precipitated from the hydrolysed protein. The amino acids from the hydrolysed protein were separated by TLC and detected with ninhydrin reagent. The remaining pulp contained 7.60 % of the TRR (0.037 mg/kg) crude cellulose.

Two extractions on yellow seeds were performed with an 351 days interval between the two extractions. The data from the second extraction were used for quantitation of residues because it contained a more complete analysis. The data from the first extraction contain a similar extraction profile with further analysis of the extracted protein.

The soybean plants were harvested a few days before all of the seeds had dried on the plants. This was done because the dried leaves falling off the plants were contaminating the surrounding area. The seeds were divided into yellow (mature) and green (immature) seeds. The green and yellow seeds were used to determine the magnitude of the residues, but the nature of the residues were determined in the yellow seeds only because the nature of the residues in an immature crop would not reflect the situation in the field where the plants would be left until all of the seeds reach maturity.

From yellow seeds, 8.9 % of the TRR (0.116 mg/kg) were extracted with hexane and 44.2 % of the TRR (0.579 mg/kg) with Tris buffer. Analysis of the buffer-extractable residues, after purification by Chelex chromatography, detected 2.6 % of the TRR as PMG (0.034 mg/kg), and 1.60 % of the TRR as AMPA (0.021 mg/kg) by HPLC. The HPLC eluent fractions were quantified by LSC. This result was confirmed with TLC analysis, which showed ^{14}C-AMPA co-migrating with the radioactivity in a purified seed extract.
Crude protein was precipitated at 24.0 % of the TRR (0.314 mg/kg) from the Tris buffer extract.
The remaining buffer-soluble residue, 40.0 % of the TRR (0.524 mg/kg), was carbohydrate and smaller protein which does not precipitate at pH 5.0. Monosaccharides were separated by TLC and detected with Bial's Reagent. Smaller proteins and other macromolecules at 3.9 % of the TRR (0.05 mg/kg) were precipitated with saturated ammonium sulfate.
The isolated protein was further characterised by dialysis. Only 3.97 % of the radiolabel (0.052 mg/kg) was detected in the dialysate, 15.23 % (0.200 mg/kg) of the protein was re-precipitated with 0.92 % (0.012 mg/kg) of the protein remaining in solution.
The bound seed residues were not directly analysed. When other bound fractions from forage straw and hull were analysed, PMG and AMPA were not found in the acid hydrolysate.
Hay (mature total aerial tissue) was not extracted and analysed. Values were calculated from data obtained for straw, hulls and seeds, assuming a hay composition of 35.0 % straw, 12.8 % hull, 19.7 % for green seeds and 32.6 % % for yellow seed.

Table 6.2.1-98: Extraction of the radioactive residues of glyphosate-trimesium in soybean forage, straw, hulls and green and yellow seed following application of glyphosate-trimesium at a dose rate of 1x 8.40 kg a.s./ha – pilot extraction #1

	Soybean forage		Soybean straw		Soybean hulls		Soybean seed, green		Soybean seed, yellow	
Days after treatment (DAT)	31		97		97		97		97	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**1.76**	**100**	**0.859**	**100**	**0.487**	**100**	**0.772**	**100**	**1.31**	**100**
Hexane extract	-	-	-	-	-	-	*0.004*	0.5	*0.060*	4.6
Aqueous extract[3]	*0.727*	41.3	*0.263*	30.6	*0.290*	59.6	*0.733*	95.0	*1.347*	102.8
Total extractable	***0.727***	**41.3**	***0.263***	**30.6**	***0.290***	**59.6**	***0.737***	***95.5***	***1.407***	***107.4***
Solids (unextracted)	NR	NR	NR	NR	NR	NR	*0.021*	2.7	*0.202*	15.4
Acid hydrolysate (carbohydrates)	*0.377*	21.4	*0.133*	15.5	*0.091*	18.6	-	-	-	-
Solids	NR	NR	NR	NR	NR	NR	-	-	-	-
Base Hydrolysate (protein and lignin)	*0.338*	19.2	*0.136*	15.8	*0.071*	14.5	-	-	-	-
Precipitate (crude cellulose)	*0.155*	8.8	*0.079*	9.2	*0.037*	7.7	-	-	-	-
ERR[1]	***1.441***	***81.9***	***0.532***	***61.9***	***0.451***	***92.7***	***0.737***	***95.5***	***1.407***	***107.4***
RRR[2]	***0.155***	**8.80**	***0.079***	**9.2**	***0.037***	**7.7**	***0.021***	**2.7**	***0.202***	**15.4**
Total sum	***1.596***	**90.6**	***0.611***	**71.1**	***0.489***	**100.4**	***0.758***	**98.2**	***1.609***	**122.8**

DAT Days after treatment
TRR Total radioactive residue expressed as PMG equivalents
NR Not reported
[1] ERR Extractable radioactive residue after conventional followed by exhaustive extraction (acidic and basic hydrolysis)
[2] RRR Residual radioactive residue after conventional and exhaustive extraction
[3] Sum of aqueous, ACN, probe wash and acidic extracts
Residues are expressed as mg/kg PMG equivalents
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

Table 6.2.1-99: Extraction of the radioactive residues of glyphosate-trimesium in soybean forage, straw, hulls and green and yellow seed following application of glyphosate-trimesium at a dose rate of 1x 8.40 kg a.s./ha – pilot extraction #2

	Soybean forage		Soybean straw		Soybean hulls		Soybean seed, green		Soybean seed, yellow	
Days after treatment (DAT)	31		97		97		97		97	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.76**	**100**	**0.859**	**100**	**0.487**	**100**	**0.772**	**100**	**1.31**	**100**
Hexane extract	-	-	-	-	-	-	*0.039*	5.1	*0.075*	5.7
Aqueous extract[3]	*0.565*	32.1	*0.222*	25.8	*0.140*	28.7	*0.662*	85.7	*0.971*	74.1
Total extractable	***0.565***	**32.1**	***0.222***	**25.8**	***0.140***	**28.7**	***0.701***	***90.8***	***1.045***	***79.8***
Solids (unextracted)	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**ND**	**ND**	**ND**	**ND**
Acid hydrolysate (carbohydrates)	*0.391*	22.2	*0.176*	20.5	*0.112*	23.1	-	-	-	-
Solids	NR	NR	NR	NR	NR	NR	-	-	-	-
Base Hydrolysate (protein and lignin)	*0.181*	10.3	*0.107*	12.4	*0.063*	12.9	-	-	-	-
Solids (crude cellulose)	*0.296*	16.8	*0.107*	12.4	*0.115*	23.6	-	-	-	-
ERR[1]	**1.137**	***64.6***	***0.504***	***58.7***	***0.315***	***64.7***	***0.701***	***90.8***	***1.045***	***79.8***
RRR[2]	***0.296***	**16.8**	***0.107***	**12.4**	***0.115***	**23.6**	**ND**	**ND**	**ND**	**ND**
Total sum	***1.433***	**81.4**	***0.611***	**71.1**	***0.430***	**88.3**	**ND**	**ND**	**ND**	**ND**

DAT Days after treatment
TRR Total radioactive residue
ND Not determined
NR Not reported
[1] ERR Extractable radioactive residue after conventional followed by exhaustive extraction (acidic and basic hydrolysis)
[2] RRR Residual radioactive residue after conventional and exhaustive extraction
[3] Sum of aqueous, ACN, probe wash and acidic extracts
Residues are expressed as mg/kg PMG equivalents
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

Table 6.2.1-100: Extraction of the radioactive residues of glyphosate-trimesium in soybean forage, straw and hulls following application of glyphosate-trimesium at a dose rate of 1x 8.40 kg a.s./ha – large scale extraction

	Soybean forage		Soybean straw		Soybean hulls	
Days after treatment (DAT)	31		97		97	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**1.76**	**100**	**0.859**	**100**	**0.487**	**100**
Water extraction						
Water extract 1&2	0.685	38.9	*0.271*	31.6	*0.210*	43.2
Chelex chromatography of water extracts 1&2						
Loading eluent	*0.246*	14.0	*0.120*	14.0	*0.068*	14.0
Water	*0.171*	9.7	*0.017*	2.0	*0.007*	1.5
0.2 N HCl #1	*0.069*	3.9	*0.025*	2.9	*0.003*	0.7
0.2 N HCl #2	-	-	*0.036*	4.2	*0.014*	2.8
6.0 N HCl #1	*0.157*	8.9	*0.048*	5.6	*0.094*	19.4
Anion exchange chromatography of HCl eluate #1						
Loading, 6.0N HCl	0.125	7.1	*0.038*	4.4	*0.084*	17.3
6.0 N HCl	0.021	1.2	*0.007*	0.8	*0.021*	4.3
6.0 N HCl #2	*0.012*	0.7	*0.003*	0.4	*0.002*	0.5
Cation exchange chromatography of water extracts 1&2						
Loading eluents #1 & 2	*0.225*	12.8	*0.122*	14.2	-	-
Acid Eluents # 3-5	*0.055*	3.15	*0.046*	5.34	-	-
Acid eluents #6-8	*0.061*	3.46	*0.091*	10.6	-	-
C18 chromatography of water extracts 1&2						
Loading and water eluents	*0.294*	16.7	*0.184*	21.4	*0.170*	34.9
Methanol eluents	*0.055*	3.15	*0.060*	6.99	*0.016*	3.3
Water extract 3	0.058	3.32	*0.032*	3.70	-	-
Methanol extract 4	0.039	2.24	*0.012*	1.39	*0.023*	4.7
Probe wash	0.009	0.49	*0.006*	0.73		
Total extractable[a]	***0.791***	**44.96**	**0.321**	**37.42**	***0.233***	**47.9**
Solids (unextracted)	**1.051**	**59.7**	**0.497**	**57.9**	**0.222**	**45.7**
Acid Hydrolysis of solids						
Hydrolysate	0.445	25.3	*0.148*	17.2	*0.082*	16.9
Chelex Chromatography						
Loading Eluent	-	-	*0.078*	9.12	*0.029*	5.92
Water Eluent #1	-	-	*0.032*	3.69	*0.014*	2.87
0.2 N HCI Eluent #2	-	-	*0.019*	2.23	*0.007*	1.52
0.2 N HCI Eluent #3	-	-	*0.007*	0.86	*0.003*	0.68
6.0 N HCI Eluent #4	-	-	0.0029	0.34	*0.0016*	0.34

Table 6.2.1-100: Extraction of the radioactive residues of glyphosate-trimesium in soybean forage, straw and hulls following application of glyphosate-trimesium at a dose rate of 1x 8.40 kg a.s./ha – large scale extraction

	Soybean forage		Soybean straw		Soybean hulls	
Days after treatment (DAT)	31		97		97	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.76**	**100**	**0.859**	**100**	**0.487**	**100**
6.0 N HCl Eluent #5	-	-	-	NS		
Retained on Chelex column	-	-	0.0029	0.34	0.0016	0.34
Solids	-	NR	-	NR	-	NR
Basic hydrolysis of solids						
Supernatant	-	NR	-	NR	-	-
Precipitation at pH 1						
Lignin	0.025	1.43	0.025	2.93	-	ND
Protein (amino acids)	0.303	17.2	0.137	16.0	-	ND
Precipitate (crude cellulose)	0.278	15.8	0.187	21.8	-	ND
ERR[1]	***1.564***	***88.88***	***0.631***	***73.55***	***0.316***	***64.8***
RRR[2]	**0.278**	**15.8**	***0.187***	**21.8**	***0.140***	***28.8***
Total sum	***1.842***	**104.7**	***0.818***	**95.3**	***0.456***	**93.6**

DAT Days after treatment
TRR Total radioactive residue
[1] ERR Extractable radioactive residue after conventional followed by exhaustive extraction (acidic and basic hydrolysis)
[2] RRR Residual radioactive residue after conventional and exhaustive extraction
ND Not determined
NR Not reported
[3] Sum of aqueous extracts, methanol extracts and probe wash
Residues are expressed as mg/kg PMG equivalents
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

Table 6.2.1-101: Extraction of the radioactive residues of glyphosate-trimesium in soybean green and yellow seed following application of glyphosate-trimesium at a dose rate of 1x 8.40 kg a.s./ha – large scale extraction

	Soybean seed, yellow (First Extraction)		Soybean seed, yellow (Second Extraction)	
Days after treatment (DAT)	97		97	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.31**	**100**	**1.31**	**100**
Hexane extract	*0.098*	**7.5**	0.116	**8.9**
Solids	NR	NR	NR	NR
Tris buffer extraction of solids, pH 8				
Protein Extract #1	*0.781*	59.6	NR	NR
Protein precipitation of protein extract #1 at pH 5.0				
Supernatant #1	*0.465*	35.5	*0.515*	39.3
Chelex chromatography of supernatant #1				
Loading eluent	-	-	*0.130*	9.94
Water eluent #1	-	-	*0.014*	1.10
0.2 N HCl Eluent #2	-	-	*0.001*	0.11
0.2 N HCl Eluent #3	-	-	*0.001*	0.07
6 N HCl Eluent #4	-	-	*0.221*	16.9
Anion exchange chromatography of eluent #4				
Loading 6 N HCl			*0.280*	21.4
Elution 6 N HCl			*0.028*	2.1
6 N HCl Eluent #5		-	*0.014*	1.06
Supernatant			*0.064*	4.9
Saturation with ammonium sulfate				
Supernatant	*0.401*	30.6	-	-
Whey protein and macromolecules	*0.051*	3.9	-	-
Protein precipitate #1	*0.316*	24.1[3]	*0.314*	24.0
Dialysis of protein precipitate #1 against Tris buffer				
Dialysate	*0.052*	3.97	-	-
Dialysed Protein	NR	NR	-	-
Protein precipitation at pH 5.0				
Non-precipitated Protein Supernatant	*0.012*	0.92	-	-
Purified Protein Precipitate	0.200	15.23	-	-

Table 6.2.1-101: Extraction of the radioactive residues of glyphosate-trimesium in soybean green and yellow seed following application of glyphosate-trimesium at a dose rate of 1x 8.40 kg a.s./ha – large scale extraction

	Soybean seed, yellow (First Extraction)		Soybean seed, yellow (Second Extraction)	
Days after treatment (DAT)	97		97	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.31**	**100**	**1.31**	**100**
Solids			*0.299*	22.8
Tris buffer extraction of solids, pH 8				
Protein extract #2	*0.075*	5.7	-	-
Protein precipitation of protein extract #2 at pH 5.0				
Supernatant #2	*0.042*	3.2	-	-
Protein precipitate #2	*0.033*	2.5[4]	-	-
Total extractable	***0.605***	**46.2[5]**	***0.696***	**53.10**
Pulp	NR	NR	-	-
ERR[1]	***0.879***	***67.1***	***1.010***	**77.1**
RRR[2]	***ND***	**ND**	***0.299***	**22.8**
Total sum	***ND***	***ND***	***1.309***	**99.9**

DAT Days after treatment
TRR Total radioactive residue
ND Not determined
NR Not reported
[1] ERR Extractable radioactive residue after conventional and exhaustive extraction; for first extraction: sum of hexane extract and protein extract #1; for second extraction: sum of hexane extract + supernatants after protein precipitation + protein precipitate (%TRR in protein extract #1 not reported)
[2] RRR Residual radioactive residue after conventional and exhaustive extraction
[3] *% Protein determined indirectly from difference of extract and pH 5.0 supernatant
[4] % Protein determined indirectly from difference of protein extract #2 and supernatant #2
[5] Sum of hexane extract + supernatants (protein extracts) at pH 5.0, after protein precipitation
Residues are expressed as mg/kg PMG equivalents
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

The extraction and hydrolysis of seeds was performed twice with an interval of 351 days between the first and the second extraction. The data obtained from the second extraction was used for quantitation of residues because it contained a more complete analysis. The data from the first extraction represent a similar extraction profile with further analysis of the extracted protein.

Table 6.2.1-102: Distribution of the radioactive residues of glyphosate in soybean forage, straw and hulls following application of glyphosate at a dose rate of 1x 8.40 kg a.s./ha

	Soybean forage		Soybean straw		Soybean hulls	
Days after treatment (DAT)	31		97		97	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.76**	**100**	**0.859**	**100**	**0.487**	**100**
Extractable residues[3]	***0.791***	**44.96**	***0.321***	**37.42**	**0.233**	**47.9**
Parent (PMG)	0.058	3.30	0.005	0.57	0.020	4.10
Metabolite (AMPA)	0.100	5.70	0.023	2.70	0.007	1.50
Natural products[4]	0.634	36.0	0.293	34.13	0.206	42.3
Bound residues (after conventional extraction)	**1.051**	**59.7**	**0.497**	**57.9**	**0.222**	**45.7**
Carbohydrate	0.445	25.3	0.148	17.2	0.082	16.9
Protein	0.303	17.2	0.137	16.0	0.071[6]	14.50[6]
Lignin[c]	0.025	1.43	0.025	2.93		
Cellulose[c]	0.278	15.8	0.187	21.8	0.037[6]	7.60[6]
Bound natural products	-	n.a.	-	n.a.	-	n.a.
Total identified	***0.158***	***9.00***	***0.028***	***3.27***	***0.027***	***5.60***
Total characterised	***1.685***	***95.730***	***0.791***	***92.06***	***0.396***	***81.300***
ERR (after exhaustive extraction)[1]	***1.564***	***88.88***	***0.631***	***73.55***	***0.316[7]***	***64.8[7]***
RRR (after exhaustive extraction)[2]	**0.278**	**15.8**	**0.187**	**21.8**	***0.140[e]***	***28.8[7]***
Total sum	***1.842***	**104.7**	***0.818***	**95.3**	***0.456***	**93.6[7]/ 100.4[d]**

DAT Days after treatment
TRR Total radioactive residue
ND Not determined
n.a. Not applicable
[1] ERR Extractable radioactive residue after conventional followed by exhaustive extraction (acidic and basic hydrolysis)
[2] RRR Residual radioactive residue
[3] Sum of aqueous extracts, methanol extract and probe wash.
[4] Natural products consist of mono- and disaccharides and amino acids
[5] Determined by combustion/LSC
[6] Calculated from pilot extraction
[7] Calculated from large-scale extraction
Residues are expressed as mg/kg PMG equivalents
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

Table 6.2.1-103: Distribution of the radioactive residues of glyphosate in soybean green and yellow seed and soybean hay following application of glyphosate at a dose rate of 1x 8.40 kg a.s./ha

	Soybean green seed[4]		**Soybean yellow seed**		**Soybean hay**[5]	
Days after treatment (DAT)	97		97		97	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**0.772**	**100**	**1.31**	**100**	**0.8537**	**100**
Extractable residues	**0.410**	**53.10**[6]	**0.696**	**53.10**[6]	**0.46**	**48.49**
Parent (PMG)	0.020	2.60	0.034	2.60	0.02	2.08
Metabolite (AMPA)	0.012	1.60	0.021	1.60	0.02	1.97
Natural products	0.378	48.90	0.641	48.90	0.42	44.43
Bound residues	**0.361**	**46.80**	**0.613**	**46.80**	**0.47**	**49.95**
Carbohydrate	-	ND	-	ND	0.06	8.40
Protein	0.185	24.00	0.314	24.00	0.20	20.01
Lignin[3]	-	ND	-	ND	0.01	1.02
Cellulose[3]	-	ND	-	ND	0.07	8.60
Bound natural products	0.176	22.80	0.299	22.80	0.13	11.92
Total identified	***0.595***	***77.10***	***1.010***	***77.10***	***0.739***	***86.51***
ERR (after exhaustive extraction)[1]	***0.595***	***77.10***	***1.010***	***77.10***	***0.665***	***77.91***
RRR (after exhaustive extraction)[2]	**0.176**	**22.80**	**0.299**	**22.80**	***0.175***	***20.52***
Total sum	***0.771***	**99.9**	***1.309***	**99.9**	***0.841***	**98.43**

DAT Days after treatment
TRR Total radioactive residue
ND Not determined
[1] ERR Extractable radioactive residue after conventional followed by exhaustive extraction (acidic and basic hydrolysis)
[2] RRR Residual radioactive residue
[3] Determined by combustion/LSC
[4] Distribution of residues determined from analysis of yellow seed.
[5] calculated from data obtained for straw, hulls and seeds, assuming a hay composition of 35.0 % straw, 12.8 % hull, 19.7 % for green seeds and 32.6 % % for yellow seed.
[6] Sum of hexane and aqueous extract
Residues are expressed as mg/kg PMG equivalents
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

C. Storage stability

The samples remained frozen (-20 °C) prior to analysis. First samples (immature plants) were taken on January 29, 1990. A pilot characterisation for storage stability in soybean forage, straw, hull and seed was performed on June 13, 1990. Large scale extraction and hydrolysis procedures on soybean forage and straw were performed on December 17, 1990, hull was extracted on July 23, 1991, while large scale seeds extraction was performed on July 11, 1990 (first extraction) and June 27, 1991 (second extraction), respectively. All extractions showed similar profiles as outlined in Table 6.2.1-104 and Table 6.2.1-105. There did not appear to be any degradation over the storage span during the study.

Table 6.2.1-104: Extraction and hydrolysis profile of [^{14}C-PMG] glyphosate-trimesium in soybean forage, straw and hull

Tissue	Extraction, Date	Extractable (%)	Carbohydrate (%) - Acid hydrolysate	Protein & Lignin (%) – Base hydrolysate	Cellulose (%)	Recovery (%)
Control plants						
Forage	Pilot, 13.06.90	32.1[1]	22.2	10.3	16.8[2]	81.4
Straw	Pilot, 13.06.90	25.8[1]	20.5	12.4	12.4[2]	71.1
Hull	Pilot, 13.06.90	28.7[1]	23.1	12.9	23.6[2]	88.3
Treated plants						
Forage	Pilot, 13.06.90	41.3[1]	21.4	19.2	8.8[2]	90.6
	Large-scale, 17.12.90	45.0[3]	25.3	Protein 17.2 Lignin 1.4[b]	15.8[2]	104.7
Straw	Pilot, 13.06.90	30.6[1]	15.5	15.8	9.2[b]	71.1
	Large-scale, 17.12.90	37.4[c]	17.2	Protein 16.0 Lignin 2.9[b]	21.8[2]	95.3
	Large-scale, 23.07.1991	34.2[3]	ND	ND	ND	96.2
Hull	Pilot, 13.06.90	59.6[1]	18.6	14.5	7.7[2]	100.4
	Large-scale, 23.07.1991	47.9[3]	16.9	ND	ND	93.6

1 Sum of aqueous, ACN, probe wash and acidic extracts.
2 Determined by combustion/LSC.
3 Sum of aqueous, methanol extracts and probe wash.
Minor deviations may occur due to rounding.

Table 6.2.1-105: Extraction and hydrolysis profile of [^{14}C-PMG] glyphosate-trimesium in soybean seed

Tissue	Extraction, Date	Hexane (%)	Aqueous (%)	Protein (%)	Bound (%)	Recovery (%)
Control plants						
Seed, green	Pilot, 13.06.90	5.1	85.7	NR	ND	ND
Seed, yellow	Pilot, 13.06.90	5.7	74.1	NR	ND	ND
Treated plants						
Seed, green	Pilot, 13.06.90	0.5	95.0	NR	2.7[a]	98.2
Seed, yellow	Pilot, 13.06.90	4.6	102.8	NR	15.4[a]	122.8
	Large-scale, 11.07.1990	7.5	38.7[b]	26.5*	ND	ND
	Large-scale, 27.06.1991	8.9	44.2[b]	24.0*	22.8[a]	99.9

Table 6.2.1-105: Extraction and hydrolysis profile of [^{14}C-PMG] glyphosate-trimesium in soybean seed

ND Not determined
NR Not reported
a Determined by combustion/LSC.
b Supernatant at pH 5.0, after protein precipitation
Minor deviations may occur due to rounding

The reference standards were analysed periodically throughout the study by TLC. There did not appear to be any degradation which would be indicated by a change in R_f or multiple spots.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in fruits at the end of this chapter.

III. Conclusions

The nature of the residues in plants following the use of glyphosate was studied in soybean. [^{14}C-PMG]glyphosate-trimesium was applied at a rate of 8.40 kg a.s./ha by soil drench method within two hours after planting the seeds.

The TRRs were 1.76 mg/kg in forage sampled 31 days after the application, 0.859 mg/kg in straw, 0.487 mg/kg in hulls, 0.772 mg/kg in green seeds and 1.31 mg/kg in yellow seeds, respectively, sampled 97 days after application.
44.96 % (0.791 mg/kg), 37.42 % (0.321 mg/kg), 47.90 % (0.233 mg/kg) and 53.1 % (0.410 mg/kg and 0.696 mg/kg, respectively) of the radioactive residues in forage, straw, hulls and green and yellow seeds were extractable.

In extractable residues of forage, PMG was identified at 3.30 % of the TRR (0.058 mg/kg) and AMPA at 5.70 % of the TRR (0.100 mg/kg). The remaining fractions of the extractable residue (36.0 % of the TRR, 0.634 mg/kg) were shown to be radiolabelled natural products consisting of mono and disaccharides and amino acids.
The unextractable (bound) residues consisted of natural products, 25.3 % of the TRR (0.445 mg/kg) carbohydrates, 1.43 % of the TRR (0.025 mg/kg) lignin, 17.2 % of the TRR (0.303 mg/kg) protein and 15.8 % of the TRR (0.278 mg/kg) crude cellulose.

Extractable residues of straw showed 0.57 % of the TRR as PMG (0.005 mg/kg) and 2.70 % of the TRR of the AMPA (0.023 mg/kg). The remaining fractions of the extractable residue (34.13 % TRR, 0.293 mg/kg) consisted of natural products, mono and disaccharides and amino acids.
The unextractable (bound) residues were consistent with natural products. Acid hydrolysis liberated carbohydrates, 17.2 % of the TRR (0.148 mg/kg).
The base hydrolysate contained 2.93 % of the TRR (0.025 mg/kg) lignin and 16.0 % of the TRR (0.137 mg/kg) protein. The remaining pulp contained 21.8 % of the TRR (0.187 mg/kg) crude cellulose.

In extractable residues of hull, PMG was identified at 4.10 % of the TRR (0.020 mg/kg) and AMPA at 1.50 % (0.007 mg/kg). The remaining fractions of the extractable residue (42.3 % of the TRR, 0.206 mg/kg) were shown to be radiolabelled natural products consisting of mono and disaccharides and amino acids.
The unextractable (bound) residues were consistent with natural products. Acid hydrolysis liberated carbohydrates,16.9 % of the TRR (0.082 mg/kg).
The base hydrolysate from the pilot extraction contained 14.5 % of the TRR (0.071 mg/kg) protein and lignin. The remaining pulp contained 7.60 % of the TRR (0.037 mg/kg) crude cellulose.

From yellow seeds, 8.9 % of the TRR (0.116 mg/kg) were extracted with hexane. Subsequent extraction with Tris buffer released 44.2 % of the TRR (0.579 mg/kg), of which 2.6 % of the TRR were shown to be PMG (0.034 mg/kg) and 1.60 % of the TRR AMPA (0.021 mg/kg).
Crude protein was precipitated at 24.0 % of the TRR (0.314 mg/kg) from the Tris buffer extract.
The remaining fractions of the buffer-soluble residue (40.0 % of the TRR, 0.524 mg/kg) were shown to be carbohydrate and smaller protein which does not precipitate at pH 5.0.

In conclusion, in soybeans treated with [^{14}C-PMG]glyphosate-trimesium ion at planting only minor levels of glyphosate or AMPA were found in the various plant parts. Most of the radioactivity was incorporated into natural products like carbohydrates and proteins.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate-trimesium in soybean has been previously evaluated at EU level and was considered to be acceptable (see glyphosate trimesium monograph; 1998; Renewal Assessment Report, 2015). It was performed under GLP and is still considered to be scientifically valid. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501.

Assessment and conclusion by RMS:

Miscellaneous

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/016
Report author	[redacted]
Report year	1975
Report title	CP-67573, Residue and Metabolism. Part 24: The Metabolism of CP-67573 in Coffee Plants
Report No	344
Document No	M-649024-01-1
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Application rate for foliar, stem and hydroponic treatment is given as total amount of radioactivity applied or total amount of radioactivity per plant or per leaf and not as mg a.s./ha. • In the bean producing coffee tree phytotoxic symptoms of chlorosis and leaf drop were observed at this treatment rate. These symptoms could have been due to the large number of beans that developed on this tree • No information of the storage stability for all major components of the total radioactive residues • No description of conditions and duration of storage of samples • Residues after solvent extraction (RRR) were not further measured or examined (the RRRs were calculated assuming total equal to

	100 % and that there were no losses during extraction and purification). • No full accountability reported. • For some matrices after hydroponic treatment less than 90 % of TRR was identified and characterised due to low extraction rates. In these cases, no attempts of exhaustive extraction have been performed. For matrices after foliar treatment at least 90 % was identified and characterised.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

In this study the uptake and translocation of ^{14}C-radiolabelled glyphosate and ^{14}C-AMPA were investigated following foliar, stem, hydroponic or soil treatment to coffee plants. The nature of residues was studied after foliar and hydroponic treatment.

For the soil treatment stocking solutions were sprayed at rates equivalent to 4.5 kg glyphosate or AMPA per ha. After 4, 6 and 8 weeks plant samples were collected.

For the uptake via the stem a ^{14}C-glyphosate solution was applied to the stem. The plant was kept in a hydroponic solution for 5 weeks. After this timeframe samples of leaves, untreated stems, treated stems and roots were collected.

The behaviour of glyphosate following foliar application was investigated by treating eight leaves with a solution of ^{14}C-glyphosate. After 3-5 weeks treated and untreated leaves, stems and roots were sampled.

In a second experiment leaves of bean producing coffee plants were treated. Each 4 weeks samples of coffee beans were collected and analysed for radioactive residues.

Hydroponic treatment of coffee plants was conducted with 1.1, 3.6 or 11.1 mg/L glyphosate in the hydroponic solution. The treated plants were grown for 21 days before samples were collected.

In coffee plants treated *via* soil, stem, foliar or hydroponic application the uptake and translocation of radioactivity was minimal. After the soil treatment only 0.052 and 0.061 mg/kg (0.017-0.038 % applied radioactivity (AR)) of ^{14}C-glyphosate and ^{14}C-AMPA, respectively, was found in aerial part of the tree 8 weeks after the treatment. After the stem treatment the TRR of the treated stem was 97.41 mg/kg (87.2 % AR), whereas the TRR of leaves, untreated stems and roots was 0.050 to 0.687 mg/kg, resulting totally in 2.72 % AR.

For the foliar uptake of glyphosate several experiments with ^{13}C- and ^{14}C-glyphosate were used with different formulations and application techniques. For the experiment using a mixture of ^{13}C- and ^{14}C-glyphosate identification of the residue revealed only unchanged parent. In all samples, glyphosate was the major residue present (>71.7 to 95.0 % of the TRR). AMPA/N-methyl AMPA accounted for <0.7-<1 % of the TRR.

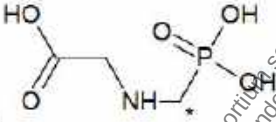
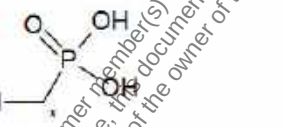
Coffee trees carrying beans were also foliar treated with ^{14}C-glyphosate. The beans were grown to maturity within 23 weeks after the treatment and analysed for the recovered radioactivity and its composition. In the immature beans 0.02 to 0.05 % of the applied radioactivity was found after 4 to 20 weeks after treatment, increasing to 0.94 % and to 0.68 % of applied radioactivity in green beans and pods as well as in ripe beans, respectively. Glyphosate was the major component of residue in all investigated bean matrices, comprising 91.2 to 98.0 % of the TRR, AMPA/N-methyl AMPA amounted to 0.98 to 5.0 % TRR.

After hydroponic treatment for three weeks most of the AR was recovered in roots and in the remaining hydroponic solution. Only 0.1 to 0.2 % AR and 4.3 to 11.7 % AR were found in aerial parts and roots,

respectively. Up to 86 and 90 % of the TRR (corresponding to 0.039 and 5.64 mg/kg after the 1.1 mg/L treatment) could be extracted from aerial parts and roots, respectively. The significant part of the residue aerial parts and roots was identified as the unchanged parent (up to 74.0 and 81.9 % of the TRR, 0.093 and 9.65 mg/kg, respectively). Additionally, a considerable amount of AMPA in aerial parts and roots was found which accounted for up to 14.0 and 8.1 % of the TRR (0.081 and 1.81 mg/kg), respectively. Thus, in coffee plants treated via soil, stem, foliar or hydroponic application the uptake and translocation of radioactivity was minimal. Identification of the recovered radioactivity revealed mainly unchanged parent, followed by AMPA at much lower levels.

I. Materials and methods

A. Materials

Test Material:	a, b) : N-(phosphonomethyl- ^{14}C)-glycine, (CP-67573- ^{14}C) c): N- (phosphonomethyl- ^{13}C)-glycine, (CP-67573- ^{13}C) d): aminomethyl- ^{14}C-phosphonic acid, (^{14}C-AMPA, CP-50435-^{14}C)
Chemical structure:	 a, b), c) * Position of ^{14}C or ^{13}C radiolabel  d) * Position of ^{14}C-radiolabel
Radiochemical purity:	a) >99 % (TLC) b) 97 % (TLC)[1] c) 97.5 % (GLPC with internal standartisation) d) >99 % (TLC)
Specific activity:	a) ^{14}C-glyphosate: 1.98 MBq/mg or 9.07 mCi/mmol (high specific activity, Code 245) b) ^{14}C-glyphosate: 0.41 MBq/mg or 1.87 mCi/mmol (medium specific activity, Code 240) c) ^{13}C-glyphosate, (Code 1311) specific activity not specified d) ^{14}C-AMPA (Code 236) 1.33 MBq/mg or 4.00 mCi/mmol
CAS No:	a-c) 1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt) d) 1066-51-9 (AMPA)
Log $P_{o/w}$	a-c) - 3.2, pH 7 at 25°C d) - 1.63, PH 7 at 20°C

[1] After subsequent purification, two impurities removed and only spot visible on TLC

Test system:

Soil: Hydroponic solution:	Drummer silty clay loam soil for seedling coffee plants (about 0.50 m high) 1:1 Michigan peat moss and sand for 4 – 5 year old plants (2-2.5 m high) Modified Tanaka solution
Crop:	Coffee plants
Botanical name:	*Coffea arabica L.*
Crop part(s):	Leaves, treated and untreated leaves, stems, beans (green and ripe), pods, roots

B. Study design

1. In-life phase

In this study the uptake and translocation of ^{14}C-glyphosate and ^{14}C-AMPA were investigated following soil stem, foliar, or hydroponic treatment to coffee plants.

Soil treatment
Six (15 × 15 cm) pots of soil grown coffee plants were selected. Three of these were used as controls, and three pots were each treated on the soil surface with 8.01 mg (2.05 x 10^8 dpm, 3.42 MBq) of ^{14}C-glyphosate in 0.1 N NH_4HCO_3 This treatment approximates an application rate of 4.5 kg a.s./ha. The pots were watered from the top daily for the duration of the experiment.
Additionally, six pots of soil grown coffee plants were selected. Three of these were used as controls, and three pots were each treated on the soil surface with 8.17 mg (6.43 × 10^8 dpm, 10.72 MBq) of ^{14}C-AMPA in 0.1 N NH_4HCO_3. This treatment approximates an application rate of 4.5 kg AMPA/ha or 3.0 glyphosate/ha.

Stem treatment
Two coffee plants were placed in 4 L glass jars filled with Tanada coffee nutrient solution (pH 4.6). A total of 1.9 mg ^{14}C-glyphosate in commercial formulation containing isopropylamine and G3780A adjuvant in water was applied to each of the two plants, uniformly coating three of the lower segments of the stems of each plant. The applied radioactivity corresponded to 2.98 × 10^7 dpm (0.497 MBq). The plant was kept in a hydroponic solution for 5 weeks.

Foliar treatment, experiment 1
Four coffee tree plants (about 0.5 m high) growing in hydroponic tanks were treated. A total of 1.9 mg ^{14}C-glyphosate in commercial formulation containing isopropylamine and G3780A adjuvant in water was applied to the leaf surface. Either both the top and bottom leaf surfaces (2 plants) or only the top leaf surfaces (1 plant) or only the bottom leaf surfaces (1 plant) were treated. Eight leaves on each plant were treated with 10 µl/treated surface. A total of 7.7 × 10^6 dpm (0.128 MBq, 0.32 mg) or 1.54 × 10^7 dpm (0.257 MBq, 0.64 mg) was applied, dependent if one side or both side of the leaves was treated.
Additionally, one coffee plant was treated with 0.608 mg ^{14}C-glyphosate in 0.1 N NH_4HCO_3. Both surfaces of eight leaves were treated with 10 µL/leaf surface. A total of 1.54 × 10^7 dpm (0.257 MBq) was applied.

Foliar treatment, experiment 2
One mature 20 cm coffee tree in a 23 liter pail containing a mixture of 1:1 peatmoss and sand was treated foliarly with ^{14}C-glyphosate. The coffee tree had bloomed about 1 month before this treatment and had set a large number of beans. A total of 1.9 mg ^{14}C-glyphosate in commercial formulation containing isopropylamine and G3780A adjuvant in water was applied per plant to the leaf surface. The commercial formulation was prepared by combining isopropylamine, ^{14}C-glyphosate, G3780A adjuvant and water. A total of 15 µL of test solution was applied to the lower surface of each of 100 leaves on 10 different lower branches. The applied radioactivity corresponded to 7.12 × 10^8 dpm (11.87 MBq).

Foliar treatment, experiment 3
Twenty coffee plants growing in two of the large hydroponic tanks were treated. One additional plant was maintained as a control. The test solution contained isopropylamine, ^{13}C-glyphosate, ^{14}C –glyphosate, (0.41 MBq/mg corresponding to 3.8 mg/mL), water and G3780A adjuvant.
164 µL of the test solution was applied to each of the plants, 8 leaves on each plant were treated on the lower surfaces only. The applied ^{14}C-radioactivity corresponded to 3.9×10^7 dpm (0.650 MBq).

Hydroponic treatment
Three coffee plants (40-50 cm) were placed in 4 L glass jars filled with 2 L Tanada coffee nutrient solution (pH 4.6). To each jar 2.11 mg of ^{14}C-glyphosate was added, thus the applied radioactivity in each jar corresponded to 5.0×10^7 dpm. Additionally, 0, 5 and 20 mg of unlabelled glyphosate was added to each jar, corresponding to 1.1, 3.6 or 11.1 mg/L glyphosate in the hydroponic solution. The hydroponically treated plants were grown for 21 days after treatment. The plants were exposed to the labelled compound throughout the duration of the experiment. The solution level in the jars was maintained by addition of distilled water.

2. Sampling

Soil treatment
4, 6, and 8 weeks after treatment one treated and one control plant were cut off 2.5 cm above the soil, and the wet weight measured. Each sample was then frozen, lyophilised, the dry weight measured and the sample was ground.

Stem treatment
After 5 weeks of treatment the plants were divided into the following parts: leaves, untreated stems, treated stems, and roots. The plant parts were weighed, frozen, lyophilised, reweighed and ground.

Foliar treatment, experiment 1
Three weeks after treatment one plant which had been treated on both leaf surfaces with the commercial formulation and the plants which had been treated on only the top or bottom leaf surfaces were harvested. They were divided into the following parts: roots, treated leaves, untreated leaves, and stems. The plant parts were weighed, frozen, lyophilised, reweighed and ground.
Five weeks after treatment the other two plants were harvested and analysed in the same manner as those harvested at 3 weeks after treatment.

Foliar treatment, experiment 2
Every four weeks after treatment a random sample of beans was removed from the tree. Leaves which had wilted and fallen off during each 4 week period were collected. The samples were, weighed, frozen, lyophilised, reweighed, and ground.

Foliar treatment, experiment 3
Five weeks after treatment the plants were divided into treated leaves, stems and branches, untreated leaves and roots. The samples were, weighed, frozen, lyophilised, reweighed, and ground.

Hydroponic treatment
After 21 days of treatment the aerial portions of the plants were removed. The roots were each washed three times with 1N NH_4OH. The plant parts were then weighed, frozen, and lyophilised. The dry weight was determined, and each plant sample was ground. At the time that the plants were harvested the volume of the hydroponic solutions was measured, and triplicate aliquots were taken from each jar.

3. Analytical procedures

The total radioactive residues were determined in the lyophilised plant samples by liquid scintillation counting (LSC) after combustion. The samples of hydroponic solutions were directly subjected to LSC.

Plant samples were extracted two times with water and three times with 0.5 N NH_4OH. The combined supernatants were acidified with HCl to pH 2 cooled to 4°C overnight followed by centrifugation and filtration. The filtrate was assayed by LSC. The plant residue after extraction was lyophilised, and non-extractable radioactivity was assayed by combustion. The lyophilised combined extracts were re-dissolved in water. After purification by cation exchange chromatography (AG50W-X4, AG50W-X8 (H^+-form), anion exchange chromatography (AG1-X8, AG-1-X4 (HCO_3^--form)) and gel-filtration (Bio-gel P2), the radioactive fractions were analysed routinely by two dimensional TLCs on cellulose plates.

For the identification of metabolites, a derivatisation to n-butyl-esters of N-trifluoroacetylated compounds by reacting the desired compound with trifluoroacetic acid/trifluoroacetic anhydride (4:1) followed by removal of solvent under N_2, addition of n-butanol, and finally, addition of ethereal diazo-n-butane was included. Afterwards, GLPC followed by mass spectrometry (MS), two-dimensional TLC (with Beta-Camera analysis, spraying with Ninhydrin for the determination of amino acids and its analogues, with Hanses reagent to detect phosphorous containing compounds) as well as NMR (^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR) were used and the results compared with reference substances.

II. Results and discussion

A. Total radioactive residues (TRRs)

Soil treatment

After the soil treatment only 0.052 and 0.061 mg/kg (0.017-0.038 % applied radioactivity (AR)) of ^{14}C-glyphosate and ^{14}C-AMPA, respectively, was found in aerial part of the tree 8 weeks after the treatment. Clearly the uptake resulting from soil treatment is so low that it is not amenable to metabolism studies. The high residues in control (up to 0.031 mg/kg expressed as glyphosate equivalents, 0.01 % AR) may be discussed due to microbial degradation of ^{14}C-glyphosate applied to soil to $^{14}CO_2$ which was then available for photofixation by all the plants. In addition to ruling out soil uptake for metabolic studies, these results clearly suggest that only a small percentage of the glyphosate applied to the soil and weeds or its major metabolite AMPA will ever be incorporated into the aerial portions of coffee plants. Any residues observed in the aerial parts or the fruit are not likely to originate from glyphosate applied to the soil.

Table 6.2.1-106: Total radioactive residues in coffee plants following application to soil of ^{14}C-glyphosate or ^{14}C-AMPA 4 to 8 weeks before sampling

Sample description	Treatment	Weeks after treatment	TRR		
			% AR	dpm	mg/kg
^{14}C-glyphosate, 2.05 x 10^8 dpm, soil application					
Aerial part of the tree	control	4	0.0063	12662	*0.015*
	treated		0.0033	6614	*0.021*
	control	6	0.0068	13663	*0.023*
	treated		0.0113	2267	*0.003*
	control	8	0.0115	2297	*0.004*
	treated		0.0169	33823	*0.052*
^{14}C-AMPA, 6.43 x 10^8 dpm, soil application					
Aerial part of the tree	control	4	0.0060	39302	*0.027 (0.018)*
	treated		0.0157	102924	*0.047 (0.031)*
	control	6	0.0060	39027	*0.015 (0.010)*
	treated		0.0222	145402	*0.039 (0.026)*
	control	8	0.0119	77915	*0.019 (0.013)*
	treated		0.0381	255560	*0.061 (0.040)*

TRR Total radioactive residue
AR Applied radioactivity

The TRRs are given in the report as % applied radioactivity (% AR) or dpm and were recalculated based on the specific radioactivity of 0.41 MBq/mg (^{14}C-glyphosate) or 1.31 MBq/mg (^{14}C-AMPA). The TRRs are expressed as glyphosate. The TRRs resulting after application of ^{14}C AMPA are additionally of expressed as AMPA (in brackets). The values recalculated upon

dossier compilation are given in *italics*.

Stem treatment

Since some of the formulated ^{14}C-glyphosate which is applied by directed spraying may be deposited on the trunks of the coffee trees, the propensity for uptake from the trunks was investigated. The TRR of the treated stem was 97.41 mg/kg (87.2 % AR), whereas the TRR of leaves, untreated stems and roots was 0.050 to 0.687 mg/kg, resulting totally in 2.72 % AR. Assuming that less than 5 % of the total applied by directed spraying would be deposited on the trunk, less than 0.1 % would be taken up into the leaves and stems. These data indicate that uptake via trunk application will be minimal and that any residues observed will probably result from foliar application

Table 6.2.1-107: Total radioactive residues in coffee plants following 5 weeks stem treatment

Sample description	Treatment duration	TRR		
		% AR	dpm	mg/kg
^{14}C-glyphosate, 2.98 x 10^7 dpm				
Treated Stems	5 weeks	87.2	25970000	*97.41*
Leaves		0.54	160000	*0.081*
Untreated Stems		1.68	500000	*0.687*
Roots		0.50	150000	*0.050*
Hydroponic Solution		1.14	340000	

TRR Total radioactive residue
AR Applied radioactivity
The TRRs are given in the report as % applied radioactivity (% AR) or dpm and were recalculated based on the specific radioactivity of 0.41 MBq/mg. For hydroponic treatments the dilution of ^{14}C-glyphosate with non-labeled glyphosate was considered. The values recalculated upon dossier compilation are given in *italics*.

Foliar treatment

After the foliar application of the ^{14}C-glyphosate the highest residues were found in leaves accounting for (19.20 – 53.59 mg/kg (65.2 - 93.8 % of the applied radioactivity (AR)). Although the majority of the ^{14}C applied remained on the treated leaves, substantial uptake and translocation had occurred. The majority of the translocated ^{14}C-activity was associated with the roots and stems accounting for 0.22 - 3.66 mg/kg (1.3 - 10.4 % AR) and 0.54 - 7.73 mg/kg (2.1 - 17.9 %AR) respectively.

Residues in ^{13}C/^{14}C-experiment, which were further extracted and characterised were similar to other foliar experiments: 33.31 mg/kg (43.6 %AR) in leaves, 4.30 mg/kg (26.5 % AR) in roots, 4.10 mg/kg (11.5 % AR) in stems, 3.55 mg/kg (0.1 % AR) in wilted lower leaves and 0.63 mg/kg (2.8 % AR) in untreated leaves.

In the bean uptake experiment the residues in coffee beans were very low and slightly increased with time, amounting to 0.147, 0.212 and 0.433 mg/kg in ripe coffee beans, pods and green beans and pods, respectively. This corresponded to only 0.66 to 0.94 % AR. In the bean producing coffee tree phytotoxic symptoms of chlorosis and leaf drop were observed at this treatment rate. These symptoms could have been due to the large crop of beans that developed on this tree.

Table 6.2.1-108: Total radioactive residues in coffee plants following foliar treatment 3 and 5 weeks before sampling

Sample description	Weeks after treatment	TRR		
		% AR	dpm	mg/kg
^{14}C-glyphosate, 7.7 x 10^6 dpm, upper leaf surface treated				
Treated leaves	3 weeks	86.3	6648000	*19.20*
Roots		1.3	100000	*0.22*
Stems		6.2	477000	*1.31*
Untreated leaves		2.7	208000	*0.64*

Table 6.2.1-108: Total radioactive residues in coffee plants following foliar treatment 3 and 5 weeks before sampling

Sample description	Weeks after treatment	TRR		
		% AR	dpm	mg/kg
^{14}C-glyphosate, 7.7 x 10^6 dpm, lower leaf surface treated				
Treated leaves	3 weeks	65.2	5023000	*26.89*
Roots		8.5	654000	*3.66*
Stems		17.9	1381000	*7.73*
Untreated leaves		0.8	65000	*0.34*
^{14}C-glyphosate, 1.54 x 10^6 dpm, upper and lower leaf surface treated				
Treated leaves	3 weeks	73.3	11290000	*44.74*
Roots		10.4	1609000	*2.00*
Stems		8.0	1227000	*2.12*
Untreated leaves		0.6	88000	*0.11*
^{14}C-glyphosate, 1.54 x 10^7 dpm, upper and lower leaf surface treated				
Treated leaves	5 weeks	75.1	11567000	*31.08*
Roots		10.2	1565000	*2.05*
Stems		13.8	2119000	*3.96*
Untreated leaves		4.1	627000	*1.10*
^{14}C-glyphosate (unformulated), 1.54 x 10^7 dpm, upper and lower leaf surface treated				
Treated leaves	5 weeks	93.8	14445000	*53.59*
Roots		4.3	667000	*0.45*
Stems		2.1	331000	*0.54*
Untreated leaves		1.8	274000	*0.25*
^{13}C- and ^{14}C-glyphosate, 3.9 x 10^7 dpm, upper and lower leaf surface treated				
Treated leaves	5 weeks	43.6	16996000	*33.31*
Roots		26.5	10342000	*4.30*
Stems		11.5	4472000	*4.10*
Untreated leaves		2.8	1100000	*0.63*
Wilted lower leaves		0.1	39000	*3.55*
Hydroponic Solution		3.4	1315000	-
^{14}C-glyphosate, 7.12x 10^8 dpm, lower leaf surface of lower branches treated				
Coffee beans	4 weeks	*0.02*	142000	*0.093*
Coffee beans	8 weeks	*0.02*	120000	*0.056*
Coffee beans	12 weeks	*0.03*	202000	*0.108*
Coffee beans	16 weeks	*0.05*	389000	*0.183*
Coffee beans	20 weeks	*0.04*	285700	*0.147*
Ripe coffee beans	23 weeks	*0.66*	4670000	*0.147*
Ripe pods	23 weeks	*0.68*	4831000	*0.212*
Green beans and pods	23 weeks	*0.94*	6721000	*0.433*

TRR Total radioactive residue
AR Applied radioactivity
The TRRs are given in the report as % applied radioactivity (% AR) or dpm and were recalculated based on the specific radioactivity of 1.98 MBq/mg in bean experiment and 0.41 MBq/mg in all other experiments, For foliar treatments with both ^{14}C and ^{13}C glyphosate, the dilution of ^{14}C-glyphosate with ^{13}C glyphosate was considered. The values recalculated upon dossier compilation are given in *italics*.

Hydroponic treatment

After hydroponic treatment for three weeks most of the AR was recovered in roots and in the remaining hydroponic solution. Thus, the TRR in roots after 1.1, 3.6 and 11.1 mg/L treatment were 6.27, 12.19 and 29.64 mg/kg, which corresponds to 11.7, 4.3 and 5.0 % AR, respectively. The TRR of the aerial parts of the plants were only 0.045, 0.155 and 0.842 mg/kg (0.10-0.20 % AR). The major radioactivity was found in hydroponic solutions and root washes, the sum of both amounted to 62.2, 99.0 and 88.0 % AR, respectively.

Table 6.2.1-109: Total radioactive residues in coffee plants following 3 weeks hydroponic treatment

Sample description	Treatment duration	TRR		
		% AR	dpm	mg/kg
^{14}C-glyphosate, 5.0 x 10^7 dpm + 0 mg glyphosate, (1.1 mg/l total glyphosate)				
Roots	3 weeks	11.7	6850000	*6.27*
Aerial		0.1	60000	*0.045*
Root washes		38.7	19330000	
Hydroponic solution		23.5	11750000	
^{14}C-glyphosate, 5.0 x 10^7 dpm + 5 mg glyphosate, (3.6 mg/l total glyphosate)				
Roots	3 weeks	4.3	2450000	*12.19*
Aerial		0.1	50000	*0.155*
Root washes		15.2	7600000	
Hydroponic solution		83.8	41900000	
^{14}C-glyphosate, 5.0 x 10^7 dpm + 20 mg glyphosate, (11.1 mg/l total glyphosate)				
Roots	3 weeks	5.0	2950000	*29.64*
Aerial		0.2	110000	*0.842*
Root washes		10.5	5260000	
Hydroponic solution		77.5	38750000	

TRR Total radioactive residue
AR Applied radioactivity
The TRRs are given in the report as % applied radioactivity (% AR) or dpm and were recalculated based on the specific radioactivity of 0.41 MBq/mg. For hydroponic treatments the dilution of ^{14}C-glyphosate with non-labeled glyphosate was considered. The values recalculated upon dossier compilation are given in *italics*.

B. Extraction and characterisation of residues

The foliar treatment experiment using a mixture of ^{13}C- and ^{14}C-glyphosate was succeeded by extraction and identification of residues. After extraction with water and 0.5 N NH_4OH up to 92 % of the TRR (30.65 mg/kg) could be extracted from leaves, 90.9 % of the TRR (3.73 mg/kg) from stems and 96.0 % of the TRR (4.13 mg/kg) from roots. The identification of the residue revealed unchanged parent (>99 % AR), thus amounting to >90.0 to >95 % of the TRR, corresponding to >3.73 to >30.65 mg/kg in treated leaves, stems and roots. In untreated leaves, 72.4 % of radioactivity was extracted and >71.7 % of the TRR was identified as glyphosate. In all of the tested commodities only <0.7 - 0.9 % of the TRR (<0.004 - <0.30 mg/kg) was defined as AMPA (see Table 6.2.1-110).

Additionally, the coffee beans with pods (week 4 and 8) as well as ripe beans and ripe pods (week 23) after the foliar treatment with ^{14}C-glyphosate were extracted and the residues were identified. After extraction with water and 0.5 N NH_4OH 96 to 99 % of the TRR (0.055 to 0.210 mg/kg) could be extracted.

The identification of the residue revealed unchanged parent accounting for 91.2 - 98.0 % of the TRR (0.054 - 0.199 mg/kg). Only 0.98 - 5.0 % of the TRR (<0.001 - 0.011 mg/kg) was defined as AMPA (see Table 6.2.1-111).

The extraction and identification of residues were performed also with trees which were hydroponically treated with 1.1, 3.6 and 11.1 mg/L glyphosate. The aerial parts and roots of the coffee tree plants were extracted with water and 0.5 N NH_4OH. Up to 86 and 90 % of the TRR (corresponding to 0.039 and 5.64 mg/kg after the 1.1 mg/L treatment) could be extracted from aerial parts and roots, respectively. The significant part of the residue aerial parts and roots was identified as the unchanged parent (up to 74.0 and 81.9 % of the TRR, 0.093 and 9.65 mg/kg, respectively). Additionally, a considerable amount of AMPA in aerial parts and roots was found which accounted for up to 14.0 and 8.1 % of the TRR (0.081 and 1.81 mg/kg), respectively.

For the full dataset please refer to Table 6.2.1-112 and Table 6.2.1-113.

Table 6.2.1-110: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in different tree parts following foliar treatment

	Treated leaves		Stems		Untreated leaves		Roots	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Weeks after treatment	5		5		5		5	
TRR	**100**	***33.31***	**100**	***4.10***	**100**	***0.63***	**100**	***4.30***
Parent (Glyphosate)	*>91.1* (>99)	*>30.35*	*>90.0* (>99)	*>3.69*	*>71.7* (>99)	*>0.45*	*>95.0* (>99)	*>4.09*
AMPA/*N*-methyl-AMPA[1]	*<0.9* (<1)	*<0.30*	*<0.9* (<1)	*<0.04*	*<0.7* (<1)	*<0.004*	*<1* (<1)	*<0.04*
Total identified	**92.0**	***30.65***	**90.9**	***3.73***	**72.4**	***0.46***	**96.0**	***4.13***
ERR	**92.0**	***30.65***	**90.9**	***3.73***	**72.4**	***0.46***	**96.0**	***4.13***
RRR	***8.0***	***2.66***	***9.1***	***0.37***	***27.6***	***0.17***	***4.0***	***0.17***
Total sum	**100**	***33.31***	**100**	***4.10***	**100**	***0.63***	**100**	***4.30***

In brackets residues expressed as % of extracted radioactivity are given. Residues expressed as % TRR were recalculated based on the data available within the report.
[1)] N-methyl-AMPA named as CP-70948 in the report
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue; was calculated under assumption that the total sum amounts to 100 % and that there were no losses during extraction and purification.

Table 6.2.1-111: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in coffee beans following foliar treatment

	Beans with pods		Beans with pods		Ripe beans		Ripe pods	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Weeks after treatment	4		8		23		23	
TRR	**100**	***0.093***	**100**	***0.056***	**100**	***0.147***	**100**	***0.212***
Parent (Glyphosate)	*98.0* (99)	*0.091*	*97.0* (99)	*0.054*	*91.2* (95)	*0.134*	*94.0* (95)	*0.199*
AMPA/N-methyl-AMPA[1]	*0.99* (1)	*0.001*	*0.98* (1)	*0.001*	*4.8 (5)*	*0.007*	*5.0* (5)	*0.011*
Total identified	**99**	*0.092*	**98**	***0.055***	**96**	***0.141***	**99**	***0.210***
ERR	**99**	*0.092*	**98**	***0.055***	**96**	***0.141***	**99**	***0.210***
RRR	***1***	*0.001*	***2***	***0.001***	***4***	***0.006***	***1***	***0.002***
Total sum	**100**	*0.093*	**100**	***0.056***	**100**	***0.147***	**100**	***0.212***

In brackets residues expressed as % of extracted radioactivity are given. Residues expressed as % TRR were recalculated based on the data available within the report.
1) N-methyl-AMPA named as CP-70948 in the report
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

Table 6.2.1-112: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in aerial parts of coffee trees following hydroponic treatment

	1.1 mg/l total glyphosate applied		**3.6 mg/l total glyphosate applied**		**11.1 mg/l total glyphosate applied**	
	% TRR	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Weeks of treatment	**3**		**3**		**3**	
TRR	**100**	***0.045***	**100**	***0.155***	**100**	***0.842***
Parent (Glyphosate)	*74.0* (86)	*0.033*	*59.9* (81)	*0.093*	*38.4* (80)	*0.323*
AMPA	*12.0* (14)	*0.005*	*14.1* (19)	*0.022*	*9.6* (20)	*0.081*
N-methyl-AMPA[1]	-		-		-	
Total identified	***86.0***	*0.039*	***74.0***	***0.115***	***48.0***	***0.404***
ERR	**86**	*0.039*	**74**	***0.115***	**48**	***0.404***
RRR	***14***	*0.006*	***26***	***0.040***	***52***	***0.438***
Total sum	***100***	*0.045*	***100***	***0.155***	***100***	***0.842***

In brackets residues expressed as % of extracted radioactivity are given.
Values calculated upon dossier compilation and are given in *italics*.
[1] N-methyl-AMPA named as CP-70948 in the report
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

Table 6.2.1-113: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in roots of coffee trees following hydroponic treatment

	1.1 mg/l total glyphosate applied		**3.6 mg/l total glyphosate applied**		**11.1 mg/l total glyphosate applied**	
	% TRR	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**
Weeks of treatment	**3**		**3**		**3**	
TRR	**100**	***6.27***	**100**	***12.19***	**100**	***29.64***
Parent (Glyphosate)	*81.9* (91)	*5.71*	*79.2* (91)	*9.65*	*31.9* (84)	*9.46*
AMPA	*8.1* (9)	*0.51*	*7.8* (9)	*0.95*	*6.1* (16)	*1.81*
N-methyl-AMPA[1]		-	-	-	-	-
Total identified	***90.0***	*5.64*	***87.0***	*10.61*	***38.0***	*11.26*
ERR	**90**	*5.64*	**87**	*10.61*	**38**	*11.26*
RRR	***10***	*0.63*	***13***	*1.58*	***62***	*18.38*
Total sum	***100***	*6.27*	***100***	*12.19*	***100***	*29.64*

Table 6.2.1-113: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in roots of coffee trees following hydroponic treatment

In brackets residues expressed as % of extracted radioactivity are given. Residues expressed as % TRR were recalculated based on the data available within the report.
[1] N-methyl-AMPA named as CP-70948 in the report
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

C. Storage stability

Storage period is not specified within the study, the date of analysis and sampling dates are not given. Nevertheless, the study duration is from October, 1973 to December, 1974. Therefore, as the worst case assumption, the storage time would be below 455 days (15 months). Considering the time necessary to launch such extensive study and the time to write the final report, it is likely to be a huge overestimation. Within the study oil-rich matrices (coffee beans) and water-rich matrices (roots, aerial part of the tree, stems, leaves) were investigated.

Storage stability of frozen samples of high oil matrices in metabolism studies has been tested in canola seeds and soybean seeds for 16.7 months (501 days) (Rogers MSL_13318.)

Storage stability of frozen samples of high water content in metabolism study has been shown in carrot tops for 15 months (McMullan_MSL_9810).

Therefore, the storage stability is covered.

D. Degradation pathway

Please refer to the pathway of glyphosate crop group pulses and oilseeds presented further below.

III. Conclusion

The extent to which glyphosate and its metabolites are taken up by coffee plants (beans, foliage, roots and stems) was determined and the nature of residues was studied. For this purpose, different experiments were performed: soil uptake, trunk treatment, foliar applications and hydroponic uptake experiments.

In coffee plants treated *via* soil, stem, foliar or hydroponic application the uptake and translocation of radioactivity was minimal. After the soil treatment only 0.052 and 0.061 mg/kg (0.017-0.038 % applied radioactivity (AR)) of ^{14}C-glyphosate and ^{14}C-AMPA, respectively, was found in aerial part of the tree 8 weeks after the treatment. After the stem treatment the TRR of the treated stem was 97.41 mg/kg (87.2 % AR), whereas the TRR of leaves, untreated stems and roots was 0.050 to 0.687 mg/kg, resulting totally in 2.72 % AR.
For the foliar uptake of glyphosate several experiments with ^{13}C- and ^{14}C-glyphosate were used with different formulations and application techniques. For the experiment using a mixture of ^{13}C- and ^{14}C-glyphosate identification of the residue revealed only unchanged parent. In all samples, glyphosate was the major residue present (>71.7 to 95.0 % of the TRR). AMPA/N-methyl AMPA accounted for <0.7-<1 % of the TRR.

Coffee trees carrying beans were also foliar treated with ^{14}C-glyphosate. The beans were grown to maturity within 23 weeks after the treatment and analysed for the recovered radioactivity and its composition. In the immature beans 0.02 to 0.05 % of the applied radioactivity was found after 4 to 20 weeks after treatment, increasing to 0.94 % and to 0.68 % of applied radioactivity in green beans and pods as well as in ripe beans, respectively. Glyphosate was the major component of residue in all investigated bean matrices, comprising 91.2 to 98.0 % of the TRR, AMPA/N-methyl AMPA amounted to 0.98 to 5.0 % TRR.

After hydroponic treatment for three weeks most of the AR was recovered in roots and in the remaining

hydroponic solution. Only 0.1 to 0.2 % AR and 4.3 to 11.7 % AR were found in aerial parts and roots, respectively. Up to 86 and 90 % of the TRR (corresponding to 0.039 and 5.64 mg/kg after the 1.1 mg/L treatment) could be extracted from aerial parts and roots, respectively. The significant part of the residue aerial parts and roots was identified as the unchanged parent (up to 74.0 and 81.9 % of the TRR, 0.093 and 9.65 mg/kg, respectively). Additionally, a considerable amount of AMPA in aerial parts and roots was found which accounted for up to 14.0 and 8.1 % of the TRR (0.081 and 1.81 mg/kg), respectively.
Thus, in coffee plants treated via soil, stem, foliar or hydroponic application the uptake and translocation of radioactivity was minimal. Identification of the recovered radioactivity revealed mainly unchanged parent, followed by AMPA at much lower levels.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in coffee plants has been previously evaluated at EU level. It was not performed under GLP (as in 1975 GLP was not yet established at the test facility). The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with some deficits: no information of the storage stability for all major components of the total radioactive residues; no description of conditions and length of storage of samples; for some matrices resulting after hydroponic treatment less than 90 % of TRR was identified and characterised due to low extraction rates. In these cases, no attempts of exhaustive extraction or solubilisation have been performed. Nevertheless, after foliar treatment in the matrices of interest as ripe coffee beans, ripe pods, beans with pods, treated leaves, stems and roots the sum of identified and characterised radioactivity was above 90 %. Thus, the RRRs (calculated under the assumption that there were no losses during purification and extraction) in these matrices are below 10 %.

Justification for storage stability:
As the worst case assumption, the duration of storage of sample cannot exceed the study duration, which was 455 days (15 months). Within the study oil-rich matrices (coffee beans) and water-rich matrices (roots, aerial part of the tree, stems, leaves) were investigated. Most of residue was attributed to glyphosate, also metabolite AMPA/N-methyl-AMPA was found. Storage stability of frozen samples of high oil content has been shown in canola seeds for 16.7 months (501 days) ([redacted]_13318). Storage stability of frozen samples of high water content has been shown in carrot tops for 15 months (McMullan_MSL_9810). Thus, storage stability has been addressed and covered.
The study is considered to be reliable for the assessment of the metabolic behaviour of glyphosate in coffee because it provides data on the distribution of glyphosate-derived radioactivity within the coffee plant and on the formation of the metabolites in coffee beans and leaves.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/017
Report author	Anonymous
Report year	1976
Report title	Glyphosate residue and metabolism studies in sugarcane and soils
Report No	RD93
Document No	M-651454-02-1

Guidelines followed in study	None
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: The study consists of different experiments with limited relevance on metabolism (field residue studies using non-radiolabelled glyphosate and analysis for known analytes (glyphosate and AMPA); processing of sugarcane juice into refined sugar after spiking of radiolabelled glyphosate and spiking of raw sugar by a mixture of radiolabelled and non-labelled glyphosate followed by processing into refined sugar. Both processing experiments were analysed for the fate of radioactive residues only. The analytical method used yielded low recoveries (< 70 %). The root and foliar absorption experiments which were performed using radiolabelled glyphosate do not follow the current guideline in major terms: • The radiochemical purity of the test item is not clearly specified • Physical facility and environmental conditions insufficiently described • Developmental stages of the crop at application and harvesting are not reported. • The sampled RACs (raw agricultural commodities) were not appropriate, no sugarcane sample investigated • Radioactive residues are expressed in % of applied activity and TRR in mg/kg dry matter • No release and characterisation and/or identification was attempted. • No details on radioactive counting data • No description of conditions and duration of storage of samples
Previous evaluation	Yes, accepted as informative in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The study consists of different experiments using either non-labelled glyphosate or ^{14}C-labelled glyphosate.

Within the first experiment the non-labelled active substance was applied either before pre-planting (11.2 kg a.s./ha), or as an interline broadcast treatment (3.4 or 6.7 kg a.s./ha) or post-emergent treatment along the field edges (5.6 or 11.2 kg a.s./ha).

The results from the pre-plant soil treatment indicate the uptake of residues from soil was low. Residues of glyphosate and its metabolite were below the limit of quantification in all samples (0.05 mg/kg in sugarcane, bagasse and refined sugar; 0.5 mg/kg in molasses).

Immediately after interline broadcast treatment the residues of glyphosate in sugarcane ranged between 0.74 and 3.0 mg/kg after application of 3.4 kg a.s./ha and between 1.85 and 6.9 mg/kg after application of 6.7 kg a.s./ha. Over time glyphosate residues decreased and were below 0.05 mg/kg after 165 or 183 days except for one sample with 0.09 mg/kg after 165 days. Residues of AMPA in sugarcane were below the LOQ of 0.05 mg/kg at all sampling intervals.

In the three tests where Roundup was applied to the ground along the field edge according to normal practice, the residues of glyphosate and its metabolite AMPA were all <0.05 mg/kg in the sugarcane 42-47 days after application. In the two trials where the cane foliage was purposely sprayed to maximize residues, the residues of glyphosate ranged between 0.14 to 0.24 mg/kg 40-44 days after application of 5.6 kg a.s./ha and between 0.28 to 0.34 mg/kg 40-44 days after application of 11.2 kg a.s./ha, while residues of AMPA were <0.05 mg/kg in all cases.

Processing of sugar cane following commercial practice indicated that glyphosate residues and its metabolite do not appear or concentrate in any of the processed fractions from applications where residues are <0.05 mg/kg in or on the sugarcane. In the two trials where deliberate contact of foliage was made, residues of glyphosate decreased during processing in the bagasse and raw sugar, while residues in molasses increased. Residues of AMPA remained below the respective LOQ in any of the processed samples.
An additional experiment for processing of mixed sugarcane juice performed with ^{14}C-labelled glyphosate showed that 36 % of the original glyphosate was removed in the liming solids. Most of the radioactive residues which were in the clarified juice found their way into the molasses fraction. The molasses film on raw sugar carried some portion of the radioactivity, and minor portions of molasses were occluded in the sugar crystal. Refining removed this minor residue by adsorption on the bone char resulting in no radioactivity detectable in refined sugar.

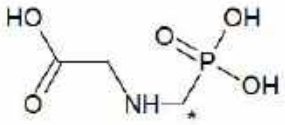
These results were confirmed after processing of sugar with an aqueous solution of labelled and unlabelled glyphosate into refined sugar. During processing the main part of the radioactivity remained in the bone char (84 %). Further 6 % were recovered in the filter cake from liming, 3 % in refined molasses (<0.5 mg/kg) and 7 % remained unaccountable, while radioactive residues in refined sugar were <0.05 mg/kg.

The results of the root absorption experiment where sugarcane plants were grown in a hydroponic solution of ^{14}C-methane-glyphosate showed that glyphosate was absorbed from nutrient culture solution into sugarcane roots to an amount of 13 % after 12 weeks. 8 % of the applied radioactivity remained fixed in the roots; the stalk carried 1 % and the leaves 3 %. 81 % of the applied radioactivity had disappeared, probably as $^{14}CO_2$. Concentrations of 5 mg/kg (dry basis) occurred in roots, apical meristem, and the unexpanded leaf spindle, the regions of high metabolic growth activity but not of photosynthesis or sugar storage. Thin layer chromatography showed the residues to be in both the metabolite and parent forms with a possible predominance of metabolite aminomethylphosphonic acid (AMPA).

In the experiment investigating the foliar absorption of ^{14}C-glyphosate, 18.6 % of residues applied were determined after 12 weeks. The treated leaves retained 4.6 %, roots accumulated 4.6 % and the primary stalk accumulated 4.3 %. Secondary suckers carried 3.2 % and the untreated leaves and spindle about 1.5 %. Accumulation took place in untreated younger leaves, spindle, primary apical meristem, stalk and roots. There was evidence of considerable translocation within the plant, probably in the phloem. The major translocated species was glyphosate, with only a minor contribution of the metabolite.

I. Materials and methods

A. Materials

Test Material:	Round up (residue and corresponding processing experiments), non-labelled N-(phosphono-^{14}C-methyl)glycine (^{14}C-methane glyphosate (root and foliar absorption and sugarcane juice and raw sugar processing experiments)
Chemical structure:	 * Position of radiolabel
Radiochemical purity:	Not stated
Specific activity:	9.07 mCi/mM (1.96 MBq.mg^{-1})[1] (raw sugar experiment, root absorption experiment) [1] calculated based on a molecular weight of 169.07 g/mol not stated for sugarcane juice processing and foliar application experiment
CAS No:	1071-83-6
Log $P_{o/w}$:	- 3.2

Test system:

Soil:	Pre-plant soil treatment: Low humic Latosol/Torrox (Hawaiian soil)
Crop:	Sugarcane
Botanical name:	*Saccharum officinarum*
Crop part(s):	Sugarcane, leaves, tops as well as processed commodities (bagasse, molasses, sugar)

B. Study design

1. In-life phase

The study consists of several experiments. Only those which may be considered as informative in context of metabolism and residue behaviour are detailed in the following. Additional experiments, e.g. determination of soil adsorption, dissipation and half-life of glyphosate in soil, soil residue determination or irrigation ditch treatment are not relevant to this section and are not summarised further.

In the first part of experiments of this study the behaviour of glyphosate (applied as non-labelled Roundup formulation) in sugar cane following different methods of treatment was investigated in the USA. Selected samples were processed into bagasse, molasses and sugar and all samples were analysed for glyphosate and its metabolite AMPA:

Pre-plant treatment to soil: a single application (11.2 kg a.s./ha) of the Roundup formulation of glyphosate was applied one week prior to planting of sugarcane seed pieces. Sugarcane seed pieces were planted in the irrigation furrow at a depth of ~10-15 cm below the surface. No symptoms of stunting or phytotoxicity occurred compared with an untreated check.

In the interline post-emergent directed broadcast treatment glyphosate was applied in two Hawaiian locations to six month old sugarcane at a rate of 3.4 or 6.7 kg a.s./ha. The application was done by hand knapsack. The sprays were directed near ground level between crop rows to avoid most of the crop foliage. The two locations represent dry - irrigated (Oahu Sugar Company) and wet - unirrigated (Mauna Kea Sugar Company) conditions. The 6.7 kg a.s./ha application at Mauna Kea was made to maximize residues and produced considerable toxic symptoms, as the result of more foliage spray than at the Oahu Sugar test site. The zero day residue results reflect this difference.

Post-emergent treatment: Spray applications were performed at five locations (Florida (2), Louisiana (1) and Hawai (2)) at a rate of 5.6 or 11.2 kg a.s./ha. The two Florida tests were performed to maximize residues. The spray was directed to the sugarcane foliage, confining the spray to the ground immediately adjacent to the field edge.

Additional processing experiments were performed. In the first one, sugarcane juice was spiked with ^{14}C-labelled glyphosate and processed into refined sugar. In the second one, raw sugar was spiked with an aqueous solution of labelled and unlabelled glyphosate; 500 g sugar was spiked with 1 mg/kg unlabelled glyphosate and 4.5 µCi labelled glyphosate (9.07 mCi/mmM) and dissolved in water.

Two experiments investigating the root and foliar absorption after 12 weeks were performed. ^{14}C-methane-glyphosate was applied to the roots via a nutrient culture solution or to the leaves by foliar treatment:

Root absorption: Single vegetative buds of sugarcane stalks, cultivar H 50-7209, were pre-germinated in soil/vermiculite (1/1), and transplanted individually after five weeks into glazed porcelain crocks containing 3000 mL of aerated complete nutrient solution. Each of the established plants at 8 weeks, about 30 cm in height, were treated with a single increment of 3.0 mg of ^{14}C-glyphosate (9.07 mCi/mM) to provide an initial concentration of 1.0 mg/L. Each plant received 160.1 pCi of radioactivity.

Foliar absorption: approximately 0.5 mg (26.2 µCi) of glyphosate-^{14}C in 0.1 M NH_4HCO_3 solution containing 0.5 % surfactant (Tergitol 15-S-9) was placed on 20 to 30 cm^2 of sugarcane leaf surface near the midsection of each of 4 leaves. An actual amount of 1.96 mg was placed on each of ~122-183 cm tall plants, which were exposed to outdoor conditions during the day and covered (indoors) at night.

2. Sampling and processing

In the pre-plant soil treatment experiment sugarcane samples were sampled six months after treatment. A second sample was taken one year after treatment and was processed into sugar, molasses and bagasse.
In the tests of interline post-emergent directed broadcast treatment sample were taken at 2-3 months and 6 months.
In the tests of post-emergent directed treatment along field edges the interval before harvest in each case was about six weeks. Samples were taken only from the edge of the field.
Selected samples were processed in the Hawaiian Sugar Planters' Association mini-factory which produces a washed raw sugar (washed with water and saturated sugar syrup), molasses, and bagasse fiber.

Processing of mixed sugarcane juice: A portion of mixed sugarcane juice was spiked with 0.107 mg/kg glyphosate. The spiking solution was composed of 1.27 mg of unlabelled glyphosate in the form of the commercial isopropylamine salt and 73.1 µg of ^{14}C-glyphosate-(9.07 mCi/mM). To the mixed solution was added live steam and $Ca(OH)_2$ to a pH of 8.0. The hot limed juice was allowed to settle, the clarified juice drawn off, and a portion evaporated under vacuum to a syrup of 64.5 % solids. The filtered mud solids were analysed for radioactivity and discarded. The sugar syrup was crystallised to raw sugar and

molasses, and the raw sugar was partially refined by a single washing with saturated refined sugar syrup to remove adhering molasses. Since the resulting washed sugar, after centrifuging, showed no detectable radioactivity, no carbon column purification or recrystallisation was carried out.

Processing experiment for sugar refining: The aqueous spiked sugar solution was treated with phosphoric acid and lime, heated, and filtered with Celite 545 filter aid. The clear syrup was then equilibrated with bone char at 80 °C and filtered again with Celite 545. The colorless liquid was evaporated and crystallised to a white sugar and a "refiner's molasses," which is used commercially to add to brown sugars or syrups.

Root absorption: Plant tissue from individual plants were taken at 1, 4, 8, and 12 weeks after treatment. Plant roots were washed before analysis to remove nutrient medium, and were air dried.

Foliar absorption: Samples consisting of individual plants were harvested at 1, 4, 8, and 12 weeks after treatment. Each treated leaf was sectioned to be able to determine residues remaining at the treatment site, as well as those translocated distally and proximally in the same leaf. Untreated portions of the plant were assayed to show movement of labelled residues out of the treated leaves. Although the leaves were green and active at the time of treatment, leaf No. 1 was the top expanded leaf in all cases, they became senescent and detached normally before the experiment was completed. For this reason "fresh" and "dry" weights of these dried leaves may be identical or nearly so. The same condition applies to the roots, which were washed free of soil and air dried before analysis.

3. Analytical procedures

Pre-plant soil treatment, post-emergent directed broadcast treatment and post-emergent directed treatment along field edges: The combined residue of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) that may result from the use of glyphosate in or near sugarcane crops were extracted from the homogenised (Hobart chopper) crop, leafy tops and bagasse with distilled water. Sugar and molasses were dissolved in water. The aqueous extracts were eluted from an appropriate ion exchange resins (AG 1-X8 anion resign in the bicarbonate form) to separate the chemical residues from substances that interfere in subsequent analysis. Darco G-60 was added to the NH_4HCO_3 eluate followed by filtration and evaporation to dryness (50°C, water bath). The remainder was reconstituted in water and further cleaned using a cation exchange resign (AG 5QW-X8). NH_4HCO_3 was added to the aqueous eluates and each fraction was evaporated to dryness.
For the determination of glyphosate and its metabolite AMPA the residues were derivatised with trifluoroacetic anhydride (TFAA) and trifluoroacetic acid (TFA). Samples were analysed using GC-FPD (using the phosphorous mode).
The reported method LOQ for glyphosate and AMPA was 0.05 mg/kg for sugarcane, sugarcane tops and bagasse and 0.5 mg/kg for molasses. However, the recovery experiments at fortification levels between 0.05 mg/kg and 4 mg/kg were often unsatisfactory since most of the recoveries were < 70 %.

Root absorption: Plant tissue from individual plants were analysed for radioactivity after combustion. Aqueous extracts of leaves, containing the largest proportion of the radioactivity with the exception of the roots, were chromatographed on microcrystalline cellulose prekote plates. Extract clean-up consisted of anion exchange on AG1-X8 resin in the bicarbonate form and elution with 0.2 M NH_4HCO_3 and evaporation of the solution to dryness. Two dimensional chromatography separated the parent glyphosate and the metabolite AMPA.

Foliar absorption: Plant preparation and combustion analysis followed the same procedure as in the root absorption study. Aqueous extracts of the treated leaves were chromatographed in the same manner as extracts from root treatment. However, to avoid the swamping effect of excess surface residues, the extracts were prepared from portions of the treated leaves not including the treatment site. The extracts therefore represent translocated residues.

II. Results and discussion

a) Field study and processing study results:

Pre-plant soil treatment, post-emergent directed broadcast treatment and post-emergent directed treatment along field edges:

Samples of sugarcane and its processed products (sugarcane, bagasse, tops and leaves) were extracted with water (sugar and molasses were dissolved in water) and analysed after purification for residues of glyphosate and aminomethylphosphonic acid (AMPA).

The uptake of radioactivity observed in sugar cane following pre-plant treatment to soil at a rate of 11.2 kg a.s./ha, is summarised in the table below. Residues of glyphosate and its metabolite AMPA in sugarcane sampled at 6 months of age were less than 0.05 mg/kg. A second sample at 1 year of age, processed to sugar, molasses, and bagasse showed no detectable residues in any fraction (<0.05 mg/kg, except in molasses : <0.5 mg/kg).

Glyphosate, applied in 2 Hawaiian locations (Oahu and Mauna) at 3.4 or 6.7 kg a.s./ha as a directed broadcast spray to 6 month old sugarcane, resulted in residues of glyphosate which decreased over time. The results showing maximum residue values from each location.

Directly after the application residues of glyphosate ranged between 0.74 and 3.0 mg/kg after application of 3.4 kg a.s./ha and ranged between 1.85 and 6.9 mg/kg after application of 6.7 kg a.s./ha. Over time glyphosate residues decreased and were below 0.05 mg/kg after 165 or 183 days except for one sample with 0.09 mg/kg after 165 days. Residues in processed commodities were <0.05 mg/kg (<0.5 mg/kg for molasses) except one sample with residues of glyphosate of 0.96 mg/kg in molasses and 0.08 mg/kg in sugar.

Residues of AMPA were below the respective LOQ (<0.05 or <0.5 mg/kg (molasses only)) in all samples at all sampling intervals.

Post-emergent treatment: Spray applications were performed at five locations (Florida (2), Louisiana (1) and Hawai (2)) at a rate of 5.6 or 11.2 kg a.s./ha. The results showing maximum residue values from each location of samples taken from the edges.

In the three tests where Roundup was applied normally, i.e., to the ground along the field edge, residues of glyphosate and its metabolite were all less than 0.05 mg/kg in the sugarcane. In Florida (Pahokee and Clewiston), where the cane foliage was purposely sprayed to maximize residues, residues of glyphosate ranged between 0.14 to 0.24 mg/kg after application of 5.6 kg a.s./ha and between 0.28 to 0.34 mg/kg after 11.2 kg a.s./ha, while residues of AMPA were <0.05 mg/kg in all cases (except molasses with a method LOQ of 0.5 mg/kg).

Residues of glyphosate and its metabolite did not appear or concentrate in any of the processed fractions from applications where residues are <0.05 mg/kg in or on the sugarcane.

In the cases (Pahokee and Clewiston) where deliberate contact of foliage was made, residues of glyphosate and its metabolite AMPA in the bagasse and raw sugar were less than the amount present in the sugarcane. Where residues in the cane were present residues of glyphosate concentrated into molasses (1.6 to 4.5 mg/kg). Analyses of tops and leaves from the two sites where the foliage was purposely sprayed had higher residues amounting up to 2.0 mg/kg at 11.2 kg a.s./ha spray rate.

An additional processing experiment of mixed sugarcane juice performed with ^{14}C-labelled glyphosate showed that 36 % of the original glyphosate was removed in the liming solids. These consist of lime salts and coagulated colloids, bits of fibre, and soil which was not removed in the cane washer. Most of the radioactive residues which were in the clarified juice found their way into the molasses fraction. The molasses film on raw sugar carried some portion of the radioactivity, and minor portions of molasses were occluded in the sugar crystal. Refining removed this minor residue by adsorption on the bone char resulting in no radioactivity detectable in refined sugar.

To demonstrate the fate of any residue that may appear in raw sugar during refining operation an additional experiment (processing of sugar into refined sugar) was performed. After spiking of raw sugar with an aqueous solution of labelled and unlabelled glyphosate (1 mg/kg), the main part of the radioactivity remained in the bone char (84 %). Further 6 % were recovered in the filter cake from liming, 3 % in refined molasses (<0.5 mg/kg) and 7 % remained unaccountable, while radioactive residues in refined sugar were <0.05 mg/kg.

Table 6.2.1-114: Residues in sugarcane, bagasse, molasses and sugar sampled/processed after treatment with glyphosate following pre-plant application to soil at rates equivalent to 11.2 kg a.s./ha (results expressed in mg/kg; highest residue value of replicates) - non-labelled glyphosate

Location	Kunia				Oahu			
Sample	Sugar-cane	Bagasse	Molasses	Sugar	Sugar-cane	Bagasse	Molasses	Sugar
DALT	195				354			
Glyphosate	<0.05	-	-	-	<0.05	<0.05	<0.5	<0.05
AMPA	<0.05	-	-	-	<0.05	<0.05	<0.5	<0.05

DALT days after last treatment

Table 6.2.1-115: Residues in sugarcane, bagasse, molasses and sugar sampled/processed after treatment with glyphosate following interline directed broadcast spraying at rates equivalent to 3.4 or 6.7 kg a.s./ha (results expressed in mg/kg; highest residue value of replicates) - non-labelled glyphosate

Location	Oahu											
Sample	Sugarcane			Bagasse			Molasses			Sugar		
DALT	0	91	183	0	91	183	0	91	183	0	91	183
Application rate 0 kg a.s./ha												
Glyphosate	0.20	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
AMPA	<0.05	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
Application rate 3.4 kg a.s./ha												
Glyphosate	0.74	0.13	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
AMPA	<0.05	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
Application rate 6.7 kg a.s./ha												
Glyphosate	1.85	0.11	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
AMPA	<0.05	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
Location	**Mauna**											
Sample	**Sugarcane**			**Bagasse**			**Molasses**			**Sugar**		
DALA	**0**	**73**	**165**	**0**	**73**	**165**	**0**	**73**	**165**	**0**	**73**	**165**
Application rate 0 kg a.s./ha												
Glyphosate	0.15	0.03	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
AMPA	< 0 .05	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
Application rate 3.4 kg a.s./ha												
Glyphosate	3.0	0.40	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
AMPA	<0.05	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05
Application rate 6.7 kg a.s./ha												
Glyphosate	6.9	0.48	0.09	-	-	<0.05	-	-	0.96	-	-	0.08
AMPA	<0.05	<0.05	<0.05	-	-	<0.05	-	-	<0.5	-	-	<0.05

DALT days after last treatment

Table 6.2.1-116: Residues in sugarcane, bagasse, molasses and sugar sampled/processed after treatment with glyphosate to the ground along the field edges at rates equivalent to 5.6 or 11.2 kg a.s./ha (results expressed in mg/kg; highest residue value of replicates) - non-labelled glyphosate

Location	Pahokee				
Sample	**Sugarcane**	**Bagasse**	**Molasses**	**Sugar**	**Tops and leaves**
DALT	**40**				
Application rate 0 kg a.s./ha					
Glyphosate	<0.05	<0.05	-	-	0.1
AMPA	<0.05	<0.05	-	-	<0.05
Application rate 5.6 kg a.s./ha					
Glyphosate	0.14	0.09	3.0	0.08	0.30
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 11.2 kg a.s./ha					
Glyphosate	0.28	0.14	4.5	0.13	1.9
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Location	**Clewiston**				
Sample	**Sugarcane**	**Bagasse**	**Molasses**	**Sugar**	**Tops and leaves**
DALT	**44**				
Application rate 0 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	0.20
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 5.6 kg a.s./ha					
Glyphosate	0.24	0.21	1.6	<0.05	1.0
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 11.2 kg a.s./ha					
Glyphosate	0.34	0.25	3.0	0.14	2.0
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Location	**Franklin**				
Sample	**Sugarcane**	**Bagasse**	**Molasses**	**Sugar**	**Tops and leaves**
DALT	**47**				
Application rate 0 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	-	<0.05
AMPA	<0.05	<0.05	<0.5	-	<0.05
Application rate 5.6 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 11.2 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Location	**Waialua**				
Sample	**Sugarcane**	**Bagasse**	**Molasses**	**Sugar**	**Tops and leaves**
DALT	**42**				
Application rate 0 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 5.6 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 11.2 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05

Table 6.2.1-116: Residues in sugarcane, bagasse, molasses and sugar sampled/processed after treatment with glyphosate to the ground along the field edges at rates equivalent to 5.6 or 11.2 kg a.s./ha (results expressed in mg/kg; highest residue value of replicates) - non-labelled glyphosate

Location	Oahu				
Sample	**Sugarcane**	**Bagasse**	**Molasses**	**Sugar**	**Tops and leaves**
DALT	44				
Application rate 0 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 5.6 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05
Application rate 11.2 kg a.s./ha					
Glyphosate	<0.05	<0.05	<0.5	<0.05	<0.05
AMPA	<0.05	<0.05	<0.5	<0.05	<0.05

DALT days after last treatment

Table 6.2.1-117: Residues in sugarcane juice processed fractions after spiking with ^{14}C-labelled glyphosate

Process	**mg/kg**
Mixed Juice	0.107
Liming process	
Solids	0.89
Clarified Juice	0.083
Evaporation	
Condensate water	n.d.
Syrup	0.45
Crystallisation/centrifugation	
Molasses	0.84
Raw sugar	0.22
Washing with saturated syrup	
Partially refined sugar	n.d.

n.d. no radioactivity detectable

b) Root and foliar absorption experiments:

The results of the root absorption experiment showed that ^{14}C-methane-glyphosate was absorbed from nutrient culture solution into sugarcane roots over time to an amount of 3 % after 1 week to 13 % after 12 weeks. During this period 81 % of the applied radioactivity had disappeared, probably as $^{14}CO_2$. Of the 13 % absorbed ^{14}C, 8 % remained fixed in the roots; the stalk carried 1 % and the leaves 3 %. Concentrations of 5 mg/kg (dry basis) occurred in roots, apical meristem, and the unexpanded leaf spindle, the regions of high metabolic growth activity but not of photosynthesis or sugar storage. Results are summarised in the following table.

Thin layer chromatography showed the residues to be in both the metabolite and parent forms with a possible predominance of metabolite aminomethylphosphonic acid (AMPA).

Table 6.2.1-118: Distribution of radioactivity in sugarcane after root absorption of ^{14}C-glyphosate

Root absorption								
	1 week		4 weeks		8 weeks		12 weeks	
	% AR	mg/kg DM	% AR	mg/kg DM	% AR	mg/kg DM	% AR	mg/kg DM
Green leaves								
Expanded after treatment	np	np	0.47	0.55	1.30	0.96	2.09	0.05
Present at treatment	0.13	0.16	0.27	0.31	0.21	0.36	np	np
Spindle (unexpanded)	0.02	0.27	0.15	1.92	0.32	2.57	0.35	5.01
Apical meristem	<0.01	0.62	0.03	2.16	0.05	5.05	0.05	4.94
Dry leaf trash	np	np	0.56	1.53	0.72	1.93	0.55	0.96
Stalk	0.07	0.32	0.60	0.59	1.17	0.75	1.16	0.45
Roots	2.03	9.23	3.86	5.67	7.87	8.34	7.85	5.31
Vegetative seedpiece	0.53	1.29	0.56	1.69	0.35	0.55	0.21	0.90
Secondary sugars	np	np	0.36	0.91	np	np	np	np
Whole plant	**2.79**	**1.58**	**6.90**	**1.46**	**13.89**	**2.15**	**12.55**	**1.63**
Nutrient culture solution	**71.4**		**47.4**		**21.9**		**6.53**	
Unaccountable	**25.8**		**45.8**		**64.2**		**80.6**	

AR = applied radioactivity
np = not present
DM = dry matter

In the experiment investigating the foliar absorption of ^{14}C-glyphosate, 18.6 % of residues applied were determined after 12 weeks. The treated leaves retained 4.6 %, roots accumulated 4.6 % and the primary stalk accumulated 4.3 %. Secondary suckers carried 3.2 % and the untreated leaves and spindle about 1.5 %.
Accumulation took place in untreated younger leaves (3.2 mg/kg dry basis), spindle (4.0 mg/kg dry basis), primary apical meristem (5.8 mg/kg dry basis), stalk (2.6 mg/kg dry basis) and roots (0.9 mg/kg dry basis). Results are summarised in the table below.
There was evidence of considerable translocation within the plant, probably in the phloem, major translocated species is the parent compound, with only a minor contribution of the metabolite.

Table 6.2.1-119: Distribution of radioactivity in sugarcane after foliar application of ^{14}C-glyphosate

Foliar absorption								
	1 week		4 weeks		8 weeks		12 weeks	
	% AR	mg/kg DM	% AR	mg/kg DM	% AR	mg/kg DM	% AR	mg/kg DM
Treated leaf 1								
Treated area	24.6	773	0.36	34.2	0.27	15.3	0.36	8.0
Distal portion	0.39	15.5	0.28	25.7	0.06	5.4	0.05	2.6
Proximal portion	1.82	6.6	0.79	2.6	0.50	3.2	0.71	2.3
Treated leaf 2								
Treated area	2.57	109	0.84	14.2	0.49	14.8	0.52	9.9
Distal portion	0.44	29.3	0.16	5.9	0.07	2.4	0.05	1.4
Proximal portion	0.90	2.7	0.36	1.2	0.92	3.0	1.90	nr
Treated leaf 3								
Treated area	2.01	65.2	0.50	8.4	0.65	27.2	0.44	13.9
Distal portion	0.32	13.3	0.10	2.7	nr	4.0	0.03	1.2
Proximal portion	0.87	3.2	0.24	1.0	0.83	2.9	0.19	0.54
Treated leaf 4								
Treated area	1.69	66.2	1.63	46.4	0.24	12.8	0.22	7.8
Distal portion	0.80	26.7	0.17	4.2	0.01	1.4	0.03	1.4

Table 6.2.1-119: Distribution of radioactivity in sugarcane after foliar application of ^{14}C-glyphosate

Foliar absorption								
	1 week		4 weeks		8 weeks		12 weeks	
Proximal portion	1.10	5.0	0.20	0.93	0.18	0.84	0.07	0.28
Treated leaves (total)	***37.51***		***5.63***		***>4.22***		***4.57***	
Untreated distal								
Leaves	0.25	1.1	0.77	2.5	0.80	5.8	1.02	3.2
Untreated proximal								
Leaves	1.97	5.6	np	np	np	np	np	np
Spindle	0.94	6.4	0.33	9.9	0.67	11.7	0.51	4.0
Apical meristem	0.53	20.1	0.12	8.3	0.39	10.6	0.13	5.8
Dry leaf trash	3.39	2.4	0.10	0.21	0.06	0.19	0.24	0.31
Stalk	4.10	8.1	1.80	2.8	2.71	3.6	4.29	2.6
Roots	1.27	1.6	6.65	1.6	4.37	1.5	4.59	0.88
Vegetative								
Seedpiece	0.23	0.46	0.18	0.40	0.17	0.46	0.07	0.20
Secondary suckers								
Leaves	np	np	1.60	0.38	1.75	0.29	1.69	0.18
Stalk	np	np	2.04	0.37	1.55	0.35	1.44	0.12
Spindle	2.49	1.26	0.09	0.54	0.14	0.54	0.06	0.19
Apical meristem	-	-	0.07	2.6	0.02	1.0	0.01	0.44
Secondary suckers (total)	***2.49***		***3.80***		***3.46***		***3.20***	
Whole plant	**52.1**	**8.82**	**19.78**	**2.07**	**17.0**	**1.0**	**18.6**	**0.54**
Unaccountable	**47.3**		**80.2**		**83.0**		**82.8**	

AR = applied radioactivity
np = not present
nr = value not readable in the report
DM = dry matter
Values in *italics* were calculated during dossier compilation

C. Storage stability

Storage intervals for frozen samples and extracts are not reported. No information on storage stability is reported.

D. Degradation pathway

Based on the experiments and results of this study no pathway can be proposed. Please refer to the overall pathway of glyphosate in plants at the end of this chapter.

III. Conclusions

The non-labelled active substance was applied either before pre-planting (11.2 kg a.s./ha), as an interline broadcast treatment (3.4 or 6.7 kg a.s./ha) or post-emergent treatment along the field edges (5.6 or 11.2 kg a.s./ha). The results from the pre-plant soil treatment indicate the uptake of residues from soil was low. Residues of glyphosate and its metabolite were <0.05 mg/kg in all samples (sugarcane and processed products (bagasse, molasses and refined sugar)).

Immediately after interline broadcast treatment, the residues of glyphosate in sugarcane ranged between 0.74 and 3.0 mg/kg after application of 3.4 kg a.s./ha and between 1.85 and 6.9 mg/kg after application of 6.7 kg a.s./ha. Over time glyphosate residues decreased and were below 0.05 mg/kg after 165 or 183 days except for one sample with 0.09 mg/kg after 165 days. Residues in processed commodities were <0.05 mg/kg (<0.5 mg/kg for molasses) except one sample with residues of glyphosate of 0.96 mg/kg in molasses and 0.08 mg/kg in sugar.

Residues of AMPA were below the respective LOQ (<0.05 or <0.5 mg/kg (molasses only)) in all samples at all sampling intervals.

In the three tests where Roundup was applied to the ground along the field edge according to normal practice, the residues of glyphosate and its metabolite AMPA were all <0.05 mg/kg in the sugarcane. In the two trials where the cane foliage was purposely sprayed to maximize residues, the residues of glyphosate ranged between 0.14 to 0.24 mg/kg after application of 5.6 kg a.s./ha and between 0.28 to 0.34 mg/kg after application of 11.2 kg a.s./ha, while the residues of AMPA in the sugarcane were below the method LOQ in all cases.

Processing of sugar cane following commercial practice, as well as processing of sugar cane juice indicated that glyphosate residues and its metabolite do not appear or concentrate in any of the processed fractions with the exception of molasses where radioactive residues increased. During processing of raw sugar into refined sugar the main part of the radioactivity remained in the bone char with lower amounts in the filter cake from liming and in refined molasses. The radioactive residues in refined sugar were <0.05 mg/kg.

The results of the root absorption experiment showed that ^{14}C-methane-glyphosate was absorbed from nutrient culture solution into sugarcane roots to an amount of 13 % after 12 weeks. Of the 13 % absorbed ^{14}C, 8 % remained fixed in the roots; the stalk carried 1 % and the leaves 3 %. 81 % of the applied radioactivity had disappeared, probably as $^{14}CO_2$.
Concentrations of 5 mg/kg (dry basis) occurred in roots, apical meristem, and the unexpanded leaf spindle, the regions of high metabolic growth activity but not of photosynthesis or sugar storage.
Thin layer chromatography showed the residues to be in both the metabolite and parent forms with a possible predominance of metabolite aminomethylphosphonic acid (AMPA).

In the experiment investigating the foliar absorption of ^{14}C-glyphosate, 18.6 % of residues applied were determined after 12 weeks. The treated leaves retained 4.6 %, roots accumulated 4.6 % and the primary stalk accumulated 4.3 %. Secondary suckers carried 3.2 % and the untreated leaves and spindle about 1.5 %. Accumulation took place in untreated younger leaves, spindle, primary apical meristem, stalk and roots. There was evidence of considerable translocation within the plant, probably in the phloem. The major translocated species was glyphosate, with only a minor contribution of the metabolite.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study consists of several experiments, which may be considered as supportive in context of metabolism and residue behaviour of glyphosate and its residues in sugarcane. It was previously evaluated at EU level.
The different experiments were not performed under GLP. The experiments performed mainly do not follow the current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 (field residue studies were performed using non-radiolabelled glyphosate and analysis for known analytes (glyphosate and AMPA); processing of sugarcane juice into refined sugar after spiking of radiolabelled glyphosate and spiking of raw sugar by a mixture of radiolabelled an non-labelled glyphosate followed by processing into refined sugar. Both processing experiments were analysed for the fate of radioactive residues only. The analytical method used yielded low recoveries (< 70 %). The root and foliar absorption experiments which were performed using radiolabelled glyphosate do not follow the current guideline in major terms: the radiochemical purity of the test item is not clearly specified, physical facility and environmental conditions insufficiently described, developmental stages of the crop at application and harvesting are not reported, the sampled RACs (raw agricultural commodities) were not appropriate, no sugarcane sample investigated, radioactive residues are expressed in % of applied activity and TRR in mg/kg dry matter, no release and characterisation and/or identification was attempted, no details on radioactive counting data, no description of conditions and duration of storage of samples).
However the different experiments give general information on the uptake of glyphosate into plant after

different application scenarios, information on behaviour of glyphosate related residues during sugar processing and on translocation of glyphosate-related residues within the plant.
Therefore, the study is considered supportive for uses of glyphosate in/on sugar cane and similar crops.

Assessment and conclusion by RMS:

Genetically modified plants

CP4 EPSPS, CP4 EPSPS and GOX modification

Root and tuber vegetables

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/018
Report author	[redacted]
Report year	2000
Report title	Metabolism of Glyphosate in Roundup Ready Sugarbeet
Report No	861W
Document No	MSL-16247
Guidelines followed in study	EC Directive 91/414/EEC EPA Residue Chemistry Test Guidelines, OPPTS 860.1300 - Nature of the Residue - Plants, Livestock
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • The radioactive balances for sugar beet tops extractions were below 90 % (total sum recovered 86.29 and 88.46 % of TRR for metabolite characterisation / identification and 78.9 and 81.8 % of TRR for storage stability)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The nature of the residues in plants following the use of glyphosate was studied in sugar beet line 77, which was modified to express CP4 EPSPS. N-(phosphonomethyl)glycine (glyphosate), labelled in the phosphonomethyl-moiety with ^{12}C, ^{13}C or ^{14}C, respectively, was applied either pre-emergent at a target rate of 0.9 kg glyphosate acid equivalents/ha or twice post-emergent at a target rate of 1.08 kg glyphosate acid equivalents /ha per treatment.

TRRs determined after pre-emergent treatment were very low (0.006 mg/kg / 0.005 mg/kg in tops and 0.009 mg/kg / 0.008 mg/kg in roots).

After post-emergent treatment, TRRs were much higher (sugar beet tops 3.561 mg/kg / 3.437 mg/kg and roots 1.256 mg/kg / 1.396 mg/kg).

Glyphosate was the major component of the residue in both sugar beets tops and roots, accounting for 79.65 % (2.74 mg/kg) and 95.31 % (1.33 mg/kg) of the TRR, respectively. The metabolite aminomethylphosphonic acid (AMPA) accounted for 1.84 % (0.06 mg/kg) and 3.79 % (0.05 mg/kg) of the TRR in tops and roots, respectively.

Trace levels of glyphosate/AMPA acetylated conjugates (0.80 % of the TRR (0.03 mg/kg) in tops and 0.55 % of the TRR (0.01 mg/kg) in roots) and small amounts of ^{14}C-labelled natural products (1.38 % of the TRR (0.05 mg/kg) in tops and 1.22 % of the TRR (0.02 mg/kg) in roots) were also found after post-emergent treatment.

The unextracted (bound) radioactive residues in the post-emergence sugar beet tops and roots were 1.81 % (0.062 mg/kg) and 1.32 % (0.018 mg/kg) of the TRR, respectively. Acid hydrolysis of extracted tops released the majority of the bound radioactivity (1.53 % of the TRR, 0.053 mg/kg).

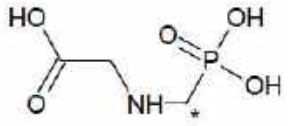
In summary it can be concluded that uptake of glyphosate from soil is very low in sugar beets; very low TRRs (0.006 mg/kg / 0.005 mg/kg in tops and 0.009 mg/kg / 0.008 mg/kg in roots) were found after pre-emergent application. After post-emergent application residues were present in treated parts of the plants and a translocation into the roots is observed. In CP4 EPSPS sugar beets the metabolism of glyphosate was limited with unchanged parent posing the major residue ~75 % TRR in all matrices.
The results of this study demonstrate that the metabolism of glyphosate in sugar beet containing the CP4 EPSPS gene is the same as that found in other tolerant and non-tolerant crops. Glyphosate is slowly degraded to aminomethylphosphonic acid (AMPA), which is the primary plant metabolite. AMPA is further metabolised to low levels of conjugates. In addition to conjugation, the results indicated that glyphosate is further degraded to one carbon fragments that become incorporated into natural products and plant constituents.

I. Materials and Methods

A. Materials

Test Material: N-(phosphonomethyl)glycine; mixture of
a) N-(phosphono-^{14}C-methyl)glycine (53.24 mg)
b) N-(phosphono-^{13}C-methyl)glycine (123.1 mg)
c) N-(phosphono-^{12}C-methyl)glycine (103.3 mg)
Code Nr. C-2420.2

Chemical structure:



* Position of label

Radiochemical purity: a) Batch No. C-1750.22; 97.7 % radiochemical purity
b) Batch No. GLP-9907-9741-A; 98 % chemical purity
c) Batch No. GLP-9606-7189-A; 99.9 % chemical purity

Specific activity: a) 39.36 mCi/mmol (8.61 MBq/mg)
specific activity of mixture C-2420.2: 7.09 mCi/mmol (93080 dpm/µg or 1.55 MBq/mg, respectively)

CAS No: 1071-83-6

Log $P_{o/w}$: -3.2

Test system:

Soil:	Loamy sand (*Arnold Loamy Sand*; pH: 6.1; cation exchange capacity: 11.4 meq./100 g; bulk density: 1.31 g/cm^3; organic matter: 2.2 %; sand: 83 %; silt: 10 %; clay: 7 %; textural class (USDA): loamy sand)
Crop:	Roundup-Ready® sugarbeet line 77 (variety HM Empire RR, lot no. 57010-40101002, modified to express CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase))
Botanical name:	*Beta vulgaris subsp. vulgaris convar. vulgaris var. altissima*
Crop part(s):	Tops, roots

B. Study design

1. In-life phase

The in-life phase of this study was conducted by PTRL West, Inc. Richmond, CA 94806, USA. The sugar beet plants were grown in confined plots at Plant Sciences, Inc. in Watsonville, CA.
The test substance consisted of an isotopic mixture of ^{13}C-glyphosate with glyphosate that was ^{13}C-, ^{14}C-labelled in the phosphonomethylene carbon (N-(phosphono-^{13}C-methyl)glycine and N-(phosphono-^{14}C-methyl)glycine). The specific activity of the resulting radiolabelled test substance was 7.09 mCi/mmol (93080 dpm/µg or 1.55 MBq/mg, respectively) and the radiochemical and chemical purities were 98.2 % and 98 %, respectively. For all applications, glyphosate was applied as the isopropylamine salt formulated as MON 52276 herbicide (equivalent to Roundup® Ultra herbicide in US). The formulation was produced by mixing a portion of the radiolabelled test substance stock solution (45.547 g of solution containing 244.815 mg of glyphosate acid) with 87.5 mg of isopropylamine and 126.3 mg of MON 8153 surfactant. The formulated application solution was shown to contain a concentration of 5.32 mg of ^{14}C-glyphosate acid/g of solution (4.95×10^8 dpm/g, or 8.25 MBq/g). The radiochemical purity of the application solution was found to be 98.2 %. Application rates in kg a.s./ha are expressed as glyphosate acid equivalents.
Two ^{14}C-treated test plots and two untreated control plots were used in this study. The crops were grown in above ground soil containers outdoor inside a screened enclosure.
One ^{14}C-treated test group received a pre-emergence application at a target rate of 0.9 kg a.s./ha one day after sowing. The second ^{14}C-treated test group received two sequential post-emergence applications of the test substance, first at a target rate of 1.08 kg a.s./ha when the majority of plants were at the 2-4 true leaf stage (BBCH 12-14), 35 days after planting. The second post-emergence application was at a target rate of 1.08 kg a.s./ha when the plants were at the 12-14 leaf stage (BBCH 19), 68 days after planting.
A pre-emergent treatment rate of 0.9 kg a.s./ha and a post-emergent rate of 1.08 kg a.s./ha corresponds to 1.2 kg glyphosate isopropylamine salt/ha and 1.4 kg glyphosate isopropylamine salt/ha and 2.5 L/ha and 3.0 L/ha of MON 52276 herbicide, respectively.

The achieved application rates were 0.93 kg a.s./ha (65.28 mg glyphosate acid equivalents/plot, corresponding to 6075965625 dpm or 101.27 MBq per plot, respectively) for the pre-emergent treatment, and 1.15 kg a.s./ha (80.32 mg glyphosate acid equivalents/plot, corresponding to 7476353471 dpm or 124.61 MBq per plot, respectively) and 1.08 kg a.s./ha (75.45 mg glyphosate acid equivalents/plot, corresponding to 7022600487 dpm or 117.04 MBq per plot, respectively) for the two post-emergent treatments, respectively.

For the pre-emergence application, the soil surface of the plot was sprayed uniformly with the dosing solution one day after planting. For the post-emergence applications, weighed portions of the dosing solutions were sprayed using several passes directed towards the plant canopy.
During post-emergence applications the soil between the rows of sugar beet was covered with plastic-backed absorbent paper to minimise the contact between the soil and ^{14}C-glyphosate.

Stability of the test substance at application was established before each application by a purity check of the formulated test substance and after each application using a retain sample of the spray solution. The purity of the test substance varied from 97.2 % to 97.7 %.

2. Sampling

Sugar beet was sampled from the control and ^{14}C-treated plots at the final mature harvest stage, at 158 days after treatment for the pre-emergent treatment and at 91 days after the last of two post emergent treatments.
At the time of harvest the mature crop was separated into tops and roots. The root crown was combined with the tops as per commercial practice. Samples were stored frozen until shipment on dry ice to PTRL. Crop samples ground with dry ice at PTRL were shipped with dry ice to Monsanto Company. Samples were received in good condition, with dry ice present.

3. Analytical procedures

Grinding of plant materials, combustion and liquid scintillation counting (LSC), as well as the storage stability study were performed by PTRL West, Inc. Extraction, characterisation and identification of residues in the processed samples was performed by Monsanto.

Samples were ground in the presence of dry ice using a Hobart Cutter Mixer (HCM) to a fine consistency. The total radioactive residues in sugar beet raw agricultural commodity were determined by combustion analysis of the ground samples prior to shipment under frozen conditions to the Monsanto laboratory.
At the time of sample extraction at Monsanto, combustions were conducted using a Packard System 387 Automated Sample Preparation Unit. All reported mg/kg residue levels determined for extraction and characterisation / identification of the residues are based on the latter determinations.

Moisture content of ground sugar beet roots and tops was determined by weighting before and after heating at approximately 110 °C for 24 hours.

Portions of ground treated RAC were extracted four times with water with the exception of tops and roots harvested from the pre-emergence group which were extracted three times. The resulting extracts were weighed and analysed by LSC. Portions of the extracted plant material after air drying were analysed by combustion and LSC for bound residue determination.

The aqueous extracts of post-emergence sugar beet commodities were each fractionated by a sequence of chromatographic separations. The first step of isolation scheme used a Chelex® 100 column (iron form) to separate phosphonate-containing compounds that are bound to the resin from non-retained non-phosphonate-containing compounds.
The retained phosphonate-containing compounds, in the form of their iron salts, were then eluted from the Chelex® column with hydrochloric acid. Iron was removed from the eluate by passage through AG 1-X8 anion exchange resin (chloride form). The phosphonate-containing compounds were then separated on a cation exchange column with AG 50W-X8 resin (hydrogen form) to afford three main fractions: designated glyphosate fraction, AMPA fraction, and conjugate fraction.

Aqueous extracts, concentrates, combustion solutions and HPLC fractions were analysed by Liquid Scintillation Counting (LSC).

High performance liquid chromatography systems employed in this study were strong anion exchange chromatography (SAX HPLC), cation exchange chromatography (CX HPLC), amino column chromatography (Amino HPLC), reversed phase paired ion chromatography (RP-PIC HPLC) and reversed phase chromatography (RP HPLC).
The primary method of radioactive detection (HPLC/LSC) consisted of fraction collection of the HPLC eluate with subsequent quantitation of the fractions by LSC. A second method (HPLC/RAD) applied a Flo-One radioactive flow detector (RAD).

The nature of the radioactive residues was determined by co-chromatography with authentic ^{14}C-labelled standards. For determining HPLC retention times and for the preparation of derivatives as a reference for MS analyses, pure standards of glyphosate and aminomethylphosphonic acid were used.

In addition to reference substances, ratios of ^{12}C and ^{13}C were used to identify incorporated glyphosate residues or degradation products by mass spectroscopy.

Gas Chromatography/Electron Impact/Mass Spectrometry (GC/EI/MS) and RP HPLC/RAD were performed after derivatisation with trifluoroethanol/trifluoroacetic anhydride. Glyphosate and AMPA were identified by mass spectral analysis of their ester derivatives.

Liquid chromatography coupled with mass spectrometry was applied for analysis of the test substance and to control the results of the derivatisation reaction with trifluoroethanol/trifluoroacetic anhydride.

II. Results and discussion

A. Total radioactive residues (TRRs)

Following the first post-emergent treatment at the 2 - 4 true leaf stage, the treated plants looked similar to those in the control except for a few plants which appeared less vigorous. These plants were removed at the time of the next thinning. This weakness of a few plants could have been due to a minor degree of phytotoxicity. These symptoms were not observed following the second post-emergent application when plants were at the 12-14 leaf stage. During the course of the study control and treated plots developed in a similar manner.
The total radioactive residue (TRR) in sugar beet tops and roots are summarised in Table 6.2.1-120. TRRs in treated sugar beet tops and roots were determined by PTRL prior to shipment to Monsanto and also at the Monsanto laboratory, while determination of TRRs in untreated sugar beet commodities was performed only at PTRL.

The highest TRR values were detected in sugar beet tops (3.561 mg/kg / 3.437 mg/kg) and roots (1.256 mg/kg / 1.396 mg/kg) treated post-emergence. TRRs for sugar beets treated pre-emergence were 0.006 mg/kg / 0.005 mg/kg (tops) and 0.009 mg/kg / 0.008 mg/kg (roots). There was very limited uptake of $^{14}CO_2$ by the control plants. Control TRR values for pre-emergent treatment were <0.001 mg/kg for the tops (e.g. radioactivity < twice the background) and 0.002 mg/kg for the roots. Control TRR values for post-emergent treatment were <0.001 mg/kg for the tops and 0.001 mg/kg for the roots.

Table 6.2.1-120: Total radioactive residues in sugar beet commodities

Sample description	DALT	TRR determined at PTRL (mg eq./kg)	TRR determined at Monsanto laboratory (mg eq./kg)
Single pre-emergence treatment at 0.9 kg a.s./ha			
Untreated tops	-	<0.001	n.a.[1]
Pre-emergence tops	158	0.006	0.005
Untreated roots	-	0.002	n.a.[1]
Pre-emergence roots	158	0.009	0.008
Two post-emergence treatments at 1.08 kg a.s./ha			
Untreated tops	-	<0.001	n.a.[1]
Post-emergence tops	91	3.561	3.437
Untreated roots	-	0.001	n.a.[1]
Post-emergence roots	91	1.256	1.396

DALT Days after last treatment
TRR Total radioactive residue, expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents
[1] = n.a.: Not applicable. Untreated control plants were not combusted at Monsanto since they were not extracted.

B. Extraction and characterisation of residues

Only sugar beet roots and tops from the treated plots were extracted and analysed. The TRRs in control samples were less than 0.01 mg/kg and therefore these samples were neither analysed nor extracted.
All reported mg/kg residue levels determined for extraction and characterisation / identification of the residues are based on the TRR values determined at the Monsanto laboratory; TRRs determined by PTRL are presented in the table above only for the sake of completeness.

Table 6.2.1-121 summarises the results for extraction of treated sugar beet roots and tops. 158 days after pre-emergence treatment, 59.22 % of the TRR in sugar beet tops (0.003 mg/kg) and 85.56 % of the TRR in sugar beet roots (0.007 mg/kg) were extractable with water.
Unextractable residues accounted for 49.99 % of the TRR (0.003 mg/kg) in sugar beet tops and for 20.43 % of the TRR (0.002 mg/kg) in sugar beet roots. Recovery was 109.21 % of the TRR in sugar beet tops and 105.99 % of the TRR in sugar beet roots (0.006 mg/kg and 0.009 mg/kg, respectively).
The aqueous extracts from the pre-emergence treatment RAC were not analysed further due to their low radioactivity levels (<0.01 mg/kg).

91 days after the last of two post-emergent treatments, 86.65 % of the TRR in sugar beet tops (2.978 mg/kg) and 103.30 % of the TRR in sugar beet roots (1.442 mg/kg) were extractable with water.
Unextractable residues accounted for 1.81 % of the TRR (0.062 mg/kg) in sugar beet tops and for 1.32 % of the TRR (0.018 mg/kg) in sugar beet roots.
Recovery was 88.46 % of the TRR in sugar beet tops and 104.62 % of the TRR in sugar beet roots (3.040 mg/kg and 1.460 mg/kg, respectively).

Aqueous extracts of sugar beet tops and roots from post-emergent treatment were analysed by strong anion exchange (SAX HPLC) and cation exchange (CX HPLC) high performance liquid chromatography.
The nature and magnitude of radioactivity found in tops and roots extracts with both HPLC systems are summarised in the table below.
In tops extracts, glyphosate was found at 78.85 % of the TRR (2.71 mg/kg) with SAX HPLC and at 80.45 % of the TRR (2.77 mg/kg) with CX HPLC. The mean of both determinations was 79.65 % of the TRR (2.74 mg/kg). AMPA was found at 1.84 % of the TRR (0.06 mg/kg) with CX HPLC; with SAX HPLC, AMPA was not resolved. Glyphosate/AMPA acetylated conjugates were detected with SAX HPLC at 0.80 % of the TRR (0.03 mg/kg) and were not resolved on CX HPLC. The amount of natural products was calculated from SAX HPLC data (natural product plus AMPA peak) and CX HPLC data (AMPA peak) to be 1.38 % of the TRR (0.05 mg/kg).

In roots extracts, glyphosate was found at 95.04 % of the TRR (1.33 mg/kg) with SAX HPLC and at 95.58 % of the TRR (1.33 mg/kg) with CX HPLC. The mean of both determinations was 95.31 % of the TRR (1.33 mg/kg). AMPA was found at 3.79 % of the TRR (0.05 mg/kg) with CX HPLC; with SAX HPLC, AMPA was not resolved. Glyphosate/AMPA acetylated conjugates were detected with SAX HPLC at 0.55 % of the TRR (0.01 mg/kg) and were not resolved on CX HPLC. The amount of natural products was calculated from SAX HPLC data (natural product plus AMPA peak) and CX HPLC data (AMPA peak) to be 1.22 % of the TRR (0.02 mg/kg).

The aqueous extract of sugar beet roots was further characterised by fractionation and clean-up applying a sequence of Chelex®, anion exchange and cation exchange resins as described above.
Separation of phosphonate-containing compounds from non-phosphonate-containing compounds on Chelex® resulted in 1.54 % of the TRR (0.021 mg/kg) in the water rinses, which represent non-phosphonate compounds not bound to the resin. While the 0.1 N HCl eluate contained no radioactivity, elution with 6 N HCl and collection in three fractions recovered 95.71 % of the TRR (1.336 mg/kg) in fractions # 1 and # 2, containing the phosphonate-containing compounds.
After purification of the combined fractions #1 and #2 by anion exchange chromatography and separation into fifty fractions by cation exchange chromatography, the radioactivity was confined to three areas, fractions # 5-6 (conjugate fraction, 0.81 % of the TRR, 0.011 mg/kg), fractions #7-11, (glyphosate fraction, 84.89 % of the TRR, 1.185 mg/kg) and fractions #36-41 (AMPA fraction, 3.32 % of the TRR, 0.046 mg/kg).
AMPA and glyphosate fractions were further characterised by HPLC. Because of the low radioactivity level contained in fractions # 5-6 (roots conjugate fraction), this fraction was not further characterised. SAX HPLC/RAD showed that the glyphosate fraction contained one major peak which co-chromatographed with ^{14}C-glyphosate reference standard. Eluates containing the AMPA fraction were analysed by SAX HPLC/RAD. The AMPA fraction contained one component, which co-chromatographed with ^{14}C-AMPA reference standard.

A large scale extraction and fractionation of sugar beet tops from the post-emergence test group was performed to isolate sufficient quantities of the metabolites for identification and characterisation of AMPA and glyphosate by mass spectroscopy. 82.28 % (2.828 mg/kg) of the TRR were extractable with water. After concentration and centrifugation, three fractions of radioactivity in an aliquot of the concentrated extract were characterised as natural products plus AMPA (2.95 % of the TRR, 0.10 mg/kg), glyphosate (75.93 % of the TRR, 2.61 mg/kg) and glyphosate/AMPA acetylated conjugates (0.69 % of the TRR, 0.02 mg/kg) by SAX HPLC.

Chelex® chromatography of the remaining aqueous extract after a dilution and centrifugation step characterised 0.96 % of the TRR (0.033 mg/kg) as non-phosphonate natural products not retained on the Chelex® resin. The fraction eluted with 0.1 N HCl contained only 0.16 % of the TRR and was not analysed further. Elution of the phosphonate-containing compounds with 6 N HCl and collection as three fractions recovered 77.83 % of the TRR (2.675 mg/kg) in fraction # 1.

After purification of fraction #1 by anion exchange chromatography and separation by cation exchange chromatography steps the radioactivity could be characterised as three radioactive fractions: Conjugate Fraction, 1.02 % of the TRR (0.035 mg/kg), glyphosate fraction, 62.66 % of the TRR (2.154 mg/kg), and AMPA fraction, 10.78 % of the TRR (0.371 mg/kg). The HPLC/RAD profile of the AMPA fraction indicated these fractions contained glyphosate in addition to AMPA. After a second cation exchange step, it was shown that the radioactivity in this fraction was confined to two regions fractions, #6-11 (glyphosate fraction, 9.39 % of TRR / 0.323 mg/kg) and fractions #21-32 (AMPA fraction, 1.33 % of TRR / 0.046 mg/kg). The glyphosate fractions from both cation exchange steps were combined (72.05 % of TRR / 2.476 mg/kg), analysed by SAX HPLC/LSC and shown to contain one major peak which showed co-chromatography with ^{14}C-glyphosate reference standard. GC/CI/MS analysis after derivatisation with trifluoroethanol/trifluoroacetic anhydride was consistent with the TFE/TFAA derivative of glyphosate.

The AMPA-containing fractions were analysed by SAX and CX HPLC/LSC and contained one major peak which showed co-chromatography with ^{14}C-AMPA reference standard under both anion and cation exchange HPLC conditions. The GC and HPLC retention times and mass spectral data of the TFAA/TFE derivative of sugar beet tops AMPA fraction matched those of the TFAA/TFE derivative of the AMPA reference standard, which was prepared in the same manner.

The conjugate fraction generated in the first sugar beet tops extraction represented a total of 0.8 % of the TRR in tops (0.03 mg/kg).
RP-PIC HPLC/LSC demonstrated one major radiolabelled peak accounting for 71.50 % of the radioactive distribution (0.57 % of the TRR, 0.020 mg/kg). After isolation of this radioactive component by preparative RP-PIC HPLC and clean-up of the isolate by cation exchange chromatography, analysis by SAX HPLC showed one major broad peak with numerous minor components. The major component of the purified conjugate fraction accounted for 66.42 % (0.5 % of the TRR, 0.02 mg/kg) of radioactive distribution.
Acid hydrolysis of an aliquot of the purified conjugate fraction using 1 M HCl at 95-100 °C for 5 hours yielded two major products, AMPA and glyphosate. Glyphosate was found not to undergo hydrolysis to AMPA under these conditions. The identity of glyphosate was confirmed by SAX HPLC co-chromatography using ^{14}C-glyphosate reference standards. Sugar beet tops conjugate fraction on SAX HPLC co-eluted with AMPA/glyphosate acetylated conjugate standard substance isolated during the wheat metabolism study (Mehrsheik, 2000, CA 6.2.1/019), where the corresponding isolated metabolite gave N-acetyl-AMPA in addition to AMPA and glyphosate under mild hydrolysis conditions (HCl, room temperature, two days).

Radioactive compounds contained in the aqueous extract that were not retained on the Chelex® column were characterised as non-phosphonate containing compounds and designated the natural product fraction. In the fractionation procedure this fraction represented 1.54 % of the TRR (0.021 mg/kg) in roots and 0.96 % of the TRR (0.033 mg/kg) in tops.

The SAX HPLC/LSC chromatograms of the concentrate of Chelex® non-retained fraction generated during the first extraction of sugar beet tops contained one major non-retained radiolabelled peak which accounted for the majority of the profiled ^{14}C-activity. Analysis by Amino HPLC revealed the presence of several radioactive peaks. The largest peak amounted to approximately 0.33 % of the TRR (0.011 mg/kg) of the profiled radioactivity. The natural product isolated from the post-emergence roots was not analysed by HPLC since it contained insufficient amount of radioactivity for HPLC analyses.

Sugar-beet tops solids contained 1.81 % of the TRR or 0.062 mg/kg of glyphosate equivalents after conventional extraction with water. Exhaustive extraction released 1.53 % of the TRR in tops (0.053 mg/kg) resulting in a final extractability of 88.18 % of the TRR.

Table 6.2.1-121: Extraction of the radioactive residues of glyphosate in sugar beet tops and roots following pre-emergent treatment at a dose rate of 1x 0.9 kg a.s./ha or post-emergent treatment at 2 x 1.08 kg a.s./ha

	Sugar beet tops pre-emergence		Sugar beet roots pre-emergence		Sugar beet tops post-emergence		Sugar beet roots post-emergence	
Days after last treatment (DALT)	**158**		**158**		**91**		**91**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**0.005**	**100**	**0.008**	**100**	**3.437**	**100**	**1.396**	**100**
Aqueous extract[1]	0.003	59.22	0.007	85.56	2.978	86.65	1.442	103.30
Concentration								
Aqueous concentrate	-	-	-	-	*2.915*	84.81	*1.408*	100.84
SAX HPLC								
Glyphosate	-	-	-	-	2.71	78.85	1.33	95.04
AMPA	-	-	-	-	-[2]	-[2]	-[2]	-[2]
Glyphosate/AMPA acetylated conjugates	-	-	-	-	0.03	0.80	0.01	0.55
RP-PIC HPLC								
Unknown 1	-	-	-	-	*0.02*	*0.57*	-	-
Other unknowns	-	-	-	-	Not quantified	Not quantified	-	-
SAX HPLC								
Unknown	-	-	-	-	0.02	0.5	-	-
Natural products + AMPA	-	-	-	-	0.11	3.22	0.07	5.01
Amino HPLC								
Unknown 1	-	-	-	-	*0.011*	0.33	-	-
Other unknowns	-	-	-	-	Not quantified	Not quantified	-	-
CX HPLC								
Glyphosate	-	-	-	-	2.77	80.45	1.33	95.58
AMPA	-	-	-	-	0.06	1.84	0.05	3.79
Natural products + conjugates	-	-	-	-	0.06	1.76	0.02	1.79
Chelex® chromatography								
Aqueous eluate							0.021	1.54
0.1 N HCl							0.0	0.0
6 N HCl Fractions #1&2							*1.336*	95.71
Anion exchange chromatography								
6 M HCl							*1.252*	89.70
Cation exchange chromatography								
Loading							*1.243*	89.02

Table 6.2.1-121: Extraction of the radioactive residues of glyphosate in sugar beet tops and roots following pre-emergent treatment at a dose rate of 1x 0.9 kg a.s./ha or post-emergent treatment at 2 x 1.08 kg a.s./ha

	Sugar beet tops pre-emergence		**Sugar beet roots pre-emergence**		**Sugar beet tops post-emergence**		**Sugar beet roots post-emergence**	
Days after last treatment (DALT)	**158**		**158**		**91**		**91**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Eluate 1 (Conjugate fractions # 5-6)							0.011	0.81
Eluate 2 (Glyphosate fractions # 7-11)							1.185	84.89
Eluate 3 (AMPA fractions # 36-41)							0.046	3.32
6 N HCl Fraction #3							n.r.[3]	n r.[3]
Precipitate	-	-	-	-	n r.[3]	n.r.[3]	n.r.[3]	n r.[3]
RRR	0.003	49.99	0.002	20.43	0.062	1.81	0.018	1.32
Acid hydrolysis								
Hydrolysate	-	-	-	-	0.053	1.53	-	-
Solids	-	-	-	-	*0.0096*	*0.28*	-	-
ERR	**0.003**	**59.22**	**0.007**	**85.56**	***3.031[4]***	***88.18[4]***	**1.442**	**103.30**
Final residue	**0.003**	**49.99**	**0.002**	**20.43**	***0.0096[4]***	***0.28[4]***	**0.018**	**1.32**
Accountability	**0.006**	**109.21**	**0.009**	**105.99**	**3.040**	**88.46**	**1.460**	**104.62**

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] = Sum of aqueous extracts, bottle and filter wash
[2] = Not resolved
[3] = n.r.: Not reported
[4] = after conventional and exhaustive extraction
Minor deviations may occur due to rounding
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-122: Extraction of the radioactive residues of glyphosate in sugar beet tops and roots after post-emergent treatment at 2 x 1.08 kg a.s./ha

	Sugar beet tops (large-scale extraction)	
Days after last treatment (DALT)	**91**	
	mg/kg	**% TRR**
TRR	**3.437**	**100**
Aqueous extract[1]	*2.828*	82.28
Concentration, centrifugation		
Aqueous concentrate	*2.768*	80.54
SAX HPLC		
Natural products + AMPA	0.10	2.95
Glyphosate	2.61	75.93
Glyphosate/AMPA acetylated conjugates	0.02	0.69
Dilution, centrifugation		
Supernatant 1	n r. [2]	n.r. [2]
Chelex® chromatography		
Aqueous eluate (natural product fraction)	*0.033*	0.96
0.1 N HCl eluate	*0.005*	0.16
6 N HCl fraction #1	*2.675*	77.83
Anion exchange chromatography		
6 N HCl eluate	*2.672*	77.74
Concentration, centrifugation		
Supernatant 2	*2.557*	74.40
Cation exchange chromatography		
Eluate 1 (Conjugate fraction, fractions # 10-12)	0.035	1.02
Eluate 2 (Glyphosate fraction, fractions # 13-18)	*2.154*	62.66
Eluate 3 (AMPA fraction, fractions # 44-62)	*0.371*	10.78
Cation exchange chromatography		
First eluate (Glyphosate fraction, fractions # 6-11)	*0.323*	9.39
Second eluate (AMPA fraction, fractions # 21-32)	*0.046*	1.33
Precipitate 2	n r. [2]	n.r. [2]
6 N HCl fraction #2 & 3(tops)	-	-
Precipitate 1	n r. [2]	n.r. [2]
RRR	*0.138*	4.01

Table 6.2.1-122: Extraction of the radioactive residues of glyphosate in sugar beet tops and roots after post-emergent treatment at 2 x 1.08 kg a.s./ha

	Sugar beet tops (large-scale extraction)	
Days after last treatment (DALT)	**91**	
	mg/kg	**% TRR**
TRR	**3.437**	**100**
ERR	***2.828***	**82.28**
Final residue	***0.138***	**4.01**
Accountability	***2.966***	***86.29***

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR: Extractable radioactive residue
RRR: Residual radioactive residue
[1] = sum of three extractions with water
[2] = n.r.: Not reported
Minor deviations may occur due to rounding
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-123: Distribution of the radioactive residues of glyphosate in sugar beet tops and roots following post-emergent application at 2 x 1.08 kg a.s./ha

	Sugar beet tops post-emergence		Sugar beet roots post-emergence	
Days after last treatment (DALT)	**91**		**91**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
TRR	**3.437**	**100**	**1.396**	**100**
Extractable residues[1]	2.978	86.65	1.442	103.30
Parent (PMG)[2]	2.74	79.65	1.33	95.31
Parent (PMG)[3]	2.77	80.45	1.33	95.58
Parent (PMG)[4]	2.71	78.85	1.33	95.04
Metabolite (AMPA)[3]	0.06	1.84	0.05	3.79
Glyphosate/AMPA acetylated conjugates[4]	0.03	0.80	0.01	0.55
Natural products[5]	0.05	1.38	0.02	1.22
RRR (after conventional extraction)	**0.062**	**1.81**	**0.018**	**1.32**
Hydrolysate[6]	0.053	1.53	-	-
Solids	*0.0096*	*0.28*	-	-
Total identified	***2.80***	***81.49***	***1.38***	***99.1***
Total characterised	***0.133***	***3.71***	***0.03***	***1.77***
ERR	***3.031***[7]	***88.18***[7]	**1.442**	**103.30**
Final residue	***0.0096***	***0.28***	**0.018**	**1.32**
Total sum	**3.040**	**88.46**	**1.460**	**104.62**

Table 6.2.1-123: Distribution of the radioactive residues of glyphosate in sugar beet tops and roots following post-emergent application at 2 x 1.08 kg a.s./ha

	Sugar beet tops post-emergence	Sugar beet roots post-emergence
Days after last treatment (DALT)	**91**	**91**

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] = sum of aqueous extracts, bottle and filter wash
[2] = mean of results from SAX HPLC/LCS and CX HPLC-LSC
[3] = determined by CX HPLC-LSC
[4] = determined by SAX HPLC/LSC
[5] = calculated from SAX HPLC data (natural product + AMPA peak) and CX HPLC data (AMPA peak)
[6] = hydrolysate: after acid hydrolysis of bound residues
[7] = after conventional and exhaustive extraction
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

C. Storage stability

Samples of sugar beet tops and roots were extracted and analysed by SAX-HPLC at Monsanto within 29 days after harvest. Separate extractions and SAX-HPLC was conducted from aliquots of sugar beet tops and roots samples in parallel to the metabolism investigations to assess the stability of the radioactive compounds in the samples during the duration of the study.

The results are summarised in the table below. Tops and roots storage stability samples were stored for 40 days. The residue concentrations found in the extracts and the HPLC profiles were nearly identical at the beginning and end of the storage period indicating stability of the major radioactive components during storage.

Table 6.2.1-124: Extraction of the radioactive residues of glyphosate in sugar beet tops and roots following post-emergent treatment at 2 x 1.08 kg a.s./ha – storage stability assessment

	Sugar beet tops post-emergence						Sugar beet roots post-emergence					
DALT	**91**						**91**					
Storage interval	**0 days**			**40 days**			**0 days**			**40 days**		
	mg/kg	**% TRR[2]**		**mg/kg**	**% TRR[2]**		**mg/kg**	**% TRR[2]**		**mg/kg**	**% TRR[2]**	
TRR	**3.561[1]**	**100**		**3.561[1]**	**100**		**1.256[1]**	**100**		**1.256[1]**	**100**	
Aqueous extract # 1	1.983	55.7		1.909	53.6		0.839	66.8		0.736	60.2	
Aqueous extract # 2	0.705	19.8		0.669	18.8		0.306	24.4		0.382	30.4	
Aqueous extract # 3	0.224	6.3		0.231	6.5		-	-		0.111	8.8	
Total extraction recovery	2.912	81.8		2.809	78.9		1.145	91.2		1.249 / 1.138[3]	99.4 / 90.6[3]	
	mg/kg	**% TRR[2]**	**% Area[2]**	**mg/kg**	**% TRR[2]**	**% Area[2]**	**mg/kg**	**% TRR[2]**	**% Area[2]**	**mg/kg**	**% TRR[2]**	**% Area[2]**
Unknown	0.122	3.4	4.2	0.118	3.3	4.2	0.054	4.3	4.7	0.076	6.1	6.1
Glyphosate	2.790	78.3	95.8	2.691	75.6	95.8	1.092	86.9	95.4	1.174	93.5	94.0

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
[1] = TRR determined at PTRL
[2] = as reported by PTRL
[3] = Sum of aqueous extracts #1 and # 2 only to allow for comparison with day 0 extraction.
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

The stability of the test substance during the two post-emergence applications was also established by a pre- and post-application purity determination on retain samples of the formulated product. HPLC assay of the application solution before and after the two sequential post-emergence applications indicated radiochemical purity in the range of 97.2 to 97.7 %, demonstrating the stability of the test substance during the application.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in pulses and oilseeds at the end of this chapter.

III. Conclusions

The nature of the residues in plants following the use of glyphosate was studied in sugar beet line 77, which was modified to express CP4 EPSPS. N-(phosphonomethyl)glycine (glyphosate), labelled in the phosphonomethyl-moiety with ^{12}C, ^{13}C or ^{14}C, respectively, was applied either pre-emergent at a target rate of 0.9 kg glyphosate acid equivalents/ha or twice post-emergent at a target rate of 1.08 kg glyphosate acid equivalents /ha per treatment.

TRRs determined after pre-emergent treatment were very low (0.006 mg/kg / 0.005 mg/kg in tops and 0.009 mg/kg / 0.008 mg/kg in roots).
After post-emergent treatment, TRRs were much higher (sugar beet tops 3.561 mg/kg / 3.437 mg/kg and roots 1.256 mg/kg / 1.396 mg/kg).

Glyphosate was the major component of the residue in both sugar beets tops and roots, accounting for 79.65 % (2.74 mg/kg) and 95.31 % (1.33 mg/kg) of the TRR, respectively. The metabolite AMPA accounted for 1.84 % (0.06 mg/kg) and 3.79 % (0.05 mg/kg) of the TRR in tops and roots, respectively.

Trace levels of glyphosate/AMPA acetylated conjugates (0.80 % of the TRR (0.03 mg/kg) in tops and 0.55 % of the TRR (0.01 mg/kg) in roots) and small amounts of ^{14}C-labelled natural products (1.38 % of the TRR (0.05 mg/kg) in tops and 1.22 % of the TRR (0.02 mg/kg) in roots) were also found after post-emergent treatment.

In summary it can be concluded that uptake of glyphosate from soil is very low in sugar beets; very low TRRs (0.006 mg/kg / 0.005 mg/kg in tops and 0.009 mg/kg / 0.008 mg/kg in roots) were found after pre-emergent application. After post-emergence application residues were present in treated parts of the plants and a translocation into the roots is observed. In CP4 EPSPS sugar beets the metabolism of glyphosate was limited with unchanged parent posing the major residue >75 % TRR in all matrices.
The results of this study demonstrate that the metabolism of glyphosate in sugar beet containing the CP4 EPSPS gene is the same as that found in other tolerant and non-tolerant crops. Glyphosate is slowly degraded to aminomethylphosphonic acid (AMPA), which is the primary plant metabolite. AMPA is further metabolised to low levels of conjugates. In addition to conjugation, the results indicated that glyphosate is further degraded to one carbon fragments that become incorporated into natural products and plant constituents.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in sugar beet has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with some deficits (slightly below 90 % of the total radioactive residue (TRR) were identified / characterised in sugar beet tops after two post-emergent treatments at 1.08 kg a.s./ha each at BBCH 12 – 14 and BBCH 19, respectively, radioactive balances for sugar beet tops extractions were below 90 % (total sum recovered 86.29 and 88.46 % of TRR for metabolite characterisation / identification and 78.9 and 81.8 % of TRR for storage stability).
Considering that the sample aliquots extracted for metabolite characterisation / identification are much larger than those used for determination of TRR via combustion (extraction: 25 -50 g; combustion: 0.1 – 0.37 g), it can be expected that extraction results are more representative of the actual residue situation in the commodities analysed. Therefore, the results of characterisation / identification are regarded as reliable for the assessment of the metabolic behaviour of glyphosate in glyphosate-tolerant sugar beet.
Moreover, post-emergence treatment represents a worst case, leading to considerably higher residues compared to the intended GAP which only comprises pre-emergence uses. The maximum intended rate for sugar beets is 1 to 3 applications with a minimum interval of 28 days at maximum 1.08 kg a.s./ha post-harvest, pre-sowing, pre-planting with a maximum amount of 2.16 kg a.s./ha in any 12 months period.
Pre-emergent treatment with glyphosate was performed in this study at 0.9 kg a.s./ha one day after sowing and resulted in a TRR of <0.01 mg/kg in sugar beet tops and thus, no differentiation of the radioactivity was needed. Radioactive residues in amounts requiring identification were only found after post-emergence application, which is not relevant for the intended uses. The characterisation / identification performed in sugar beet commodities after post-emergence application gave comprehensive information on the metabolite pattern present, which would not be feasible for sugar beet crop after pre-emergent treatment due to the very low residues expected.
The study is therefore considered to be reliable for the assessment of the metabolic behaviour of glyphosate in glyphosate-tolerant CP4 EPSPS sugar beet.

Assessment and conclusion by RMS:

Cereals

Study not previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/019
Report author	[redacted]
Report year	2000
Report title	Metabolism of Glyphosate in Roundup Ready Wheat
Report No	811W
Document No	MSL-16028
Guidelines followed in study	EC Directive 91/414/EEC EPA Residue Chemistry Test Guidelines, OPPTS 860.1300 - Nature of the Residue - Plants, Livestock
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • High unextractable residues after extraction, which were not further examined (0.49 to 2.81 mg/kg, forage, hay and straw) • Identification rate in straw was only 74.3 %, 5.7 % were characterised by extraction/distribution/chromatographic behaviour, sum of identification/characterisation in straw were about 80 %
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of residues in wheat after application of glyphosate formulated as Roundup Ultra® herbicide. The wheat was genetically modified (wheat line 25397) and was therefore tolerant to Roundup® herbicide.

The test substance consisted of a mixture of unlabelled, ^{13}C and ^{14}C-labelled glyphosate, labelled in the phosphonomethylene carbon. Two spray applications were performed, one at the 4 - 5 leaf stage (BBCH 15) and another at the pre-boot harvest stage (BBCH 43). Both applications were performed at a rate of 0.84 kg a.s./ha of glyphosate acid equivalents per treatment and were sprayed directly to the plant canopy.

Wheat forage, hay, straw and grain were collected from the test and control plots to simulate normal agricultural practices. Forage was collected 5 days following the first application (BBCH 30) and hay was

collected 30 days following the last application (BBCH 73 - 77). The mature wheat was harvested after 84 days following the last application.

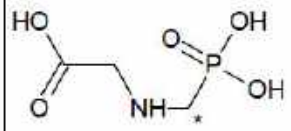
The total radioactive residues in ^{14}C-treated wheat forage, hay, straw and grain were determined by combustion followed by liquid scintillation counting and ranged from 12.12 to 34.81 mg/kg with straw containing the highest and grain the lowest level. In contrast, total radioactive residues in control samples ranged from background to 0.029 mg/kg.

Ground samples of forage, hay, straw and grain were extracted with water. Extractabilities with water ranged between 84.17 and 93.33 % TRR and 2.45, 3.86 and 8.06 % of the radioactivity remained associated with the extracted forage, hay and straw, respectively. Extracted grain (bound residue) contained 14.32 % of TRR following aqueous extractions. Acid hydrolysis of extracted grain released the majority of the bound radioactivity (10.86 % TRR). Significant amount of the acid released radioactivity was shown to be glyphosate.

Glyphosate was the major component of the residue in all wheat matrices, accounting for 18.09 mg/kg (89.44 % TRR) in forage, 23.34 mg/kg (83.86 % TRR) in hay, 24.09 mg/kg (69.19 % TRR) in straw and 8.78 mg/kg (72.40 % TRR) in grain.
AMPA, a well known plant metabolite of glyphosate, was found to be the most significant metabolite of glyphosate in wheat matrices ranging between 0.15 and 1.77 mg/kg (0.76 – 10.77 % TRR).
Grain aqueous extracts also contained N-glyceryl AMPA, glyphosate/AMPA acetylated conjugates and trace levels of other AMPA conjugates. In addition, aqueous extracts of wheat RAC contained ^{14}C-labeled natural products (<2 % of TRR). The radioactive natural products were considered to be derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from ^{14}C-glyphosate degradation into plant constituents. No single trace level metabolite accounted for greater than 2.5 % of the total radioactive residues in any raw agricultural commodity. Approximately 80-91 % of the total radioactive residues in wheat RAC was identified/characterised.

I. Materials and methods

A. Materials

Test Material:	N-(phosphonomethyl)glycine: mixture of a) N-(phosphono-^{14}C-methyl)glycine (41.2 mg) b) N-(phosphono-^{13}C-methyl)glycine (157.4 mg) c) N-(phosphono-^{12}C-methyl)glycine (141.3 mg) Code Nr. C-2410.1
Chemical structure:	 * Position of label
Radiochemical purity:	a) Batch No. C-2408; 99.6 % radiochemical purity b) Batch No. 13C-618; 98 % chemical purity c) Batch No. GLP-9606-7189-A; 99.9 % chemical purity
Specific activity:	a) 39 mCi/mmol (8.53 MBq/mg) specific activity of mixture C-2410.1: 4.59 mCi/mmol (60259 dpm/µg or 1.00 MBq/mg, respectively)
CAS No:	1071-83-6

Log $P_{o/w}$:	-3.2

Test system:

Soil:	Loamy sand (*Elkhorn Fine Sand*; pH: 6.2; cation exchange capacity: 6.3 meq./100 g; bulk density: 1.37 g/cm^3; organic matter: 1.7 %; sand: 65 %; silt: 24 %; clay: 11 %; textural class (USDA): sandy loam)
Crop:	Roundup-Ready® wheat, transgenic, line 25397 (modified to express CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase))
Botanical name:	*Triticum aestivum*
Crop part(s):	Forage, hay, straw, grain

B. Study design

1. In-life phase

The in-life phase of this study was conducted by PTRL West, Inc. Richmond, CA 94806, USA.

For the investigations, wheat plants were used, that were genetically modified (Roundup Ready® wheat line 25397, CP4 EPSPS modified) and are therefore tolerant to Roundup® herbicide when applied at commercial application rates. The crops were grown in above ground soil containers outdoor inside screened enclosures at Plant Sciences, Inc. in Watsonville, CA.

The test substance consisted of a mixture of unlabelled, ^{13}C and ^{14}C-labelled glyphosate with the ^{13}C- and ^{14}C-labels in the phosphonomethyl moiety. The specific activity of the resulting radiolabelled test substance was 1.00 MBq/mg (4.59 mCi/mmol) and the radiochemical and chemical purities were 99.2 % and 97 %, respectively.

For all applications, glyphosate was applied as the isopropylamine salt formulated as Roundup® Ultra herbicide, which is a water soluble commercial glyphosate formulation.
The formulation solution was produced by mixing a portion of the radiolabelled aqueous test substance stock solution with isopropylamine and MON 59112 surfactant. The formulated application solution had a concentration of 4.62 mg of ^{14}C-glyphosate acid/g of solution (about 2.78 x 10^8 dpm/g). Application rates in kg a.s./ha are expressed as glyphosate acid equivalents.
Wheat was grown in outdoor confined plots with dimensions of 91 cm x 76 cm x 45 cm (deep). The boxes were filled with sandy loam soil. Two above the ground containers of Roundup Ready® wheat plants received two sequential foliar applications of the test item formulated as Roundup® Ultra. The target rate was 0.84 kg a.s./ha of glyphosate acid equivalent that was achieved for all treated plots. A rate of 0.84 kg a.s./ha of glyphosate acid equivalent corresponds to 1.12 kg/ha glyphosate isopropylamine salt and 2.3 L/ha Roundup® Ultra herbicide.

Applications were performed at the 4 - 5 leaf and pre-boot harvest stages. The first application was a foliar spray application made when a majority of the plants were at the 4 - 5 leaf stage (BBCH 14-15), 30 days after planting. The second application was a foliar spray application made when approximately 10 % of the wheat plants were in the growth stage when the boot is just visibly swollen (BBCH 43), 42 days after planting.
During applications, the soil between the rows of wheat was covered with plastic backed absorbent paper to minimize the contact between the soil and ^{14}C-glyphosate. The dosing solutions were sprayed using

several passes directed towards the plant canopy using a hand-held sprayer. Following application, the application bottle was rinsed with water (1 mL) and this rinse was applied to the plants.
The empty application bottle was further rinsed with water. The actual amount applied (dpm) was determined by subtracting the dpm in the rinses from the calculated dpm in the dose solution.
Stability of the test substance was established prior to each application by a purity check of the formulated test substance and after each application using a retain sample of the dose solution which was placed on dry ice after the application process was completed. The purity of the test substance varied from 98.9 to 99.1 %.
A group of plants (control plants) received no applications and was housed in close proximity to the treated plants in order to monitor for the production and fixation of $^{14}CO_2$ and to serve as a control for the effects of glyphosate treatment on plant development.

2. Sampling

Wheat forage, hay, straw and grain samples were collected from test and control groups to simulate normal agricultural practices.
Forage (approximately 10 % of the crop in each plot) was collected 5 days following the first application. Hay was collected 24 30 days following the last application. The report states that hay was collected 30 days after treatment but these 30 days include the 6 days of drying of hay after sampling. For this, another 10 % of the crop was harvested at early boot to soft dough stage (BBCH 65 - 85) to obtain a fresh hay sample. This sample was placed on a drying rack and air dried to produce a dried hay sample.
The mature wheat was harvested after 84 days following the last application. The mature wheat was separated into grain and straw (including chaff).

Samples were stored frozen until shipment on dry ice to PTRL. Crop samples processed at PTRL were shipped with dry ice to Monsanto Company. Samples were received in good condition, with dry ice present.

3. Analytical procedures

Grinding of plant materials, combustion and liquid scintillation counting (LSC), as well as the storage stability study were performed by PTRL West, Inc. Extraction, characterisation and identification of residues in the processed samples was performed by Monsanto.

At PTRL the sample matrices were ground in the presence of dry ice using a West Bend Food Processor (forage and chopped hay), a coffee grinder (grain) or a Hobart Cutter Mixer (combined sample of straw and chaff). After processing, the samples were placed overnight in a freezer to allow the dry ice to sublime before recording the net weights.
Total radioactivity in the samples was determined by combusting aliquots of the ground samples and trapping the CO_2 generated in scintillation vials following analysis of the liquid with LSC.

All reported mg/kg residue levels determined for extraction and characterisation / identification of the residues are based on the determinations done by Monsanto.

For moisture determination, aliquots of ground samples were weighed separately into tared vials, heated at 105 – 110 °C for four hours. The samples were reweighed and the percent moisture was calculated from the difference in the mass of the samples before and after heating.

Portions of ground forage, hay, straw, and grain samples from the ^{14}C-treated group were extracted four times with a 3 - 4 fold excess of water using a Polytron tissue homogenizer with the exception of forage, which was extracted two times. The extracts were filtered after centrifugation, weighed and analysed by LSC.
For comparison, wheat hay and straw were extracted with water/acetonitrile (80/20, v/v) following similar procedures described above.

Aliquots of the extracted samples were combusted after air drying to determine unextracted radioactivity. Radioactive residues in all control samples were less than 0.05 mg/kg and therefore were neither extracted nor analysed.

Quantitative analysis by HPLC/LSC was carried out with each of the aqueous extracts or its concentrate. No single HPLC method was found that successfully separated all the components of the aqueous extracts, so they were analysed by both strong anion exchange (**SAX HPLC)** and cation exchange (**CX HPLC)** HPLC. On the strong anion exchange column, glyphosate, N-glyceryl-AMPA and AMPA/glyphosate conjugates were strongly retained and well resolved; however, AMPA was weakly retained and co-eluted with a retention time very close to that of neutral non-retained compounds. In contrast, on the cation exchange column, glyphosate and AMPA were well resolved, but N-Glyceryl-AMPA and AMPA/glyphosate conjugates co-eluted near the void volume along with the neutral non-retained compounds.
Therefore, the values for AMPA were based on CX HPLC, the values for N-glyceryl-AMPA were based on SAX HPLC and glyphosate levels were based on the average of the values calculated using SAX and CX HPLC.
For quantitative chromatographic analysis the HPLC column recoveries from SAX HPLC analysis ranged from 98 – 100 %. Recoveries from CX HPLC analysis ranged from 97 – 99 %.

For identification of metabolites, a series of column chromatography separation were used to fractionate and isolate sufficient quantities of the radioactive components. Radioactive detection was performed by liquid scintillation counting (LSC) or using a radioactive flow detector (RAD).
The first step of isolation used a Chelex® 100 resin column (iron form) to separate phosphonate-containing compounds from non-phosphonate-containing compounds. Phosphonate-containing compounds are bound to the resin, and non-phosphonate-containing materials are not retained.
Following application of the sample to the column, the column was eluted sequentially with water followed by 0.1 N HCl. The non-retained materials were called **"natural product fraction"**. The retained phosphonate-containing compounds, in the form of their iron salts, were then eluted from Chelex® column with 6 N HCl. Iron was removed from the eluate by passage through AG1-X8 anion exchange resin (chloride form). The phosphonate-containing compounds were then separated on a cation exchange column with AG50W-X8 resin (hydrogen form) to afford three main fractions: **glyphosate fraction, AMPA fraction** and **conjugate fraction**.

In the extracts of **forage, hay and straw**, AMPA and glyphosate fractions were further analysed by HPLC (SAX/CX HPLC) and glyphosate and AMPA were identified by co-chromatography experiments or comparison of the retention times with those of authentic ^{14}C-labelled reference items. Because of the low radioactivity level contained in the conjugate fractions obtained from forage hay and straw, these fractions were not further investigated. However, these fractions were characterised upon their elution behaviour.

The **extraction and fractionation of wheat grain** were carried out twice using slightly different fractionation schemes. In the first attempt (extraction A), the combined aqueous extracts of grain were subjected to the fractionation and clean up described above to provide enough material for identification of AMPA and glyphosate by mass spectroscopy. However, because of concerns regarding the stability of glyphosate and AMPA conjugate metabolites in 6 M HCI (eluate for Chelex® Column), in the second extraction/fractionation attempt (extraction B), the Chelex purification step was eliminated.
Following extraction A: The **glyphosate fraction** was analysed by SAX HPLC/LSC. For a definitive identification purpose, the glyphosate fraction was derivatised with trifluoroethanol/trifluoroacetic anhydride and an aliquot was analysed by RP HPLC/RAD. Since the results indicated the formation of two nonpolar radioactive products, a ^{14}C-glyphosate reference standard sample was derivatised in the same manner and analysed by HPLC which gave the same two radioactive peaks. GC/CI/MS analysis of the derivative mixture showed only the expected product (glyphosate TFE/TFEA derivative). HPLC coupled with mass spectral analysis (liquid chromatography/ion Spray /mass spectrometry; LC/ISP/MS)

of the corresponding reaction mixture indicated formation of an intermediate (partially) derivatised glyphosate product in addition to the expected derivative.

For identification of AMPA, the **AMPA fraction** isolated from grain and the respective ^{14}C-AMPA reference standard were analysed by CX HPLC/RAD. For a definitive identification purpose, the AMPA fraction as well as the AMPA reference were derivatised with trifluoroethanol/trifluoroacetic anhydride and analysed by RP HPLC/RAD and GC/EI/MS (gas chromatography/electron impact/mass spectrometry).

Comparison of the chromatograms from the analysis of **conjugate fraction** with that from the analysis of the initial whole aqueous extract showed that the relative concentration of two of the three trace level metabolites had decreased substantially during the purification procedures.

The fractionation for grain metabolites was therefore modified, since these minor metabolites in grain seemed to be not stable under highly acidic conditions employed in the Chelex® column. In the modified purification scheme, metabolites extracted from grain were therefore separated directly over a large AG50W-X8 cation exchange column.

Extraction B: Duplicate portions of ground grain were extracted four times with water using a Polytron tissue homogenizer. The water extracted grain pellet was dried overnight and weighed and analysed by combustion and LSC.

An aliquot of the combined extracts was analysed by SAX HPLC.

A larger aliquot of the sample was concentrated and then loaded on a large cation exchange column with AG50W-X8 resin and the column was eluted with water (in 50 fractions). The column was then eluted with 1 N HCl and 70 eluate fractions were collected. Fraction 20-28 of this fraction (conjugate fraction) was used for isolation and identification of N-glyceryl AMPA, AMPA conjugate, and glyphosate/AMPA acetylated conjugates. Fractions 31-41 (glyphosate fraction) and fractions 85-109 (AMPA fraction) were pooled and concentrated. These concentrates were used for SAX HPLC analysis.

For further characterisation and identification of components, a portion of the conjugate fraction was concentrated and two peaks (designated as peak A and B) were isolated by preparative chromatography using reversed phase paired ion chromatography (RP-PIC HPLC). The isolates were rotary evaporated to small volumes. In order to remove the tetrabutylammonium ion (TBA, the paired ion chromatography agent), the concentrated isolate was dissolved in water and passed through a small column of Bio-Rad AG50-WX8 cation exchange resin, hydrogen form.

The HPLC chromatogram of peak B of the conjugate fraction from SAX HPLC analysis showed one major broad peak with numerous minor components. Hydrolysis of an aliquot of this fraction with concentrated HCl at 97 - 100° C for 5 hours yielded two major products, AMPA and glyphosate confirmed by a SAX HPLC co-elution experiment using ^{14}C-standards. Since both AMPA and glyphosate are formed from hydrolysis it was suspected that the major component in peak B contains two structurally similar conjugates. The same conjugate fraction under mild HCl hydrolysis (room temperature 2 days) gave N-acetyl AMPA in addition to AMPA and glyphosate. The formation of N-acetyl AMPA was confirmed by co-elution experiment using ^{14}C-N-acetyl AMPA reference standard. Based on the results given above, it was speculated that peak B is comprised of two acetylated AMPA and glyphosate conjugates.

Since the **non-extracted radioactive residues** in grain was significant, an exhaustive extraction was performed. Therefore, an aliquot of the RRR was treated with 2 N HCl under reflux. The reaction mixture was filtered and the acid hydrolysate was analysed by CX HPLC and co-chromatography with ^{14}C-labeled glyphosate reference standard. HPLC analysis showed that in addition to glyphosate, the acid hydrolysate contained AMPA and a polar metabolite that was not further characterised.

The initial load and water rinses from the Chelex® column (**natural product fractions**) for hay, straw and grain were analysed by SAX HPLC/LSC and amino column chromatography (Amino HPLC/LSC). Since the natural product fraction was not retained on Chelex® resin, its radioactive components are characterised as non-phosphonate-containing compounds. The absence of the phosphonate moiety indicates that the radioactive components in the natural product fraction are natural plant constituents.

To **determine storage stability**, representative forage and grain samples were extracted and profiled by SAX HPLC/LSC soon after collection and then at the end of the experimental phase.

II. Results and discussion

A. Total radioactive residues (TRRs)

Treated plants showed no evidence of phytotoxic injury following any of the applications. During the course of the study control and treated plots developed in the same manner.

The total radioactive residue (TRR) in wheat forage, hay, straw and grain are summarised in Table 6.2.1-125. TRRs in treated wheat commodities were determined by PTRL prior to shipment to Monsanto and also at the Monsanto laboratory, while determination of TRRs in untreated plant matrices was performed only at PTRL.

The highest TRR values were detected in wheat straw (39.16 mg/kg / 34.81 mg/kg) and hay (27.72 mg/kg / 27.83 mg/kg). TRRs for wheat forage and grain were 18.30 mg/kg / 20.22 mg/kg and 12.37 mg/kg / 12.12 mg/kg. There was very limited uptake of $^{14}CO_2$ by the control plants. Control TRR values were 0.029 mg/kg for straw, 0.022 mg/kg for grain, 0.015 mg/kg for hay and <0.01 mg/kg for forage.

Table 6.2.1-125: Total radioactive residues in wheat commodities

Sample description	Test Group[1]	DALT	TRR determined at PTRL (mg eq./kg)	TRR determined at Monsanto laboratory (mg eq./kg)
two sequential foliar applications (2 x 0.84 kg a.s./ha)				
Wheat forage	1	-	<0.01	n.a.[2]
	2	5	18.30	20.22
Wheat hay	1	-	0.015	n.a.[2]
	2	24	27.72	27.83
Wheat straw	1	-	0.029	n.a.[2]
	2	84	39.16	34.81
Wheat grain	1	-	0.022	n.a.[2]
	2	84	12.37	12.12

DALT Days after last treatment
TRR Total radioactive residue, expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents
[1] = Group 1 - untreated control; Group 2 - treated with $^{13}C/^{14}C$-glyphosate
[2] = n.a.: Not applicable. Untreated control plants were not combusted at Monsanto since they were not extracted.

B. Extraction and characterisation of residues

Forage, hay, straw and grain from the treated plots were extracted and analysed. The TRRs in control samples were less than 0.05 mg/kg and therefore these samples were neither analysed nor extracted.
All reported mg/kg residue levels determined for extraction and characterisation / identification of the residues are based on the TRR values determined at the Monsanto laboratory; TRRs determined by PTRL are presented in the table above only for the sake of completeness.

Table 6.2.1-126 summarises the results for extraction of treated wheat forage, hay, straw and grain. Five days after the first application, 92.33 % of the TRR in wheat forage (18.67 mg/kg) were extractable with water. In wheat hay (24 DALT), straw (84 DALT) and grain (84 DALT) the aqueous extract contained 93.33 % of the TRR (25.97 mg/kg), 84.17 % of the TRR (29.30 mg/kg) and 89.61 % of the TRR (10.86 mg/kg), respectively.
Unextractable residues accounted for 2.45 % of the TRR (0.49 mg/kg) in wheat forage, for 3.86 % of the TRR (1.07 mg/kg) in wheat hay, for 8.06 % of the TRR (2.81 mg/kg) in wheat straw and for 14.32 % of

the TRR (1.74 mg/kg) in wheat grain. Recovery was 94.78 % of the TRR (19.16 mg/kg) in forage, 97.19 % of the TRR (27.04 mg/kg) in hay, 92.23 % of the TRR (32.11 mg/kg) in straw and 103.93 % of the TRR (12.60 mg/kg) in grain.

Aqueous extracts of the treated wheat commodities were analysed by strong anion exchange (SAX HPLC) and cation exchange (CX HPLC) high performance liquid chromatography. The nature and magnitude of radioactivity found in plant matrices extracts with both HPLC systems are shown in more detail in the tables below.
In forage extracts, glyphosate was found at 89.50 % of the TRR (18.10 mg/kg) with SAX HPLC and at 89.38 % of the TRR (18.07 mg/kg) with CX HPLC. The mean of both determinations was 89.44 % of the TRR (18.09 mg/kg). AMPA was found at 0.76 % of the TRR (0.15 mg/kg) with CX HPLC; with SAX HPLC, AMPA was not resolved. Glyphosate/AMPA acetylated conjugates were detected with SAX HPLC at 0.44 % of the TRR (0.09 mg/kg) and were not resolved on CX HPLC. The amount of natural products was calculated from SAX HPLC data (natural product plus AMPA peak) and CX HPLC data (AMPA peak) to be 0.26 % of the TRR (0.06 mg/kg).

In hay extracts, glyphosate was found at 83.78 % of the TRR (23.32 mg/kg) with SAX HPLC and at 83.93 % of the TRR (23.36 mg/kg) with CX HPLC. The mean of both determinations was 83.86 % of the TRR (23.34 mg/kg). AMPA was found at 3.45 % of the TRR (0.96 mg/kg) with CX HPLC; with SAX HPLC, AMPA was not resolved. AMPA conjugate and Glyphosate/AMPA acetylated conjugates were detected with SAX HPLC at 0.34 % of the TRR (0.09 mg/kg) and at 1.48 % of the TRR (0.41 mg/kg),respectively and were not resolved on CX HPLC. The amount of natural products was calculated from SAX HPLC data (natural product plus AMPA peak) and CX HPLC data (AMPA peak) to be 1.40 % of the TRR (0.39 mg/kg).

In straw extracts, glyphosate was found at 69.21 % of the TRR (24.09 mg/kg) with SAX HPLC and at 69.17 % of the TRR (24.08 mg/kg) with CX HPLC. The mean of both determinations was 69.19 % of the TRR (24.09 mg/kg). AMPA was found at 5.08 % of the TRR (1.77 mg/kg) with CX HPLC; with SAX HPLC, AMPA was not resolved. AMPA conjugate and Glyphosate/AMPA acetylated conjugates were detected with SAX HPLC at 1.46 % of the TRR (0.51 mg/kg) and at 2.42 % of the TRR (0.84 mg/kg),respectively and were not resolved on CX HPLC. The amount of natural products was calculated from SAX HPLC data (natural product plus AMPA peak) and CX HPLC data (AMPA peak) to be 1.68 % of the TRR (0.58 mg/kg).

In grain extracts, glyphosate was found at 73.26 % of the TRR (8.88 mg/kg) with SAX HPLC and at 71.54 % of the TRR (8.67 mg/kg) with CX HPLC. The mean of both determinations was 72.40 % of the TRR (8.78 mg/kg). AMPA was found at 10.77 % of the TRR (1.31 mg/kg) with CX HPLC; with SAX HPLC, AMPA was not resolved. N-glyceryl AMPA, AMPA conjugate and Glyphosate/AMPA acetylated conjugates were detected with SAX HPLC at 0.34 % of the TRR (0.04 mg/kg), at 0.63 % of the TRR (0.08 mg/kg) and at 0.65 % of the TRR (0.08 mg/kg), respectively and were not resolved on CX HPLC. The amount of natural products was calculated from SAX HPLC data (natural product plus AMPA peak) and CX HPLC data (AMPA peak) to be 0.57 % of the TRR (0.06 mg/kg).

The aqueous extracts were further characterised by fractionation and clean-up applying a sequence of Chelex®, anion exchange and cation exchange resins as described above. The results are summarised in the table below.
Separation of phosphonate-containing compounds from non-phosphonate-containing compounds on Chelex® resulted in 0.26 – 1.54 % of the TRR (0.05 -0.19 mg/kg) in the water rinses for forage, hay, straw and grain, which represent non-phosphonate compounds not binding to the resin. While the 0.1 N HCl eluates contained 0.05 - 0.18 % of the TRR (0.01 – 0.06 mg/kg), elution with 6 N HCl recovered 78.23 – 89.09 % of the TRR (15.82 – 24.79 mg/kg).
After purification of the respective 6 N HCl fraction of forage, hay, straw and grain by anion exchange chromatography and separation into sixty to seventy fractions by cation exchange chromatography, the

radioactivity was confined to three areas, the conjugate fraction, the glyphosate fraction and the AMPA fraction.
AMPA and glyphosate fractions of all matrices were further characterised by HPLC. SAX HPLC/RAD showed that the glyphosate fractions contained one major peak which co-chromatographed with ^{14}C-glyphosate reference standard. The AMPA fractions were analysed by SAX HPLC/RAD detecting one component, which co-chromatographed with ^{14}C-AMPA reference standard.

Grain solids contained 14.32 % of the TRR or 1.74 mg/kg of glyphosate equivalents after conventional extraction with water (first extraction). Additional exhaustive extraction (acid hydrolysis) released 10.86 % of the TRR in grain (1.32 mg/kg) resulting in a final extractability of 100.47 % of the TRR.

A large scale extraction and fractionation of wheat grain was performed to isolate sufficient quantities of the metabolites for identification and characterisation of AMPA and glyphosate by mass spectroscopy. 89.97 % (10.90 mg/kg) of the TRR were extractable with water. After concentration and centrifugation, five fractions of radioactivity in an aliquot of the concentrated extract were characterised as natural products plus AMPA (11.34 % of the TRR, 1.37 mg/kg), glyphosate (73.50 % of the TRR, 8.91 mg/kg), N-glyceryl AMPA (0.33 % of the TRR, 0.04 mg/kg), AMPA conjugate (0.58 % of the TRR, 0.07 mg/kg) and glyphosate/AMPA acetylated conjugates (0.75 % of the TRR, 0.09 mg/kg) by SAX HPLC.

After concentration and centrifugation of the remaining aqueous extract and separation by cation exchange chromatography steps the radioactivity could be characterised as three radioactive fractions: Conjugate fraction, 3.34 % of the TRR (0.40 mg/kg), glyphosate fraction, 69.39 % of the TRR (8.41 mg/kg), and AMPA fraction, 10.40 % of the TRR (1.26 mg/kg). The glyphosate fraction was analysed by SAX HPLC/LSC and contained one major peak, which was determined as glyphosate.
The AMPA-containing fractions were also analysed by SAX HPLC/LSC showing one major peak in the chromatogram, which was determined as AMPA.
The Conjugate fraction was analysed by RP-PIC HPLC/LSC and the radiochromatogram displayed two major peaks (peaks A and B). Peaks A and B accounted for 1.13 % of the TRR and 1.17 % of the TRR of the radioactive distribution, respectively. These two peaks were isolated for identification and characterisation. The major components of Peak A were identified as N-glyceryl-AMPA and AMPA conjugate by HPLC co-elution with synthetic standard.

Hydrolysis of an aliquot of the peak B of Conjugate fraction (0.95 mL) using 0.05 mL of concentrated HCl at 97-100 °C for 5 hours yielded two major products, AMPA and glyphosate (glyphosate was found not to undergo hydrolysis to AMPA under this condition). The identity of glyphosate from hydrolysis was confirmed by a SAX HPLC co-elution experiment using ^{14}C-standards. Since both AMPA and glyphosate were formed from hydrolysis, it was suspected that the major component in peak B of Conjugate fraction contained two structurally similar conjugates. Peak B of conjugate fraction under mild HCl hydrolysis (room temperature 2 days) gave N-acetyl AMPA in addition to AMPA and glyphosate. The formation of N-acetyl AMPA was confirmed by co-elution experiment using ^{14}C-N-acetyl AMPA reference standard. Therefore, it could be speculated that Peak B was comprised of two acetylated AMPA and glyphosate conjugates.

Table 6.2.1-126: Extraction of the radioactive residues of glyphosate in wheat forage, hay, straw and grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
Days after last treatment (DALT)	**5**		**24**		**84**		**84**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**20.22**	**100**	**27.83**	**100**	**34.81**	**100**	**12.12**	**100**
Aqueous extract[1]	18.67	92.33	25.97	93.33	29.30	84.17	10.86	89.61

Table 6.2.1-126: Extraction of the radioactive residues of glyphosate in wheat forage, hay, straw and grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
Days after last treatment (DALT)	**5**		**24**		**84**		**84**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**20.22**	**100**	**27.83**	**100**	**34.81**	**100**	**12.12**	**100**
Concentration								
Aqueous concentrate	-	-	25.90	93.06	29.38	84.39	10.74	88.64
SAX HPLC								
Glyphosate	18.10	89.50	23.32	83.78	24.09	69.21	8.88	73.26
AMPA	-[2]	-[2]	-[2]	-[2]	-[2]	-[2]	-[2]	-[2]
N-glyceryl AMPA	-[3]	-[3]	-[3]	-[3]	-[3]	-[3]	0.04	0.34
AMPA conjugate	-[3]	-[3]	0.09	0.34	0.51	1.46	0.08	0.63
Glyphosate/AMPA acetylated conjugates	0.09	0.44	0.41	1.48	0.84	2.42	0.08	0.65
Natural products + AMPA	0.21	1.02	1.35	4.85	2.35	6.76	1.37	11.34
CX HPLC								
Glyphosate	18.07	89.38	23.36	83.93	24.08	69.17	8.67	71.54
AMPA	0.15	0.76	0.96	3.45	1.77	5.08	1.31	10.77
Natural products + conjugates	0.18	0.89	0.97	3.47	2.65	7.60	0.41	3.41
Chelex® chromatography								
Aqueous eluate	0.05	0.26	0.31	1.10	0.50	1.44	0.19	1.54
Amino HPLC								
Unknown 1 (non-phosophonate-containing compounds)	-	-	*0.08*	0.29	*0.15*	0.42	*0.08*	0.68
Other unknowns	-	-	Not quantified	Not quantified	Not quantified	Not quantified	Not quantified	Not quantified
0.1 N HCl	0.01	0.05	0.02	0.07	0.06	0.18	0.02	0.13
6 N HCl Fraction	15.82	78.23	24.79	89.09	27.26	78.32	10.11	83.42
Anion exchange chromatography								
6 M HCl eluate	*17.58*	86.95	*24.60*	88.38	*27.55*	79.14	*10.61*	87.52
Cation exchange chromatography								
Loading	*18.07*	89.38	*28.96*	104.06	*25.50*	73.26	*10.64*	87.75
Eluate 1 (Conjugate fraction)	0.09	0.44	0.33	1.17	0.92	2.63	0.14	1.19
Eluate 2 (Glyphosate fraction)	17.67	87.39	26.36	94.71	21.91	62.94	9.04	74.56
Eluate 3 (AMPA fraction)	0.19	0.95	2.03	7.30	2.28	6.54	1.33	10.99
RRR	0.49	2.45	1.07	3.86	2.81	8.06	1.74	14.32

Table 6.2.1-126: Extraction of the radioactive residues of glyphosate in wheat forage, hay, straw and grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
Days after last treatment (DALT)	**5**		**24**		**84**		**84**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**20.22**	**100**	**27.83**	**100**	**34.81**	**100**	**12.12**	**100**
Acid hydrolysis								
Hydrolysate	-	-	-	-	-	-	1.32	10.86
Solids	-	-	-	-	-	-	*0.42*	*3.46*
ERR	**18.67**	**92.33**	**25.97**	**93.33**	**29.30**	**84.17**	***12.18***[5]	***100.47***[5]
Final residue	**0.49**	**2.45**	**1.07**	**3.86**	**2.81**	**8.06**	***0.42***[5]	***3.46***[5]
Accountability	**19.16**	**94.78**	**27.04**	**97.19**	**32.11**	**92.23**	**12.60**	**103.93**

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] = Sum of aqueous extracts, bottle and filter wash
[2] = Not resolved
[3] = Not detected
[4] = n.r.: Not reported
[5] = after conventional and exhaustive extraction
Minor deviations may occur due to rounding
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-127: Extraction of the radioactive residues of glyphosate in wheat grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat grain (large-scale extraction)	
Days after last treatment (DALT)	**84**	
	mg/kg	**% TRR**
TRR	**12.12**	**100**
Aqueous extract[1]	10.90	89.97
Concentration, centrifugation		
Aqueous concentrate	*10.81*	89.18
SAX HPLC		
Natural products + AMPA	1.37	11.34
Glyphosate	8.91	73.50
N-glyceryl AMPA	0.04	0.33
AMPA Conjugate	0.07	0.58
Glyphosate/AMPA acetylated conjugates	0.09	0.75

Table 6.2.1-127: Extraction of the radioactive residues of glyphosate in wheat grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat grain (large-scale extraction)	
Days after last treatment (DALT)	**84**	
	mg/kg	**% TRR**
TRR	**12.12**	**100**
Concentration, centrifugation		
Supernatant	*10.26*	84.64
Cation exchange chromatography		
Eluate 1 (Conjugate fraction, fractions 20-28)	0.40	3.34
RP-PIC HPLC		
Unknown 1 (Peak A)	*0.14*	1.13
Unknown 2 (Peak B)	*0.14*	1.17
Other unknowns	Not quantified	Not quantified
Eluate 2 (Glyphosate fraction, fractions 31-41)	8.41	69.39
Eluate 3 (AMPA fraction, fractions 85-109)	1.26	10.40
RRR	1.78	14.68
ERR	**10.90**	**89.97**
Final residue	**1.78**	**14.68**

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR: Extractable radioactive residue
RRR: Residual radioactive residue
[1] = sum of three extractions with water
Minor deviations may occur due to rounding
Values in *italics* were calculated from reported values upon dossier compilation

Table 6.2.1-128: Distribution of the radioactive residues of glyphosate in wheat forage, hay, straw and grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
Days after last treatment (DALT)	**5**		**24**		**84**		**84**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**20.22**	**100**	**27.83**	**100**	**34.81**	**100**	**12.12**	**100**
Extractable residues[1]	18.67	92.33	25.97	93.33	29.30	84.17	10.86	89.61
Parent (PMG)[2]	18.09	89.44	23.34	83.86	24.09	69.19	8.78	72.40
Parent (PMG)[3]	18.07	89.38	23.36	83.93	24.08	69.17	8.67	71.54
Parent (PMG)[4]	18.10	89.50	23.32	83.78	24.09	69.21	8.88	73.26
Metabolite (AMPA)[3]	0.15	0.76	0.96	3.45	1.77	5.08	1.31	10.77

Table 6.2.1-128: Distribution of the radioactive residues of glyphosate in wheat forage, hay, straw and grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
Days after last treatment (DALT)	**5**		**24**		**84**		**84**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
N-glyceryl AMPA [4]	-[7]	-[7]	-[7]	-[7]	-[7]	-[7]	0.04	0.34
AMPA conjugate [4]	-[7]	-[7]	0.09	0.34	0.51	1.46	0.08	0.63
Glyphosate/AMPA acetylated conjugates[4]	0.09	0.44	0.41	1.48	0.84	2.42	0.08	0.65
Natural products[5]	0.06	0.26	0.39	1.40	0.58	1.68	0.06	0.57
RRR (after conventional extraction)	0.49	2.45	1.07	3.86	2.81	8.06	1.74	14.32
Hydrolysate[6]	-	-	-	-	-	-	1.32	10.86
Solids	-	-	-	-	-	-	*0.42*	*3.46*
Total identified	***18.24***	***90.20***	***24.30***	***87.31***	***25.86***	***74.27***	***10.13***	***83.51***
Total characterised	***0.15***	***0.70***	***0.89***	***3.22***	***1.93***	***5.56***	***1.58***	***13.05***
ERR	**18.67**	**92.33**	**25.97**	**93.33**	**29.30**	**84.17**	***12.18***[8]	***100.47***[8]
Final residue	**0.49**	**2.45**	**1.07**	**3.86**	**2.81**	**8.06**	***0.42***[8]	***3.46***[8]
Total sum	**19.16**	**94.78**	**27.04**	**97.19**	**32.11**	**92.23**	**12.60**	**103.93**

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR Extractable radioactive residue
RRR Residual radioactive residue
[1] = sum of aqueous extracts, bottle and filter wash
[2] = mean of results from SAX HPLC/LCS and CX HPLC-LSC
[3] = determined by CX HPLC-LSC (not detected by SAX HPLC)
[4] = determined by SAX HPLC/LSC (not detected by CX HPLC)
[5] = calculated from CX HPLC data (AMPA peak)from SAX HPLC (natural product + AMPA peak)
[6] = hydrolysate: after acid hydrolysis of bound residues
[7] = Not detected
[8] = after conventional and exhaustive extraction
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

C. Storage stability

It was not clear how long the duration was between harvest of the samples/processing of samples at PTRL and extraction and analysis at Monsanto. However, wheat forage and grain were extracted at PTRL 17-18 days after harvest and the extracts were analysed by Monsanto (1 to 5 days after extraction). This was performed in parallel to the metabolism investigations to assess the stability of the radioactive compounds in the samples during the duration of the study.
The results are summarised in the table below. Forage and grain storage stability samples were stored for 271 and 174 days, respectively. The residue concentrations found in the extracts and the HPLC profiles were nearly identical at the beginning and end of the storage period indicating stability of the major radioactive components during storage.

Table 6.2.1-129: Extraction of the radioactive residues of glyphosate in wheat forage and grain following two sequential foliar applications (2 x 0.84 kg a.s./ha)

	Wheat forage				Wheat grain			
DALT	**5**				**84**			
Storage interval	**0 days**		**271 days**		**0 days**		**174 days**	
	mg/kg	**% TRR[2]**	**mg/kg**	**% TRR[2]**	**mg/kg**	**% TRR[2]**	**mg/kg**	**% TRR[2]**
TRR	**18.298[1]**	**100**	**18.298[1]**	**100**	**12.374[1]**	**100**	**12.374[1]**	**100**
Aqueous extract # 1	12.900	70.5	12.699	69.4	6.113	49.4	7.796	63.0
Aqueous extract # 2	5.910	32.3	6.166	33.7	2.759	22.3	2.289	18.5
Aqueous extract # 3	-	-	-	-	1.052	8.5	0.643	5.2
Total extraction recovery	18.810	102.8	18.865	103.1	9.924	80.2	10.728	86.7

	mg/kg	% TRR[2]	% Area[2]	mg/kg	% TRR[2]	% Area[2]	mg/kg	% TRR[2]	% Area[2]	mg/kg	% TRR[2]	% Area[2]
Unknown	0.200	*1.1*	1.1	0.258	*1.4*	1.35	1.280	*10.3*	12.9	1.384	*11.2*	12.9
Glyphosate	18.498	*101.1*	98.3	18.487	*101.0*	98.1	8.455	*68.33*	85.2	9.290	*75.1*	86.6

DALT Days after last treatment
TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
[1] = TRR determined at PTRL
[2] = as reported by PTRL
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

The stability of the test substance during the two post-emergence applications was also established by a pre- and post-application purity determination on retain samples of the formulated product. HPLC assay of the application solution before and after the two sequential post-emergence applications indicated radiochemical purity in the range of 98.9 to 99.1 %, demonstrating the stability of the test substance during the application.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusions

This metabolism study was designed to determine the nature and magnitude of residues in wheat after application of glyphosate formulated as Roundup Ultra® herbicide. The wheat was genetically modified (wheat line 25397) and was therefore tolerant to Roundup® herbicide.

The test substance consisted of a mixture of unlabelled, ^{13}C and ^{14}C-labelled glyphosate, labelled in the phosphonomethylene carbon. Two spray applications were performed, one at the 4 - 5 leaf stage (BBCH 15) and another at the pre-boot harvest stage (BBCH 43). Both applications were performed at a rate of 0.84 kg a.s./ha of glyphosate acid equivalents per treatment and were sprayed directly to the plant canopy.

Wheat forage, hay, straw and grain were collected from the test and control plots to simulate normal agricultural practices. Forage was collected 5 days following the first application (BBCH 30) and hay was

collected 30 days following the last application (BBCH 73 - 77). The mature wheat was harvested after 84 days following the last application.

The total radioactive residues in ^{14}C-treated wheat forage, hay, straw and grain were determined by combustion followed by liquid scintillation counting and ranged from 12.12 to 34.81 mg/kg with straw containing the highest and grain the lowest level. In contrast, total radioactive residues in control samples ranged from background to 0.029 mg/kg.

Ground samples of forage, hay, straw and grain were extracted with water. Extractabilities with water ranged between 84.17 and 93.33 % TRR and 2.45, 3.86 and 8.06 % of the radioactivity remained associated with the extracted forage, hay and straw, respectively. Extracted grain (bound residue) contained 14.32 % of TRR following aqueous extractions. Acid hydrolysis of extracted grain released the majority of the bound radioactivity (10.86 % TRR). Significant amount of the acid released radioactivity was shown to be glyphosate.

Glyphosate was the major component of the residue in all wheat matrices, accounting for 18.09 mg/kg (89.44 % TRR) in forage, 23.34 mg/kg (83.86 % TRR) in hay, 24.09 mg/kg (69.19 % TRR) in straw and 8.78 mg/kg (72.40 % TRR) in grain.
AMPA, a well known plant metabolite of glyphosate, was found to be the most significant metabolite of glyphosate in wheat matrices ranging between 0.15 and 1.77 mg/kg (0.76 – 10.77 % TRR).
Grain aqueous extracts also contained N-glyceryl AMPA, glyphosate/AMPA acetylated conjugates and trace levels of other AMPA conjugates. In addition, aqueous extracts of wheat RAC contained ^{14}C-labeled natural products (<2 % of TRR). The radioactive natural products were considered to be derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from ^{14}C-glyphosate degradation into plant constituents. No single trace level metabolite accounted for greater than 2.5 % of the total radioactive residues in any raw agricultural commodity. Approximately 80-91 % of the total radioactive residues in wheat RAC was identified/characterised.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in wheat has not been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with minor deficits: Only about 80 % of the total radioactive residue (TRR) were identified / characterised in wheat straw after two post-emergent treatments at 0.84 kg a.s./ha each at BBCH 14-15 and BBCH 43 and high residues remained after extraction, which were not further examined (0.49 to 2.81 mg/kg, forage, hay and straw). Grain residues after extraction were further examined by acid hydrolysis and subsequent analyses, but 0.42 mg/kg remained non-extracted.
Radioactive balances for all matrices including straw were >90 % (most cases ≥95 % with the exception of straw).
Identification rates of all matrices ranged from 74 % (straw) to 90 % of TRR, with characterisation of the remaining extracted residues by extractability, solubility in organic/aqueous solvents and/or chromatographic behaviour. The study is therefore considered to be reliable for the assessment of the metabolic behavior of glyphosate in glyphosate-tolerant CP4 EPSPS wheat.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/020
Report author	[redacted]
Report year	1995
Report title	Nature of glyphosate residues in corn plants which are tolerant to Roundup® herbicide
Report No	MSL-14018
Document No	M-650178-01-1
Guidelines followed in study	Pesticide Assessment Guideline Number §171-4(a) of Subdivision O: Nature of the Residue in Plants
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • No full description of the fractionation/flow charts available for acidic hydrolysis of the remaining radioactive residues of grain after conventional (hexane/aqueous) extraction as well as saponification of corn oil for the normal scale experiments.
Previous evaluation:	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues from the sequential post-emergence application of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) formulated as Roundup® herbicide to maize/corn plants which contain the Roundup Ready gene (modified to express CP4 EPSPS and GOX proteins).

The study was designed to allow separate determination of the residues resulting from foliar absorption alone, and soil uptake and foliar absorption combined. To distinguish between foliar and root uptake duplicate experiments were conducted either covering the soil (protected) or not (unprotected) performed in two separate greenhouses. In parallel replicate plots in the same greenhouse treated with unlabelled glyphosate at identical rates were conducted to measure the incorporation of $^{14}CO_2$ formed in soil metabolism.

The test substance consisted of an isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate with ^{13}C and ^{14}C located at the carbon atom between the nitrogen and phosphonate moieties. Carbon-^{13} was incorporated into the test substance in order to facilitate mass spectral identification of metabolites that were not totally free of biological matrix. For all applications, glyphosate was applied as the isopropylamine salt formulated as Roundup® herbicide, which is a water soluble commercial glyphosate formulation.

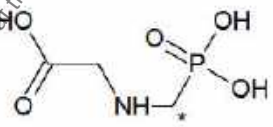
Two foliar applications were done at rates equivalent to 0.93 kg glyphosate acid equivalents/ha (5 – 6 leaf stage corresponding to BBCH 15 – 16) and 0.84 kg glyphosate acid equivalents/ha (10 – 12 leaf stage corresponding to BBCH 19, 4 weeks later).

Samples were collected immediately after each treatment and in the forage (3 DALT), silage (49 and 53 DALT) and maturity growth stage (grain and fodder, 83 DALT).

Total radioactive residue in ^{14}C-treated maize/corn forage, silage and fodder ranged from 9.11 mg/kg to 14.9 mg/kg for protected treatment, and 9.59 mg/kg to 19.1 mg/kg for non-protected treatment.
Maize/corn grain contained much lower levels of radioactivity; radioactive residues in ^{14}C-treated grain were 0.685 mg/kg and 1.04 mg/kg for protected and non-protected treatments, respectively.
Glyphosate was observed to be the major radioactive residue in forage, silage and fodder accounting for 67.1 to 83.3 % of the TRR (6.43 – 14.27 mg/kg), whereas only low levels of glyphosate were present in grain (2.6 – 7.4 % of the TRR). In contrast, AMPA was found at approximately 4.9 % to 15.9 % of the TRR in forage, silage and fodder (0.73 – 2.13 mg/kg), and 54.1 % to 60.3 % of the TRR (0.37 – 0.63 mg/kg) in grain.
Aqueous extracts also contained N-glyceryl-AMPA accounting for 0.4 % to 1.6 % of the TRR (0.05 – 0.31 mg/kg) in forage, silage and fodder and 6.9 % of the TRR (0.05 – 0.07 mg/kg) in grain and low levels (<2 % of TRR, 0.04 – 0.36 mg/kg) of other glyphosate conjugates in forage, silage and fodder, while they were not present in grain. Trace levels of other AMPA conjugates are mentioned.
In addition to this, aqueous extracts contained ^{14}C-labelled natural products (< 3.6 % of the TRR). The radioactive natural products were derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from N-(phosphono-^{14}C methyl)glycine into plant constituents.
The radioactivity in oil extracted from grain was shown to be associated with naturally occurring fatty acids.
Remaining radioactive residues after conventional extraction were less than 5.4 % of the TRR in forage, silage and fodder, while they accounted for up to 25.27 % of the TRR (0.263 mg/kg) in grain. Acid hydrolysis of extracted grain released almost all of the remaining radioactivity (90.24 % from grain). Majority of the acid-released radioactivity was shown to be glucose, derived from the incorporation $^{14}CO_2$ and other one carbon fragments of N-(phosphono-^{14}C-methyl)glycine into maize/corn starch.

I. Materials and Methods

A. Materials

Test Material:	a) N-(phosphono-^{14}C-methyl)glycine b) N-(phosphono-^{13}C-methyl)glycine c) unlabelled N-(phosphono-^{12}C-methyl)glycine
Chemical structure:	 * Position of label
Radiochemical purity:	99.29 % (N-(phosphono-^{14}C-methyl)glycine, code No. C-1736)
Specific activity (in radiodiluted and formulated treatment solution):	0.2138 mCi/g; 7.9106 MBq/g
CAS No:	1071-83-6 (glyphosate) 38641-94-0 (glyphosate isopropylamine salt)
Log P_{ow}:	Glyphosate: -3.2

Test system:

Crop:	Maize/corn (containing the Roundup Ready™ gene) genotype #599-4-2
Botanical name:	*Zea mays*

Soil:	Silt-loam soil (3.0 % OM, pH 6.3, 21 % clay, 55 % silt, 24 % sand)
Crop part(s):	Forage, silage, fodder and grain

B. Study design

1. In-life phase

In this study "Roundup ReadyTM" maize expressing both CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) and glyphosate oxidoreductase (GOX), which confer tolerance to Roundup® was treated in greenhouse with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) in two foliar spray applications at rates equivalent of glyphosate acid to 0.93 kg a.s./ha (5 – 6 leaf stage corresponding to BBCH 15-16) and 0.84 kg a.s./ha (10 – 12 leaf stage, corresponding to BBCH 194 weeks later).
To distinguish between foliar and root uptake duplicate experiments were conducted either covering the soil (protected) or not (unprotected) performed in two separate greenhouses.
The test substance consisted of an isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C-labelled glyphosate with ^{13}C and ^{14}C located at the carbon atom between the nitrogen and phosphonate moieties (N-(phosphono-^{14}C-methyl)glycine and N-(phosphono-^{13}C-methyl)glycine). ^{13}C was incorporated into the test substance in order to facilitate mass spectral identification of metabolites by providing a diagnostic doublelet pattern in the mass spectra. Mass spectra of metabolites were thus distinguished from those of plant matrix
The test substance (Code No. C-1739) was prepared by mixing 1582.6 mg of N-(phosphono-^{14}C-methyl)glycine (Code No. C-1736, specific activity of 14.4 mCi/mmol (3.15 MBq/mg), 99.29 % radiochemical purity) with 1745.8 mg of ^{13}C-glyphosate (Lot No. 3327-N, 99 % enriched in ^{13}C, 99 % chemical purity) and 546.7 mg of ^{12}C-glyphosate (Lot No. RUD-9203-3961-A, 99.8 % chemical purity). The specific activity of the resulting ^{14}C-test substance was 5.81 mCi/mmol (1.27 MBq/mg; 76272 dpm/µg).
The test substance (C-1739) was formulated as follows: to a solution of the ^{14}C-test substance in water isopropylamine and MON 0818 (an ethoxylated tallow-amine surfactant used in the commercial formulation of Roundup herbicide) was added and the solution was diluted with water.
The formulated ^{14}C-test substance had a glyphosate concentration of 6.22 mg/g (0.2138 mCi/g; 7.9106 MBq/g) and was diluted to afford individual dosing solutions for each plot/application. For soil protected treatments, the calculated amount of glyphosate in dosing solutions was 180.3 mg and 163.5 mg, for the first and second applications, respectively.
For soil non-protected treatments, the calculated amount of glyphosate in dosing solution was 177.8 mg and 161.3 mg, for the first and second applications, respectively.
The calculated concentrations of N-(phosphono-^{14}C-methyl)glycine in the dosing solution were 2.47 mg/mL for the first application and 2.24 mg/mL for the second application.
Control groups were treated with non-radiolabelled Roundup® herbicide using the similar application rates and timings as those used for ^{14}C-treated test groups and were grown in close proximity to the ^{14}C-treated test groups to allow a determination of the amount of re-incorporation of $^{14}CO_2$
Plants were grown in large steel tanks (~86 cm wide x ~236 cm long x ~58 cm deep) filled with silt-loam soil. Fifty seeds were planted in each tank. Seeds were planted in two rows (~51 cm apart) with ~5 – 8 cm between seeds.
Planting and harvesting of crop were performed manually. Fertilizers were applied before planting and during the growing season. Weeding was done manually, and no herbicides were applied. Crops were free of insect infestation. All test plots were irrigated on an as-needed basis.

2. Sampling

Samples of maize/corn forage and silage were collected approximately two hours after each treatment and the forage at DALT 3, silage at DALT 49 (non-protected soil plot) and 53 (protected soil plot). Forage and silage samples were collected in duplicate. The second samples were used for rinsing with deionised water to estimate surface residues. Fodder and grain were taken at mature crop stages (DALT 83).

The immature corn samples consisted of the entire plant and the mature crop was separated into grain, fodder, and cob. Cob samples were not analysed further. Duplicate samples of forage and silage were collected from ^{14}C-treated plots, and one sample of each was rinsed with distilled water and the rinses collected. Soil samples were collected from the ^{14}C-treated plots at each plant sampling timepoint. Plant and soil samples were stored in freezers at -20 °C.
Immature corn samples were processed in the presence of dry ice using a food processor. Maize/corn forage, silage, and fodder samples were processed in the presence of dry ice in a cutter/mixer. The forage, silage and subsamples of fodder were ground further after initial processing using a mini mill. The additional processing was performed to provide a finer, more uniform ground sample for combustion analysis. The grain samples were ground with dry ice using a grist mill. The soil samples were processed in the presence of dry ice using a blender. All samples were maintained frozen during sample preparation using dry ice. Dry ice was allowed to sublime off at -20 °C in a freezer before sample analysis. Samples were always stored frozen at or below -20 °C.

3. Analytical procedures

Ground forage and silage were four times extracted with water, fodder samples six times with water. For each extraction, the sample with water was either blended for 2-3 minutes with a polytron tissue homogenizer or shaken on a flatbed shaker for approximately 45 minutes. The slurry was centrifuged and the supernatant (extract) was collected in tared bottles.
Aliquots of extracts were counted by liquid scintillation counting (LSC). The extracted forage, silage and fodder samples were analysed by combustion for bound residue determination.
Ground grain samples were first extracted three times with hexane to remove oil. The hexane-extracted grain (meal) samples were air dried and then extracted four times with water. For each extraction, the sample was blended with solvent for 2-3 minutes with a polytron tissue homogenizer. The slurry was centrifuged and the supernatant (extract) was collected in tared bottles. The hexane and water extracts were analysed by LSC. The hexane extract was concentrated by rotary evaporation to yield crude corn oil.
The total radioactive residues (TRR) in plant samples prior extraction and in the remaining solids after extraction were determined by combustion analysis. The carbon dioxide resulting from combustion of the sample was trapped in a solution of Packard Carbo-Sorb® and Packard Permaflor® E$^+$ and then analysed by LSC. The limit of detection for plant samples was 0.005 mg/kg.
Plant moisture determinations were done in duplicate for each matrix.
Several different High Performance Liquid Chromatography systems (HPLC) were employed using UV-detection and radioactive flow detector (RAD) equipped with either a liquid cell or a solid scintillant cell detection allowing direct measurements as well as isolating material for further identification.
The second method of detection, HPLC/LSC, consisted of fraction collection of the HPLC effluent employing a fraction collector with subsequent counting of the fractions by LSC.
HPLC techniques included Chelex® 100 column, cation exchange (CX), and strong anion exchange (SAX) chromatography, reversed phase chromatography (RP), reversed phase pair ion chromatography (RP-PIC) as well as amino column chromatography.
Gas chromatography (positive ion chemical ionisation with MS detection (GC/PICI/MS) and gas chromatography (electron ionisation with MS detection (GC/EI/MS) was additionally used after derivatisation with trifluoroacetic anhydride/trifluoroethanol for identification of metabolites.
Thin layer chromatography (TLC) was used as second chromatographic method to confirm the identity of the N-glyceryl-AMPA metabolite.

Since fodder and grain from the soil non-protected experiment contained the highest levels of glyphosate residues, metabolites were isolated from the aqueous extracts for identification/characterisation.
The first step of isolation scheme used a Chelex® resin column to separate phosphonate-containing compounds from nonphosphonate-containing compounds. Phosphonate-containing compounds are bound to the resin, and nonphosphonate-containing materials are not retained, nonretained materials were called the Natural Product Fraction. The phosphonate-containing compounds were eluted from the Chelex® column and then, in a second step, separated on a cation exchange column to afford three main fractions: Conjugate Fraction, Glyphosate Fraction, and AMPA Fraction.

The Natural Product Fractions from both fodder and grain aqueous extracts (after soil non-protected treatment) were used for characterisation of natural products. Natural products were derived from the incorporation of $^{14}CO_2$ and other one carbon fragments of ^{14}C-glyphosate into plant constituents. The Conjugate and Glyphosate Fractions from fodder were used for isolation and identification of glyphosate, N-glyceryl-AMPA and conjugates of AMPA and glyphosate. The AMPA Fraction from grain extract was used for identification of the AMPA metabolite.

Large scale extraction and fractionation of fodder (after soil non-protected treatment)
The ground fodder was extracted with water. The aqueous extract was concentrated and the concentrate was analysed by SAX HPLC. The aqueous concentrate was then taken through a fractionation and clean-up scheme to provide material for identification of radioactive compounds. Clean-up of the extracts was carried out by passing the acidified extract through a column containing Chelex® 100 resin (iron form). Phosphonate-containing compounds are bound to the resin, and non-phosphonate-containing materials are not retained. The retained phosphonate-containing compounds, in the form of their iron salts, were then eluted from the column with hydrochloric acid. Iron was removed from the eluate by passage through AG1-X8 anion exchange resin (chloride form). The phosphonate-containing compounds were then separated on a cation exchange column with AG50W-X8 resin (hydrogen form) into non-retained and retained fractions. Thus, the Chelex® column fractionation separated the initial aqueous extract into two fractions: Chelex® non-retained (Natural Product Fraction) and the Chelex® retained fraction.
The Chelex® retained fraction was further fractionated by cation exchange chromatography into three main fractions: conjugate fraction (cation non-retained), glyphosate fraction and AMPA fraction (cation retained, in order of elution):
The column was eluted with water, and 85 fractions were collected. Fractions 19-29 (conjugate fraction), and fractions 34-73 (glyphosate fraction) were used for isolation and identification of glyphosate, N-glyceryl-AMPA, and AMPA/glyphosate conjugates.
The cation column was then eluted with 1 N HCl and 64 fractions were collected. Fractions 39-53 (AMPA fraction) were pooled and analysed by SAX HPLC/LSC.
Components of the conjugate fraction from fodder were isolated using RP-PIC HPLC for further identification/characterisation.
The radiochromatograms from the analysis of isolated fractions by SAX HPLC/LSC were compared with that from the analysis of the initial whole aqueous extract under the same conditions to establish that all of the major radiolabelled compounds in the whole aqueous extract were present in the isolated fractions.

Large scale extraction and fractionation of grain (after soil non-protected treatment)
Ground grain was extracted three times with hexane followed by extraction four times with water.
Hexane extracted, crude maize corn oil was refluxed under nitrogen with 3 % methanolic potassium hydroxide (KOH) for approximately 3 hours. After cooling, the reaction mixture was concentrated to near dryness and transferred with water and diethyl ether into a separatory funnel and extracted. The aqueous solution was extracted two more times with diethyl ether. The ether extracts were phase separated from the aqueous solution, combined, and concentrated to afford a non-saponifiable oil fraction (ether fraction1).
The aqueous solution, which contained the majority of the ^{14}C-activity in the crude oil was acidified to approximately pH 2 with 2 M sulfuric acid (H_2SO_4) and then extracted three times with diethyl ether. The ether extracts were phase separated from the aqueous solution, combined and concentrated to afford a saponifiable free fatty acids fraction (ether fraction 2).
The ether fractions were analysed and analytes identified by GC/MS (via mass spectra and retention time) as well as RP-HPLC/UV followed by GC-MS.

The combined aqueous extracts after hexane extraction were concentrated and acidified to pH 2 with HCl. The acidified aqueous extract was then loaded onto a Chelex® column. The column was then eluted with water. The initial load and water eluate were collected and concentrated (natural product fraction). The natural product fraction was analysed by SAX HPLC/LSC and amino HPLC.
The Chelex column was then eluted with 6 N HCl. Aliquots 1-5 were used for isolation and identification of glyphosate-derived metabolites.

The Chelex® retained fraction was concentrated and acidified with concentrated HCl and then eluted through an anion exchange column and the column was washed with 6 N HCl. The eluate was concentrated to dryness and re-dissolved in water. The sample was then placed on a cation exchange column. The column was eluted with water, and 72 fractions were collected; and the remaining aqueous eluent as a large fraction. Fractions were separately pooled to get the conjugate fraction, glyphosate fraction, and AMPA fraction. These fractions were used for isolation and identification of metabolites.
The cation column was then eluted with 1 N HCl, and 72 fractions were collected and the remaining eluent in as a large fraction. The HCl eluents contained only negligible amount of radioactivity and were not further analysed.
The radiochromatograms from the analysis of isolated fractions by SAX HPLC/LSC were compared with that from the analysis of the initial whole aqueous extract under the same conditions to establish that all of the major radiolabelled compounds in the whole aqueous extract were present in the isolated fractions.

Remaining radioactive residues (RRR) of grain (after conventional extraction with hexane followed by water) were sequentially hydrolysed using protease, amylase, and cellulose as well as by acidic hydrolysis (2 N HCl).

II. Results and discussion

A. Total radioactive residues (TRRs)

Radioactive residue in ^{14}C-treated corn forage, silage and fodder ranged from 9.11 mg/kg to 14.9 mg/kg for protected treatment, and 9.59 mg/kg to 19.1 mg/kg for non-protected treatment.
Maize/corn grain contained much lower levels of radioactivity; radioactive residues in ^{14}C-treated grain were 0.685 mg/kg and 1.04 mg/kg for protected and non-protected treatments, respectively.

Radioactive residue in control forage, silage and fodder ranged from 0.002 mg/kg to 0.043 mg/kg for protected treatment and 0.017 mg/kg to 0.129 mg/kg for non-protected treatment. Radioactive residue in control grain was 0.015 mg/kg and 0.064 mg/kg for protected and non-protected treatments, respectively.

Radioactive residues in control samples are derived from $^{14}CO_2$ re-incorporation into plant constituents. The re-incorporation of $^{14}CO_2$ is substantiated by the fact that control plant tissue from non-protected treatment had higher radioactive residue compared to tissue from protected treatment, due to presence of more $^{14}CO_2$ from soil metabolism of ^{14}C-glyphosate. The higher residues observed in control fodder and grain compared to control forage suggest a gradual re-incorporation of $^{14}CO_2$ into plant tissue over periods of time. Since control plants contained only low levels of radioactive residues, the contribution of $^{14}CO_2$ uptake to total residues in ^{14}C-treated groups is small.

Table 6.2.1-130: Total radioactive residues in maize/corn commodities

Sample description	DALT (days)	Soil protected		Soil non-protected	
		TRR (direct combustion) (mg eq./kg)[1]			
		treated	control	treated	control
Forage	37	13.3	0.002	10.8	0.017
Silage	49-53[2)]	9.11	0.011	9.59	0.049
Fodder	83	14.9	0.043	19.1	0.129
Grain	83	0.685	0.015	1.04	0.064

DALT days after last treatment
TRR Total radioactive residue (determined after combustion; mean of three replicates)
[1] Residue data are expressed as mg/kg glyphosate acid equivalents
[2] Silage sample from soil non-protected plot was collected at 49 DALT and from soil protected plot was collected at 53 DALT.

B. Extraction and characterisation of residues

Rinsing:
In the first experiment the amount of radioactivity present on the surface of forage and silage samples taken at DALT 3 and 49-53 respectively was investigated. The residues were rinsed with distilled water.
For forage from the unprotected plot 3.66 mg/kg of the radioactive residue was rinsed by water as unabsorbed surface residue. Total radioactive residues of the rinsed forage revealed that 5.35 mg/kg. For forage from the protected plot accounting the water rinse removed 4.58 mg/kg of the activity, leaving 4.87 mg/kg associated with the plant tissue. Analysis of the silage showed that 5.44 mg/kg was removed after the distilled water rinse. Total radioactive residues measured in the rinse represented 1.24 mg/kg of the radioactive residue in the silage sample. After rinsing with distilled water the silage sample from the protected plot contained 4.53 mg/kg, while 0.711 mg/kg remained in rinsed silage.
The results show that high amounts of the total radioactive residue in forage and silage were not absorbed by the corn plants; 20 %-40 % of the total radioactive residues of forage and silage were determined in the rinse).

Extraction:
In a second step the radioactive residues in forage, silage, fodder and grain were extracted after grounding. Ground forage, silage and fodder samples were extracted with water. Ground grain samples were first extracted with hexane to remove oil, and then with water.

The table below summarises the results for aqueous extraction of forage, silage, fodder and grain after treatment with soil protection.
The aqueous extract of forage (in the soil protected treatment) represented 96.2 % of the TRR (12.8 mg/kg), 93.5 % of the TRR (8.52 mg/kg) for silage, 95.2 % of the TRR (14.2 mg/kg) for fodder and 77.7 % of the TRR (0.532 mg/kg) for grain. For grain a previous extraction with hexane released 1.5 % of the TRR (0.010 mg/kg) resulting in a total extractability of 79.2 % of the TRR (0.542 mg/kg).
Unextractable residues accounted for 2.9 % of the TRR (0.379 mg/kg) for forage, 4.4 % of the TRR (0.403 mg/kg) for silage, 4.5 % of the TRR (0.676 mg/kg) for fodder and 20.9 % of the TRR (0.143 mg/kg) for grain. The sum of extracted and non-extracted residues ranged between 97.9 to 100.1 % of the TRR.

The results for aqueous extraction of forage, silage, fodder and grain after treatment without soil protection are summarised below.
The aqueous extract of forage (in the soil non-protected treatment) represented 93.0 % of the TRR (10.0 mg/kg), 86.8 % of the TRR (8.33 mg/kg) for silage, 94.4 % of the TRR (18.0 mg/kg) for fodder and 79.9 % of the TRR (0.831 mg/kg) for grain. For grain a previous extraction with hexane released 1.2 % of the TRR (0.012 mg/kg) resulting in a total extractability of 81.1 % of the TRR (0.843 mg/kg).
Unextractable residues accounted for 2.8 % of the TRR (0.307 mg/kg) for forage, 4.5 % of the TRR (0.434 mg/kg) for silage, 5.4 % of the TRR (1.03 mg/kg) for fodder and 23.2 % of the TRR (0.242 mg/kg) for grain. The sum of extracted and non-extracted residues ranged between 91.4 to 104.4 % of the TRR.

The extraction and fractionation procedure of the large scale experiment for fodder and grain (after non-protected soil treatment) is summarised below. The extracts and fractions obtained were used for compound isolation followed by identification. The radiochromatograms from the analysis of isolated fractions by SAX HPLC/LSC were compared with that from the analysis of the initial whole aqueous extract under the same conditions to establish that all the major radiolabelled compounds in the whole aqueous extract were present in the isolated fractions. In addition, the residual radioactive residues of grain after hexane and aqueous extraction were further analysed using enzymatic and acidic hydrolysis techniques. The corn oil (hexane extract) was saponified. Resulting fractions were analysed for metabolites.

Table 6.2.1-131: Extraction of the radioactive residues of ^{14}C-glyphosate in corn forage, silage, fodder and grain after soil protected treatment

	Soil protected ^{14}C-treatment							
	Forage		Silage		Fodder		Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**13.3**	**100.0**	**9.11**	**100.0**	**14.9**	**100.0**	**0.685**	**100.0**
Hexane extract	-	-	-	-	-	-	0.010	1.5
Aqueous extract	12.8	96.2	8.52	93.5	14.2	95.2	0.532	77.7
ERR	**12.8**	**96.2**	**8.52**	**93.5**	**14.2**	**95.2**	**0.542**	**79.2**
RRR	**0.379**	**2.9**	**0.403**	**4.4**	**0.676**	**4.5**	**0.143**	**20.9**
Accountability	**13.2**	**99.1**	**8.92**	**97.9**	**14.86**	**99.7**	**0.685**	**100.1**

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR Extractable radioactive residue
RRR Residual radioactive residue
Accountability Sum of radioactivity in extracts and residual radioactive residue
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

Table 6.2.1-132: Extraction of the radioactive residues of ^{14}C-glyphosate in corn forage, silage, fodder and grain after soil non-protected treatment

	Soil non-protected ^{14}C-treatment							
	Forage		Silage		Fodder		Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	10.8	100	9.59	100	19.1	100	1.04	100
Hexane extract	-	-	-	-	-	-	0.012	1.2
Aqueous extract	10.0	93.0	8.33	86.8	18.0	94.4	0.831	79.9
ERR	**10.0**	**93.0**	**8.33**	**86.8**	**18.0**	**94.4**	**0.843**	**81.1**
RRR	**0.307**	**2.8**	**0.434**	**4.5**	**1.03**	**5.4**	**0.242**	**23.2**
Accountability	**10.4**	**95.8**	**8.77**	**91.4**	**19.0**	**99.7**	**1.09**	**104.4**

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR Extractable radioactive residue
RRR Residual radioactive residue
Accountability Sum of radioactivity in extracts and residual radioactive residue
Values in *italics* were calculated from reported values upon dossier compilation
Minor deviations may occur due to rounding

The distribution of radioactive compounds identified/characterised in commodities of maize/corn (forage, silage, fodder and grain) are summarised in the tables below.

The radioactive components of aqueous extracts were mainly glyphosate and AMPA (total of 61.5 % to 90.3 % of the TRR). Glyphosate was observed to be the major radioactive residue in forage, silage and fodder accounting for 67.1 to 83.3 % of the TRR (6.43 – 14.27 mg/kg), whereas only low levels of glyphosate were present in grain (2.6 – 7.4 % of the TRR, 0.03 – 0.05 mg/kg). In contrast, AMPA was found at 4.9 % to 15.9 % of the TRR in forage, silage and fodder (0.73 – 2.13 mg/kg), and 54.1 % to 60.3 % of the TRR (0.37 – 0.63 mg/kg) in grain.
Aqueous extracts also contained N-glyceryl-AMPA accounting for 0.4 % to 1.6 % of the TRR (0.05 – 0.31 mg/kg) in forage, silage and fodder and 6.9 % of the TRR (0.05 – 0.07 mg/kg) in grain and low levels (<2 % of TRR, 0.04 – 0.36 mg/kg) of other glyphosate conjugates in forage, silage and fodder, while they were not present in grain. Trace levels of other AMPA conjugates are mentioned.

In addition to this, aqueous extracts contained ^{14}C-labelled natural products (<3.6 % of the TRR). The radioactive natural products were derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from N-(phosphono-^{14}C-methyl)glycine into plant constituents.

Corn oil (hexane extract of grain) was subjected to saponification. The ether fraction 1 (fraction after refluxing with 3 % methanolic KOH followed by ether extraction) should contained nonsaponifiable fatty acids of corn oil and was further investigated by RP HPLC/LSC followed by GC/MS. The fraction was analysed by RP-HPLC for cholesterol by comparison with retention times, indicating that some of the minor peaks may be sterols, but that the majority of the radioactivity in the fraction was not sterol related. The major radioactivity of the HPLC chromatogram was collected and analysed by GC-MS. The mass spectra of three compounds matched with the mass spectra of: 12-hydroxy-9-octadecenoic acid methylester; 9,11-octadecadienoic acid ($C_{18:2}$); and 9-octadecenoic acid methyl ester ($C_{18:1}$). Thus, the fatty acids found in this fraction could be derived from incomplete separation at the ether partitioning step. The radioactivity in ether fraction 2 should contain saponifiable fatty acids of corn oil. The fraction was found to be associated with palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$) as determined by GC/MS and RP HPLC/LSC (comparison of retention times and mass spectra). The distribution of radiolabelled fatty acids closely matched the naturally occurring composition of the major fatty acids in corn oil.

The aqueous fraction 2 refers to the polar aqueous soluble metabolite fraction. The fraction was analysed by HPLC and GC-MS after derivatisation. The fraction contained products that could be acetylated with acetic anhydride; however, the acetylated products were not amenable to thorough characterisation. However, this is acceptable based on the low radioactivity in this fraction.

Bound residue was less than 5.4 % of the TRR in forage, silage and fodder, while it was 20.9 to 23.2 % of the TRR for grain after conventional extraction. The remaining residues of grain (of the large scale experiment; PES-1) accounted for 25.27 % of TRR (0.263 mg/kg) which were sequentially hydrolysed using protease, amylase, and cellulose in a first experiment and hydrolysed under acidic conditions in a second experiment.

The enzymatic hydrolysis released 33.49 %, 19.72 %, and 19.6 % of the bound radioactivity, respectively (corresponding 8.46, 4.98, 4.95 % of the TRR (0.088, 0.052 and 0.052 mg/kg, respectively). The remaining residues after sequential enzyme hydrolysis of grain contained 34.27 % (8.66 % of the TRR, 0.090 mg/kg) of the bound radioactivity.

Since com grain contains mostly starch in mass, the starch could be hydrolysed by dilute acid and converted to glucose as a monosaccharide. In addition, starch may be slightly soluble in aqueous buffer solution during enzyme hydrolysis; therefore, several enzymatic buffer solutions may contain starch dissolved radioactivity. Thus, it was decided that the bound residues after hexane and water extraction (PES-1) should be further investigated by acid hydrolysis. No further work was done on the enzyme hydrolysates.

Hydrolysis of extracted grain by 2 M HCl for about 4 hours, resulted in the release of 90.24 % of the bound radioactivity (22.8 % of the TRR; 0.237 mg/kg) and the remaining residues 7.36 % of the bound radioactivity (accounting for 1.86 % of the TRR (0.019 mg/kg). The major radioactive component of the acid hydrolysate matched with the retention time of commercial ^{14}C-glucose. The acetyl derivative of the major hydrolysate component had similar chromatographic and mass spectral (GC/MS) properties as the acetyl derivative of ^{14}C-glucose. Thus, the majority of the acid released radioactivity was associated with glucose, resulting from the incorporation of $^{14}CO_2$ and other one carbon fragments of ^{14}C-glyphosate into starch.

Table 6.2.1-133: Large-scale extraction followed by fractionation of the radioactive residues of glyphosate in maize/corn fodder and grain after soil non-protected treatment

	Soil non-protected ^{14}C-treatment			
	Fodder		Grain	
	mg/kg	% TRR	mg/kg	% TRR
TRR	19.1	100.0	1.04	100.0
Organic extraction				
Hexane extract	-	-	0.010	0.98
Hexane concentrate	-	-	0.010	1.00
Saponification (reflux with 3 % methanolic KOH for 3 hours, N_2)				
Ether fraction 1[3]	-	-	*0.001*	0.14
Aqueous fraction 1	-	-	Not reported	Not reported
Ether fraction 2[4]	-	-	*0.011*	1.04
Aqueous fraction 2 [5]	-	-	*0.002*	0.15
Water extraction				
Water extract	16.91	88.51	0.803	77.22
Aqueous concentrate	17.33	90.72	-	-
Chelex chromatography of water extract				
Column effluent (water wash and 0.1 N HCl) Natural products	0.303	1.59	0.056	5.37
6 N HCl eluate	15.97[1]	83.61[1]	0.665[1]	63.91[1]
Anion exchange chromatography				
6 N HCl eluate	15.34	80.3	0.621	59.69
Concentrate of eluate	15.19	79.52	-	-
Cation exchange chromatography				
Elution with water/ 1 N HCl				
Conjugate Fraction[2]	0.164	0.86	0.076	7.33
Glyphosate Fraction[2]	9.82	51.39	0.019	1.8
AMPA fraction[2]	2.82	14.74	0.522	50.14
Total extractable	16.91	88.51	0.803	77.22
RRR	0.863	4.52	0.263	25.27
Sequential hydrolysis of solids				
Protease	-	-	*0.088*	*8.46* (33.49)
Amylase	-	-	*0.052*	*4.98* (19.72)
Cellulase	-	-	*0.052*	*4.95* (19.6)
Solids	-	-	*0.090*	*8.66* (34.27)
Acidic hydrolysis 2 N HCl, 4 hours of solids				
Hydrolysate[6]	-	-	*0.237*	*22.8* (90.24)
Solids	-	-	*0.019*	*1.86* (7.36)
ERR	**16.91**	**88.51**	***1.04***	***100.02***
Final residues	**0.863**	**4.52**	***0.019***	***1.86***
Accountability	***17.773***	**93.03**	***1.059***	***103.47***

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
ERR Extractable radioactive residue after conventional extraction (fodder) and conventional and exhaustive extraction (grain)
RRR Residual radioactive residue after conventional extraction
Accountability Sum of radioactivity in extracts and residual radioactive residue
(a): Refers to the first aliquot (fodder) or aliquot 1-5 (grain) after elution with 6 N HCl. The extract was used for isolation and identification of phosphate-containing metabolites.
(b): Conjugate fraction and glyphosate fraction of fodder were used for isolation and identification of glyphosate, N-glyceryl-AMPA, and AMPA/glyphosate conjugates. The AMPA fraction from grain extract was used for identification of the AMPA metabolite.
(c): The radioactivity in ether fraction 1 was found to be associated with nonsaponifiable fatty acids such as sterols, also contained radioactivity that was fatty acid related, possible due to incomplete partitioning (RP-HPLC, and GC-MS).
(d): The radioactivity in ether fraction 2 contained saponifiable fatty acids of corn oil and was found to be associated with palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$) as determined by GC/MS and RP HPLC/LSC.
(e): Aqueous fraction 2 refers to the polar aqueous soluble metabolite fraction. The fraction was analysed by HPLC and

Table 6.2.1-133: Large-scale extraction followed by fractionation of the radioactive residues of glyphosate in maize/corn fodder and grain after soil non-protected treatment

	Soil non-protected ^{14}C-treatment			
	Fodder		Grain	
	mg/kg	% TRR	mg/kg	% TRR

GC-MS after derivatisation. The fraction contained products that could be acetylated with acetic anhydride; however, the acetylated products were not amenable to thorough characterisation. However, this is acceptable based on the low radioactivity in this fraction.

(f): The acidic hydrolysate was neutralised, purified by C_{18} SPE followed by derivatisation with N, O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and analysed by GC/MS, which indicated the presence of sugars. The sample was also analysed by RP-HPLC followed by NH_2-HPLC (selected fraction). The major broad radioactive region matched with ^{14}C-glucose. Derivatisation by acetic anhydride and re-analysis by RP-HPLC, and GC-MS confirmed the presence of glucose.

Values in *italics* were calculated from reported values upon dossier compilation. Minor deviations may occur due to rounding.

Values in brackets represent percentage of radioactivity related in the residual radioactive residues (PES-1) after conventional extraction (values given in the report).

Table 6.2.1-134: Distribution of radioactive residues of glyphosate in maize/corn following soil protected treatment

	Soil protected ^{14}C-treatment							
	Forage		Silage		Fodder		Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	13.3	100.0	9.11	100.0	14.9	100.0	0.685	100.0
Parent glyphosate[1]	10.8	80.9	7.09	77.9	12.4	83.3	0.05	7.4
Metabolite (AMPA)[2]	1.25	9.4	0.82	9.0	0.73	4.9	0.37	54.1
Metabolite (N-glyceryl AMPA)[3]	0.05	0.4	0.11	1.2	0.17	1.2	0.05	6.9
Conjugates[3]	0.05	0.4	0.06	0.7	0.2	1.3	-	-
Natural products[4]	0.25	1.9	0.23	2.6	0.36	2.4	0.02	3.2
Fatty acids[5]	-	-	-	-	-	-	0.009	1.30
Starch[5]	-	-	-	-	-	-	0.13	18.80
Total identified	***12.10***	***90.7***	***8.02***	***88.1***	***13.3***	***89.4***	***0.47***	***68.4***
Total characterised	***0.30***	***2.3***	***0.29***	***3.3***	***0.56***	***3.7***	***0.159***	***23.3***
Total identified+characterised	**12.4**	**92.9**	**8.32**	**91.4**	**13.8**	**93.1**	**0.63**	**91.8**
ERR	**12.8**	**96.2**	**8.52**	**93.5**	**14.2**	**95.2**	***0.542***[6]	***79.2***[6]
RRR	**0.379**	**2.9**	**0.403**	**4.4**	**0.676**	**4.5**	**0.143**[6]	**20.9**[6]

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate equivalents))

Values in *italics* were calculated from reported values upon dossier compilation. Minor deviations may occur due to rounding.

ERR: Extractable radioactive residue after conventional aqueous extraction (including hexane phase for grain)

RRR: Residual radioactive residue after conventional

1 In case of analysis of glyphosate the values given in the table represent the average values after SAX and CX HPLC analysis:
Forage: SAX: 10.74 mg/kg; 80.8 % of the TRR and CX: 10.75 mg/kg; 80.9 % of the TRR.
Silage: SAX: 6.96 mg/kg; 76.4 % of the TRR and CX: 7.22 mg/kg; 79.3 % of the TRR.
Fodder: SAX: 12.28 mg/kg; 82.4 % of the TRR and CX: 12.53 mg/kg; 84.1 % of the TRR.
Grain: SAX: 0.04 mg/kg; 6.1 % of the TRR and CX: 0.06 mg/kg; 8.7 % of the TRR.

2 AMPA: results after CX HPLC

3 N-glyceryl AMPA and glyphosate conjugates: results after SAX HPLC (identity confirmed by TLC co-chromatography)

4 Calculated from SAX HPLC data (natural products + AMPA peak) and CX HPLC data (AMPA peak)

5 Based on the information available in the report only the remaining radioactive residues after hexane and aqueous extraction as well as the corn oil (hexane phase) of corn grain taken from the large scale experiment were analysed further. However, the report also states values for fatty acids and starch for the samples which were stated as only conventionally extracted. The given data are therefore assumed to be recalculated.

6 Results of fatty acids and starch are not considered. Residual remaining solids may be quite lower after acidic hydrolysis (as shown in the large scale experiment for grain)

Table 6.2.1-135: Distribution of radioactive residues of glyphosate in maize/corn following soil non-protected treatment

	Soil non-protected ^{14}C-treatment							
	Forage		Silage		Fodder		Grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	10.8	100.0	9.59	100.0	19.1	100.0	1.04	100.0
Parent glyphosate[1]	7.77	71.9	6.43	67.1	14.27	74.8	0.03	2.6
Metabolite (AMPA)[2]	1.72	15.9	1.26	13.1	2.13	11.2	0.63	60.3
Metabolite (N-glyceryl AMPA)[6]	0.06	0.5	0.14	1.5	0.31	1.6	0.07	6.9
Conjugates[3]	0.04	0.4	0.04	0.4	0.36	1.9	-	-
Natural products[4]	0.24	2.2	0.34	3.5	0.65	3.4	0.04	3.6
Fatty acids[5]	-	-	-	-	-	-	0.01	1.0
Starch[5]	-	-	-	-	-	-	0.22	20.9
Total identified	***9.55***	***88.3***	***7.83***	***81.7***	***16.71***	***87.6***	***0.73***	***69.8***
Total characterised	***0.28***	***2.6***	***0.38***	***3.9***	***1.01***	***5.3***	***0.27***	***25.5***
Total identified+characterised	**9.8**	**91.0**	**8.2**	**85.6**	**17.7**	**92.8**	**1.0**	**95.3**
ERR	**10.0**	**93.0**	**8.33**	**86.8**	**18.0**	**94.4**	***0.843***[6]	***81.1***[6]
RRR	**0.307**	**2.8**	**0.434**	**4.5**	**1.03**	**5.4**	**0.242**[6]	**23.2**[6]

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate equivalents))
Values in *italics* were calculated from reported values upon dossier compilation. Minor deviations may occur due to rounding.
ERR: Extractable radioactive residue after conventional aqueous extraction (including hexane phase for grain
RRR: Residual radioactive residue after conventional

[1] In case of analysis of glyphosate the values given in the table represent the average values after SAX and CX HPLC analysis:
Forage: SAX: 7.78 mg/kg; 72.0 % of the TRR and CX: 7.75 mg/kg; 71.8 % of the TRR.
Silage: SAX: 6.31 mg/kg; 65.8 % of the TRR and CX: 6.55 mg/kg; 78.6 % of the TRR.
Fodder: SAX: 13.99 mg/kg; 73.3 % of the TRR and CX: 14.55 mg/kg; 76.2 % of the TRR.
Grain: SAX: 0.01 mg/kg; 1.1 % of the TRR and CX: 0.04 mg/kg; 4.2 % of the TRR.

[2] AMPA: results after CX HPLC

[3] N-glyceryl AMPA and glyphosate conjugates: results after SAX HPLC (identity confirmed by TLC co-chromatography)

[4] Calculated from SAX HPLC data (natural products + AMPA peak) and CX HPLC data (AMPA peak)

[5] Based on the information available in the report only the remaining radioactive residues after hexane and aqueous extraction as well as the corn oil (hexane phase) of corn grain taken from the large scale experiment were analysed further. However, the report also states values for fatty acids and starch for the samples which were stated as only conventionally extracted. The given data are therefore assumed to be recalculated.

[6] Results of fatty acids and starch are not considered. Residual remaining solids may be quite lower after acidic hydrolysis (as shown in the large scale experiment for grain).

C. Storage stability

Storage stability of aqueous extract as well as stored sample material was demonstrated in this study by comparing the HPLC analyses of aqueous extracts of forage, fodder, and grain. The initial aqueous extraction for each sample was conducted shortly after harvest. The aqueous extracts were then analysed by HPLC. All initial HPLC analyses were conducted within 31 days after harvest of each sample. These initial extractions and analyses were used for the definitive quantitation in the study.

Towards the end of the study, aliquots of forage, fodder, and grain samples that had been maintained in frozen storage (-20 °C or lower) were again extracted and analysed in the same manner. In addition, aqueous extracts were re-analysed after frozen storage over periods of time for storage stability determination. Shortly after harvest and again following completion of the experimental phase of the study forage, fodder, and grain after soil-treatment were extracted. The extracts and extracted samples were analysed to determine the distribution of radioactivity. Prior to extraction, aliquots of the samples were combusted to determine initial residues. The aqueous extracts were analysed by SAX HPLC. To determine the stability of the radioactive compounds in the aqueous extracts following frozen storage, extracts stored over long periods of time were analysed by SAX HPLC/LSC and compared with the HPLC profiles of fresh extracts.

The results show that there was no significant degradation in either the stored samples or the aqueous extracts over the course of the study. New metabolite fractions were not observed to form over the course of the study, nor did the distribution of radioactivity among metabolite fractions change significantly.
Storage stability analyses thus demonstrated that glyphosate-derived residues were chemically stable up to 319, 250 and 264 days in frozen samples of forage, fodder and grain respectively and that residues in aqueous extracts are stable up to 324, 253 and 265 days for forage, fodder and grain respectively.

Table 6.2.1-136: Extraction of the radioactive residues of glyphosate in forage, fodder and grain– storage stability assessment

	Forage (protected soil)		Fodder (non-protected soil)			Grain (non-protected soil)	
Storage interval[1]	**0 days**	**319 days**	**0 days**	**36 days**	**250 days**	**0 days**	**264 days**
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
TRR	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Hexane extract	-	-	-	-	-	1.2	1.0
Aqueous extract	96.2	112.9	94.4	88.5	88.4	79.9	71.1
RRR	**2.9**	**3.3**	**5.4**	**4.5**	**4.8**	**23.2**	**24.0**
Accountability	**99.1**	**116.2**	**99.7**	**93.0**	**93.2**	**104.4**	**96.1**

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
RRR Residual radioactive residue
Accountability Sum of radioactivity in extracts and residual radioactive residue
[1] refers to the interval between first and intermediate or final hexane/water extraction.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusions

The nature of the residues in plants following the use of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) formulated as Roundup® was studied in maize/corn, which was modified to express CP4 EPSPS and GOX proteins. An isomeric mixture of glyphosate (labelled in the phosphonomethyl-moiety with ^{12}C, ^{13}C or ^{14}C), was applied as two foliar applications at rates equivalent to 0.93 kg glyphosate acid equivalents/ha (5 – 6 leaf stage corresponding to BBCH 15 – 16) and 0.84 kg glyphosate acid equivalents/ha (10 – 12 leaf stage corresponding to BBCH 19, 4 weeks later). To distinguish between foliar and root uptake duplicate experiments were conducted either covering the soil (protected) or not (unprotected).
Samples were collected immediately after each treatment and in the forage (3 DALT), silage (49 and 53 DALT) and maturity growth stage (grain and fodder, 83 DALT).
Total radioactive residue in ^{14}C-treated maize/corn forage, silage and fodder ranged from 9.11 mg/kg to 14.9 mg/kg for protected treatment, and 9.59 mg/kg to 19.1 mg/kg for non-protected treatment.
Maize/corn grain contained much lower levels of radioactivity; radioactive residues in ^{14}C-treated grain were 0.685 mg/kg and 1.04 mg/kg for soil protected and non-protected treatments, respectively.
Glyphosate was observed to be the major radioactive residue in forage, silage and fodder accounting for 67.1 to 83.3 % of the TRR (6.43 – 14.27 mg/kg), whereas only low levels of glyphosate were present in grain (2.6 – 7.4 % of the TRR). In contrast, AMPA was found at approximately 4.9 % to 15.9 % of the

TRR in forage, silage and fodder (0.73 – 2.13 mg/kg), and 54.1 % to 60.3 % of the TRR (0.37 – 0.63 mg/kg) in grain.
Aqueous extracts also contained N-glyceryl-AMPA accounting for 0.4 % to 1.6 % of the TRR (0.05 – 0.31 mg/kg) in forage, silage and fodder and 6.9 % of the TRR (0.05 – 0.07 mg/kg) in grain and low levels (<2 % of TRR, 0.04 – 0.36 mg/kg) of other glyphosate conjugates in forage, silage and fodder, while they were not present in grain. Trace levels of other AMPA conjugates are mentioned.
In addition to this, aqueous extracts contained ^{14}C-labelled natural products (<3.6 % of the TRR). The radioactive natural products were derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from ^{14}C-glyphosate into plant constituents.
The radioactivity in oil extracted from grain was shown to be associated with naturally occurring fatty acids.
Remaining radioactive residues after conventional extraction were less than 5.4 % of the TRR in forage, silage and fodder, while they accounted for up to 25.27 % of the TRR (0.263 mg/kg) in grain. However, greater than 90 % of the remaining radioactivity in grain was released by acid hydrolysis and was found to be associated with starch resulting in remaining residues of only 1.86 % of the TRR (0.019 mg/kg).

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:
The study assessing the metabolic behaviour of glyphosate maize/corn has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with minor deficits (no full description of the fractionation as well as detailed flow charts are available for acidic hydrolysis of the remaining radioactive residues of grain after conventional (hexane/aqueous) extraction as well as saponification of corn oil for the normal scale experiments. However, as details are given for a large scale experiment of grain sample the mentioned results are considered generally followable). Therefore, the study is considered reliable and supports the uses of the crop category cereal/grass crops.

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

Pulses and oilseeds

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/021
Report author	[redacted] *et al.*
Report year	1995
Report title	Nature of Glyphosate Residues in Roundup® Herbicide Tolerant Canola
Report No	MSL-13318
Document No	Not available
Guidelines followed in study	Pesticide Assessment Guideline Number 171-4(a) of Subdivision O: Nature of the Residue - Plant Study
Deviations from current test guideline	A review of this study indicates no deviations from OECD Guideline for the Testing of Chemicals, 501.

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

2. Full summary of the study according to OECD format

Executive summary

The nature of the residues in glyphosate tolerant canola following the use of glyphosate was studied. Two different treatment regimens were utilised. Each line of Roundup® herbicide tolerant canola plants (GT73 and GT200) received two different treatment regimens. The first regimen involved a single broadcast application at a rate of 0.455 kg of glyphosate/ha at BBCH 12-14 (2-4 leaf growth stage, 14 days after planting). For the second regimen, canola plants received two sequential applications of Roundup® herbicide, each at a rate of approximately 0.90 kg a.s./ha. The first application was at BBCH 12-14 (2-4 leaf growth stage, 14 days after planting), and the second was at BBCH 16 (6-leaf growth stage, 22 days after planting).

Canola seed, the only raw agricultural commodity for canola, was harvested and dried in a manner similar to normal agronomic practices. Plants that received the single application were harvested 87 days after application of the test substance. Plants that received the two sequential applications were harvested 79 days after the last application of the test substance.

The total radioactive residue (TRR) in canola seed samples taken 87 days after single early post-emergence application of 0.455 kg a.s./ha were 0.483 and 0.845 mg/kg in GT73 and GT200 lines, respectively. The control samples contained only 0.027 and 0.075 mg/kg, suggesting that $^{14}CO_2$ uptake did not make a significant contribution to the total residues in the seed samples.

The total radioactive residue (TRR) in canola seed samples taken 79 days after sequential post-emergence application 0.908 and 0.905 kg a.s./ha were 8.093 and 4.876 mg/kg in GT73 and GT200 lines, respectively. The control samples contained only 0.027 and 0.077 mg/kg.

Preliminary extractions and HPLC analyses demonstrated that there were no significant differences in glyphosate metabolism between GT73 and GT200 canola. Full identification and characterisation of residues was conducted only with samples from the commercial candidate line, GT73.

Extraction of canola seeds after early post-emergence application with hexane and water yielded 25.5 % TRR (0.123 mg/kg). In the aqueous fraction of canola seeds after early post-emergence application 7.7, 3.4, 0.9 and <2 % TRR (0.037, 0.017, 0.004 and <0.01 mg/kg) AMPA, N-glyceryl-AMPA, N-acetyl-AMPA and sucrose were identified, respectively. Moreover, 4.9 % TRR (0.024 mg/kg) was characterised as natural products.

Extraction of canola seeds after sequential post-emergence application with hexane and water yielded 26.5 % TRR (2.14 mg/kg). In the aqueous fraction 7.1, 3.9, 0.7 and 1.6 % TRR (0.58, 0.31, 0.06 and 0.13 mg/kg) AMPA, N-glyceryl-AMPA, N-acetyl-AMPA and sucrose were identified, respectively. Moreover, 3.7 % TRR (0.30 mg/kg) was characterised as natural products. In the hexane fraction 1.6 % TRR (0.13 mg/kg) were characterised as saponifiable fatty acids.

The non-extracted residues amounted to 78.8 % (6.38 mg/kg). The results of attempts to release non-extracted residues under mild conditions show that water, dilute acid (0.1 N HCl), DMF, an aqueous complexing agent (0.1 M EDTA) and an aqueous surfactant (1 % sodium lauryl sulfate with sonication) each released less than less than 5.8 % TRR (0.47 mg/kg).

Sequential enzymatic hydrolysis of the extracted meal with protease, amylase, and cellulase released 5.9, 0.9 and 1.7 % TRR (0.48, 0.07 and 0.14 mg/kg), respectively. Sequential digestion of the extracted meal with simulated gastric fluid (SGF) followed by simulated intestinal fluid (SIF) released 6.2 and 2.6 % TRR (0.51 and 0.21 mg/kg), respectively. Simulated gastric and intestinal fluid digestions, or sequential hydrolyses with protease, amylase, and cellulase, released only about 9 % TRR. These results suggest that

only a small fraction of the ^{14}C-glyphosate-derived components in extracted meal would be biologically available if ingested by animals.

Extraction with dioxane and water was used to determine the amount of radioactivity associated with lignin in the extracted meal. The results indicated that less than 5 % TRR is associated with free or bound lignin.

Hydrolysis of extracted canola meal with 6 N HCl at 100 °C for 12 hours released 13.3 % TRR (1.08 mg/kg). Analysis of the acid hydrolysate by reverse phase HPLC showed that approximately 11.6 % TRR (0.94 mg/kg) eluted as a single peak, which was derivatised with n-butanol followed by trifluoroacetic anhydride. The derivatised radioactive compounds contained in the major peak were identified and characterised as naturally occurring amino acids and organic acids. These results demonstrate that significant amounts of the acid extractable bound radioactivity are a result of incorporation of degraded one carbon units of ^{14}C-glyphosate into natural amino acids, sugars, and other biomolecules, and therefore are not of toxicological concern.

Rather harsh base hydrolysis was the only successful method for the release of significant amounts of bound radioactivity. Hydrolysis of extracted meal with 0.1 N NaOH at 100 °C released 39.9 % TRR (3.23 mg/kg). Hydrolysis of extracted canola meal with 2.5 N NaOH at 85 °C for 65 hours, followed by two extractions of the resulting hydrolysed meal with water at 85 °C, released 63.6 % TRR (5.15 mg/kg).

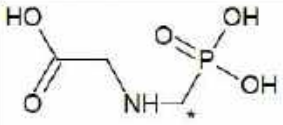
In the base hydrolysates 16.5 and 3.7 % TRR (1.34 and 0.30 mg/kg) formate and AMPA was identified, 14.9 % TRR (1.21 mg/kg) was characterised as natural products and 5.6 % TRR (0.45 mg/kg) remained unknown. 19.7 % (1.59 mg/kg) was characterised as insoluble biopolymers.

Control experiments showed that AMPA is gradually hydrolysed in base, and one of the hydrolysis products is formate. Thus, the base hydrolysis results suggest that a significant amount of the bound residues in meal are due to bound AMPA; upon hydrolysis, the AMPA is released and partially converted to formate.

Thus, results of the numerous experiments to determine the nature of radioactivity in canola meal indicate there are two types of bound radioactivity. One type is the result of incorporation of one carbon ^{14}C fragments of glyphosate into numerous natural products in the seed. The other is postulated to be bound AMPA, which is the primary metabolite of glyphosate in canola.

I. MATERIALS AND METHODS

A. Materials

Test Material:	N-(phosphonomethyl)glycine; mixture of a) N-(phosphono-^{14}C-methyl)glycine b) N-(phosphono-^{13}C-methyl)glycine c) N-(phosphono-^{12}C-methyl)glycine
Chemical structure:	 a, b * Position of label
Radiochemical purity:	98.03 %
Chemical purity:	> 99 %
Specific activity:	1.67 MBq/mg (7.7 mCi/mmol, 100190 dpm/µg)
CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate:	- 3.2

Test system:

Soil:	Silty clay loam (pH: 6.9; cation exchange capacity: 28.9 meq./100 g; bulk density: 1.05 g/cm^3; organic matter: 3.6 %; sand: 14 %; silt: 52 %; clay: 34 %; textural class (USDA): silty clay loam)
Crop:	Rapeseed (Canola) cultivar Westar plants glyphosate tolerant line GT 73 (C4EPSPS and GOXvar) GT200 (C4EPSPS and GOX)
Botanical name:	*Brassica napus*
Crop part(s):	seeds

B. Study design

1. In-life phase

The in-life portion of the study was conducted in controlled environment growth chambers. The test substance was formulated to simulate Roundup herbicide by combining the mixture of ^{12}C-, ^{13}C- and ^{14}C-glyphosate in water with isopropylamine and MON 0818 (ethoxylated tallow amine surfactant used in commercial formulations of Roundup® herbicide). The test substance was obtained by diluting 327.5 mg of ^{14}C-glyphosate (specific activity of 4.36 MBq/mg or 19.93 mCi/mmol, 98.60 % radiochemical purity) with ^{13}C-glyphosate and ^{12}C-glyphosate. The solution was then diluted with water to give the formulated test substance, with a final specific activity of 1.67 MBq/mg.
For this study, two different treatment regimens were utilised. Each line of Roundup® herbicide tolerant canola plants (GT73 and GT200) received two different treatment regimens. The first regimen involved a single broadcast application at a rate of 0.455 kg of glyphosate/ha at BBCH 12-14 (2-4 leaf growth stage, 14 days after planting). For the second regimen, canola plants received two sequential applications of Roundup® herbicide, each at a rate of approximately 0.90 kg a.s./ha. The first application was at BBCH 12-14 (2-4 leaf growth stage, 14 days after planting), and the second was at BBCH 16 (6-leaf growth stage, 22 days after planting).
Within each treatment regimen, one ^{14}C-treated test group and two ^{12}C-treated control test groups were used. The control test groups were treated with non-radiolabelled Roundup® herbicide using the same application rates and timings as those used for ^{14}C-treated test group. One group of control test plants was grown in close proximity to the ^{14}C-treated test plants to allow a determination of the amount of incorporation of $^{14}CO_2$ resulting from microbial degradation of ^{14}C-glyphosate in the soil. The second group of control plants was grown in a separate growth chamber that did not contain any radioactivity.

2. Sampling

Canola seed, the only raw agricultural commodity for canola, was harvested at maturity and dried in a manner similar to normal agronomic practices. Plants that received the single application were harvested 87 days after application of the test substance. Plants that received the two sequential applications were harvested 79 days after the last application of the test substance.
The samples were stored frozen at -10 °C or below until analysis.

3. Analytical procedures

Total radioactive residues (TRR) in all plant samples were determined by LSC following combustion. The limit of detection was 0.001 mg/kg.
Samples of the ground canola seed were extracted three times with hexane. The solid residue was dried under a stream of air, and then was extracted three times with water. The solid was dried by lyophilisation. The solid was analysed by combustion and LSC, and the extracts were analysed by LSC.
The aqueous extracts from the two GT73-90-T extractions were taken through a fractionation and cleanup scheme to provide material for identification of radioactive compounds. Cleanup of the extracts was

carried out by passing the extract through a column containing Chelex® 100 resin. Phosphonate-containing compounds were bound to the resin, and non-phosphonate containing materials were not retained. The retained phosphonate containing compounds were then eluted (in the form of their iron salts) from the column with 6 N hydrochloric acid. Iron was removed from the eluate by passage through AG1-X8 anion exchange. The phosphonate containing compounds were then separated on a cation exchange column with AG50W-X8 resin (hydrogen form) into non-retained and retained fractions.
For identification different methods were employed, such as using co-injection of authentic reference standards, isolation of peaks followed by derivatisation followed by GC-MS and HPLC/MS and comparison with authentic standards as well as mass-spectral analysis.
For some compounds, e.g. sucrose NMR analysis was employed.
To release initially unextracted residues various treatments were performed. First, the one of the following extraction solvents was added to each tube: water; 0.1 N NaOH; 0.1 N HCl; 0.1 M ethylenediamine tetraacetic acid, dipotassium salt (EDTA); dimethyl formamide (DMF); and 1 % sodium lauryl sulfate. The tubes were then shaken at room temperature for 68 hours, the tube with 1 % sodium lauryl sulfate was first sonicated and then shaken for 96 hours. The amount of released activity was determined by LSC analysis of the extracts.
Digestion of extracted meal with simulated gastric fluid (SGF) at 37°C. Additionally control sample was set with blank SGF (no enzyme). After 3 hours the tubes were centrifuged and an aliquot of the supernate was removed from each tube for LSC analysis. The tubes (containing the supernate and the canola meal residue) were allowed to stand for six hours at room temperature and then warmed to 37 °C and placed in an ultrasonic bath for 10 minutes. The samples were heated at 37 °C for 17 hours, sonicated for 10 minutes, and held at 37 °C for another 5 hours. The tubes were centrifuged, and the liquid was decanted and analysed by LSC.
To the solid residue from the SGF digestion simulated intestinal fluid (SIF) was added. The pH was adjusted to pH 7.5 with 0.2 N NaOH. To the residue from the SGF blank digestion SIF blank (no enzyme) was added. The pH was adjusted to pH 7.5 with 0.2 N NaOH. The tubes were incubated at 37 °C for 18 hours, sonicated for 10 minutes, then incubated at 37 °C for another 23 hours. The tubes were centrifuged, and the liquid was decanted and analysed by LSC.
The solid residue was lyophilised and analysed for ^{14}C-activity by combustion analysis.
Enzyme hydrolysis of extracted meal was performed with protease, amylase and cellulase. Extracted meal in 0.1 M sodium phosphate buffer (pH 7.5) was heated to 37 °C. The protease solution in sodium phosphate buffer, adjusted to pH 7.5 was added, and the resulting mixture was incubated with continuous shaking for about one hour at 37 °C. After additional addition of the protease solution incubation was continued for 24 hours. The mixture was then filtered through filter paper. The residue remaining following the protease hydrolysis was then incubated at approximately 30 °C with an amylase solution in 0.1 M sodium phosphate buffer, adjusted to pH 7.0 for about 65.5 hours. The amylase hydrolysis mixture was then filtered through filter paper. The residue remaining following the amylase hydrolysis was then incubated at about 37 °C with a cellulase solution in 0.2 M sodium acetate buffer, adjusted to pH 5.0, for a total of about 48 hours. The cellulase hydrolysis mixture was then filtered through filter paper.
Extraction of extracted meal with dioxane was performed twice with 9:1 dioxane/water solution. The dioxane/water solutions were collected by filtration through filter paper and combined. The solution contained Bjorkman lignin. The solids were then refluxed with 9:1 mixture of dioxane and 2 N HCl under an atmosphere of nitrogen for about one hour. The solution was filtered, and the solid was refluxed with another aliquot of the 9:1 dioxane/2 N HCl solution for an additional hour. The solution was filtered, and the filtrate combined with the first dioxane/2 N HCl extract solution. The remaining solids were refluxed four times with addition of aliquots of the 9:1 dioxane/2 N HCl solution for 1, 1, 14, and 3 hours until only a small amount of ^{14}C·activity was extracted. The four extract solutions were combined with the previous two dioxane/2 N HCl extract solutions. The resulting solid was reconstituted in water, concentrated, centrifuged and the aqueous solution was decanted from the precipitated solid, which was the acidolysis lignin.
Hydrolysis of extracted meal with 6 N HCl was performed at 100 °C for about 12 hours. The reaction mixture was filtered through filter paper. The aqueous filtrate (acid hydrolysate) was concentrated under nitrogen, redissolved in water, and analysed by RP HPLC. The collected HPLC fractions were concentrated to dryness under nitrogen. Hydrochloric butanol (1.0 N HCl in n-butanol) was added and

heated at approximately 90 °C for about two hours. The samples were then concentrated under nitrogen. After addition of methylene chloride and trifluoroacetic anhydride, the vials were heated at approximately 100 °C for about five minutes. After cooling, the derivatised samples were analysed by RP HPLC and GC/MS.

Mild base hydrolysis of extracted meal was performed with 0.1 N NaOH at 100 °C for 17.5 hours. The mixture was cooled and centrifuged. The supernate was decanted from the solid residue and analysed for ^{14}C-activity. An additional aliquot of fresh 0.1 N NaOH was added to the solid residue and the resulting mixture was heated at 100 °C for 66 hours. The mixture was cooled and centrifuged, and the supernatant was decanted from the solid residue and analysed for ^{14}C-activity. The combined supernates were analysed by CX HPLC/LSC.

Strong Base Hydrolysis of Extracted Meal was performed with 2.5 N NaOH, first refluxed for two hours, then stirred at 85 °C for 65 hours. The mixture was filtered, and the remaining solid was stirred in water at 85 °C for 18 hours. The mixture was cooled to room temperature and filtered. The two extracts were combined to give the hydrolysate fraction. An aliquot of the hydrolysate was acidified with HCl and ultrafiltered. The ultrafiltered solution was analysed by CX HPLC/LSC and RP HPLC/LSC. An aliquot of hydrolysate was acidified to pH 2 with 6 N HCl. The solution was ultrafiltered and then eluted through a preparative C18 reverse phase column. The C18 eluate was added to a Chelex® column which was eluted with water and 0.1 N HCl. The Chelex® eluate was extracted five times with ethyl acetate. The five ethyl acetate extracts were then sequentially extracted three times with 7 % ammonium hydroxide. The ammonium hydroxide extracts were combined, concentrated and further analysed by CX HPLC/RAD and SAX HPLC/RAD.

Column recovery was low in some cases, e.g. after CX/HPLC/LSC, it was discussed that a significant amount of the injected activity might be strongly associated with sample matrix which did not elute and was therefore retained on the cation exchange column. If the column recovery for an analysis was >90 %, the percent distribution was not corrected for column recovery. If the column recovery was <90 %, then the percent distribution was corrected for column recovery. The difference in column recoveries is attributed to the fact that the broad peak of retained radioactivity in the RP HPLC chromatogram does not elute off the cation exchange column.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in canola seed samples taken 87 days after single early post-emergence application of 0.455 kg a.s./ha were 0.483 and 0.845 mg/kg in GT73 and GT200 lines, respectively. The control samples contained only 0.027 and 0.075 mg/kg, suggesting that $^{14}CO_2$ uptake did not make a significant contribution to the total residues in the seed samples.
The total radioactive residue (TRR) in canola seed samples taken 79 days after sequential post-emergence application 0.908 and 0.905 kg a.s./ha were 8.093 and 4.876 mg/kg in GT73 and GT200 lines, respectively. The control samples contained only 0.027 and 0.077 mg/kg.

Table 6.2.1-137: Total radioactive residues in tolerant canola seeds following foliar application of ^{14}C-glyphosate

Rape seed line	DALT	TRR, mg/kg	
		^{14}C-glyphosate	^{12}C-glyphosate (control)
Single early post-emergence application of 0.455 kg a.s./ha			
GT73	87	0.483	0.027
GT200	87	0.845	0.075
Sequential post-emergence application of 0.908 and 0.905 kg a.s./ha			
GT73	79	8.093	0.027

GT200	79	4.876	0.077

All residue data are expressed as mg/kg glyphosate equivalents
DALT – days after last treatment
TRR – Total radioactive residue

B. Extraction and characterisation of residues

Preliminary extractions and HPLC analyses demonstrated that there were no significant differences in glyphosate metabolism between GT73 and GT200 canola. Full identification and characterisation of residues was conducted only with samples from the commercial candidate line, GT73.

The seed samples were extracted first with hexane to remove the oil and then with water (aqueous extract). The oil extracted with hexane contained 4.6 and 1.8 % (0.022 and 0.141 mg/kg) in the samples taken after early post-emergence and sequential post-emergence treatments, respectively. The hexane fraction of the samples taken after sequential post-emergence treatments was further analysed for saponifiable fatty acids, which accounted for 1.8 % of TRR (0.141 mg/kg). HPLC analysis of the saponified oil showed the profiled activity eluted with the same retention times as fatty acid standards. The major ^{14}C-containing HPLC peak had the same retention time as oleic and palmitic acids, and the next two smaller peaks had the same retention times as linoleic and linolenic acids. These four fatty acids are the major fatty acids in Westar variety canola with oleic acid accounting for >50 % of the total fatty acid content. Stearic acid was also found as a minor component. Isolation of the peaks followed by GC/MS analysis confirmed the identity of the fatty acids in the ^{14}C-containing HPLC fractions.
After hexane extraction the remaining solids were extracted with water. Following early post-emergence application, the aqueous fraction of canola seeds (after workup for HPLC analysis) contained 20.9 % TRR (0.101 mg/kg). The aqueous fraction was further separated in Chelex non-retained fraction (6.5 % TRR, 0.003 mg/kg) and Chelex retained fraction (14.9 % TRR, 0.072 mg/kg). AMPA, N-glyceryl-AMPA and N-acetyl-AMPA were identified at 7.7, 3.4 and 0.9 % TRR, corresponding to 0.037, 0.017 and 0.004 mg/kg. Moreover, the aqueous extract contained 4.9 % (0.024 mg/kg) natural products, of which sucrose was identified to be <2 % (<0.01 mg/kg). A total of 69.2 % TRR (0.334 mg/kg) remained non-extracted.

Canola seeds after sequential post-emergence application were extracted according to two slightly different schemes. In Extraction A the hexane extracted meal was extracted three times with water. Only the first aqueous extract (19.1 % TRR, 1.55 mg/kg) was purified further on Chelex column. The combined second and third aqueous extract (5.6 % TRR, 0.46 mg/kg) was not further investigated. In Extraction B the whole aqueous extract (21.7 % TRR, 1.757 mg/kg) was subjected to Chelex column. Thus, the non-retained Chelex fraction amounted to 4.7 and 6.3 % TRR (0.38 and 0.510 mg/kg) after Extractions A and B, respectively. The 6 N HCl eluate from Chelex column (10.8 and 14.8 % TRR, 0.87 and 1.198 mg/kg, Extractions A and B, respectively) was further purified first on anion-exchange column. The eluate amounted to 9.9 and 13.8 % TRR (0.80 and 1.117 mg/kg). The eluate from anion-exchange column was further subjected to cation-exchange column, yielding again non-retained fraction, containing AMPA conjugates (3.2 and 5.7 % TRR, 0.26 and 0.46 mg/kg) and retained fraction (5.2 and 6.7, 0.42 % TRR 0.26 and 0.54 mg/kg, Extractions A and B, respectively). The identification of compounds was done only after Extraction A. In total in aqueous extract AMPA, N-glyceryl-AMPA and N-acetyl-AMPA were identified and amounted to 7.1, 3.9 and 0.7 % TRR (0.58, 0.31 and 0.06 mg/kg, respectively). Moreover, 1.6 % TRR (0.13 mg/kg) was identified as sucrose and 3.7 % TRR (0.30 mg/kg) were characterised as natural products. A total of 78.8 and 78.6 % TRR (6.38 and 6.364 mg/kg) remained unextracted after Extractions A and B. Extensive attempts were performed to characterise the unextracted residue after Extraction A. The treatments were done in parallel, unless stated otherwise.

Initial attempts to release unextracted residues under mild conditions in GT73-90-T extracted meal included treatments with water, dilute acid (0.1 N HCl), DMF, an aqueous complexing agent (0.1 M EDTA) and an aqueous surfactant (1 % sodium lauryl sulfate with sonication), which released 2.0, 5.2, 3.5, 0.5 and 5.8 % TRR (0.166, 0.421, 0.281, 0.038 and 0.47 mg/kg), respectively.

In order to estimate the bioavailability of the bound residues, the extracted meal was treated with simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). SGF contains pepsin in pH 1.2 buffer and breaks down polypeptides. SIF consists of pancreatin in pH 7.5 buffer; it contains amylase, trypsin, lipase, ribonuclease, and protease, and digests numerous types of compounds. Sequential digestion of the extracted meal with SGF after 3h and 22h digestion followed by SIF after 41h digestion released 4.9, 6.2 and 2.6 % TRR (0.39, 0.51 and 0.21 mg/kg), respectively. Sequential digestion of the extracted meal with blank SGF and blank SIF (no enzymes present) released 3.6, 5.8 and 1.9 % of TRR (0.29, 0.47 and 0.15 mg/kg), respectively. Comparison of the amount of solubilised ^{14}C-activity in the presence and absence of enzymes shows that pepsin and pancreatin cause very little enzymatic release of bound ^{14}C-activity. These results suggest that only a small fraction of the ^{14}C-glyphosate-derived components in extracted meal would be biologically available if ingested by animals.

Sequential enzymatic hydrolysis of the extracted meal with protease, amylase and cellulase released 5.9, 0.9 and 1.7 % of TRR (0.48, 0.07 and 0.14 mg/kg), respectively. These results demonstrate that only low levels of unextracted radioactivity are associated with proteins, starch, or cellulose.
Treatment with 9:1 dioxane and water released 1.7 % TRR (0.14 mg/kg), which was associated with free (Bjorkman) lignin. The resulting residue was then exhaustively extracted with 9:1 dioxane and 2 N HCl at reflux. The bound lignin was then precipitated from the extracts and was found to contain 3.0 % TRR (0.25 mg/kg); 11.0 % TRR (0.89 mg/kg) remained in the supernate. These results indicate that less than 5 % TRR in the unextracted canola seed is associated with free or bound lignin.
Hydrolysis of extracted canola meal with 6 N HCl at 100 °C for 12 hours released 13.3 % TRR (1.08 mg/kg). Analysis of the acid hydrolysate by RP HPLC showed that approximately 11.6 % TRR (0.94 mg/kg) eluted as a single peak. Three smaller peaks were also observed, but not further investigated due to low amount of radioactivity. The major peak was derivatised with n-butanol followed by trifluoroacetic anhydride. The resulting derivatised radioactive peaks were identified and characterised as derivatives of naturally occurring amino acids and organic acids by analysis by GC/MS. These results demonstrate that the unextractable residues in meal released by acid hydrolysis result from the incorporation of degraded one carbon units of ^{14}C-glyphosate into amino acids, sugars, and other biomolecules, and therefore are not of toxicological concern.
Mild base hydrolysis with 0.1 N NaOH at 100 °C released 39.9 % TRR (3.23 mg/kg). Analysis of the base hydrolysate by cation exchange HPLC showed three major radioactive peaks. The second and third peaks (16.5 and 5.8 % TRR, 1.34 and 0.47 mg/kg) were characterised as formate and AMPA, respectively, by comparison of their CX HPLC retention times with those of authentic standards. The first peak (3.5 % TRR, 0.28 mg/kg) was not characterised further.
Strong base hydrolysis of the extracted meal with 2.5 N NaOH at 85 °C for 65 hours, followed by two extractions of the resulting hydrolysed meal with water at 85°C was conducted in order to maximize the amount of released radioactivity. The hydrolysis released 63.6 % TRR (5.15 mg/kg). The combined base hydrolysate and aqueous extracts were acidified and filtered, in order to remove solubilised matrix prior to HPLC analysis. The filtrate was found to contain 43.9 % TRR (3.56 mg/kg). Although not characterised further, the precipitate retained upon filtration (19.7 % TRR, 1.59 mg/kg) is presumed to be natural biopolymers. Analysis of the filtrate by cation exchange HPLC showed three major radioactive peaks. The non-retained peak, which accounted for 5.6 % TRR (0.45 mg/kg), was not characterised further. The other peak accounting for 16.5 % TRR (1.34 mg/kg) was isolated and identified as formate by comparison with an authentic standard. The peak that accounting for 3.7 % TRR (0.30 mg/kg) was identified as AMPA by co-injection with an authentic standard.
Analysis of the filtrate by RP HPLC revealed two non-retained radioactive peaks and a very broad peak that was retained and accounted for approximately 15 % TRR. The reverse phase HPLC radiochromatogram of this broad peak closely matched the corresponding UV chromatogram. Since this broad peak of radioactivity is strongly retained on reverse phase chromatography and is associated with the UV-absorbing matrix, it is postulated to be a complex mixture of solubilised compounds derived from the degradation of glyphosate to one carbon fragments that are incorporated into natural, insoluble plant constituents.

Under both mild and strong basic hydrolysis, about 16 % TRR was released as ^{14}C-formate, and 3-6 % TRR was ^{14}C-AMPA. Strong base hydrolysis released more of the bound ^{14}C-activity initially, but significant amounts were either lost after acidification and filtration or appeared to be associated with matrix.

Control experiments to determine the stability of AMPA showed that AMPA is gradually hydrolysed in base, and one of the hydrolysis products is formate. After 99 hours in 2.5 N NaOH at 110 °C only 27.0 % remained as AMPA. Two other peaks were present in the cation HPLC profile that corresponded to those in the base hydrolysate of meal. One (23.8 %) had the same retention time as formate, and the other (46.3 %) eluted early in the gradient.
The base hydrolysis results suggest that a significant amount of the unextracted residues in meal are due to bound AMPA. Upon hydrolysis, the AMPA is released and partially converted to formate.

Table 6.2.1-138: Extraction of the radioactive residues of glyphosate in canola seeds following foliar early post-emergence application of glyphosate

Fraction	**Residues in seeds**	
	mg/kg	**% TRR**
	GT73 (1 × 0.455 kg as/ha)	
	^{14}C treated	
DAT		87
TRR	0.483	100
Hexane extract	0.022	4.6
Aqueous extract[1]	0.101	20.9
Chelex		
Aqueous fraction 1 (non-retained)	*0.003*	6.5
Aqueous fractions 2 and 3 (retained)	0.072	14.9
AMPA[2]	0.037	7.7
N-glyceryl-AMPA[2]	0.017	3.4
N-acetyl-AMPA[2]	0.004	0.9
natural products (sucrose)[2]	0.024 (<0.01)	4.9 (<2)
RRR	0.334	69.2
Identified	***0.058***	**12.0**
Characterised	***0.046***	**9.5**
ERR	***0.123***	**25.5**
RRR	***0.334***	***69.2***
Total sum	***0.457***	***94.7***

All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
Total sum – Sum of radioactivity in extract and extracted RAC
DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue (considering combined extracts measured)
RRR – Residual radioactive residue
[1] After workup for HPLC analysis
[2] Total amount found in aqueous extract
Characterised was calculated as sum of natural products and hexane extract

Table 6.2.1-139: Extraction of the radioactive residues of glyphosate in canola seeds following foliar sequential post-emergence application of glyphosate

Fraction	**Residues in seeds**			
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
	GT73 (2 × 0.9 kg as/ha)		**GT73 (2 × 0.9 kg as/ha)**	
	Extraction A		**Extraction B**	
	^{14}C treated		**^{14}C treated**	

DALT	79		79	
TRR	8.093	100	8.093	100
Hexane extract	0.141	1.8	0.141	1.8
Saponifiable fatty acids	0.13	1.6	-	-
1st aqueous extract	1.55	19.1	-	-
Concentrated 1st aqueous extract [3]	*1.38*	17.0	-	-
2nd and 3rd aqueous extract	0.46	5.6	-	-
Aqueous extract[1]	-	-	1.757	21.7
Chelex column[3]				
Aqueous Chelex eluate Aqueous fraction 1 (non-retained, containing natural products)	0.38	4.7	*0.510*	6.3
Aqueous fraction 1 (non-retained) concentrated	-	-	0.43	5.3
6N HCl Chelex eluate	*0.87*	10.8	*1.198*	14.8
AX column				
AX Eluate	*0.80*	9.9	*1.117*	13.8
CX Column				
CX non-retained eluate Aqueous fraction 2 (AMPA conjugates)	0.26	3.2	*0.46*	5.7
Aqueous fraction 2 concentrated (AMPA conjugates)	-		0.39	4.8
CX retained eluate (aqueous fraction 3, concentrated)	0.42	5.2	0.54	6.7
AMPA[2]	0.58	7.1	-	-
N-glyceryl-AMPA[2]	0.31	3.9	-	-
N-acetyl-AMPA[2]	0.06	0.7	-	-
Sucrose[2]	0.13	1.6	-	-
Natural products[2]	*0.30*	*3.7*	-	-
RRR	6.38	78.8	6.364	78.6
Extraction under mild conditions (parallel treatments)				
Aqueous extract	*0.166*	*2.0* (2.6)	-	-
Solids	*6.199*	*76.6*	-	-
0.1 N HCl	*0.421*	*5.2* (*6.6*)	-	-
Solids	*5.941*	*73.4*	-	-
0.1 N EDTA	*0.281*	*3.5* (*4.4*)	-	-
Solids	*6.081*	*75.1*	-	-
DMF	*0.038*	*0.5* (*0.6*)	-	-
Solids	*6.323*	*78.1*	-	-
1 % sodium lauryl sulfate	0.47	*5.8* (*7.4*)	-	-
Solids	6.16	76.2	-	-
Sequential Simulated digestions of extracted meal				
SGF Supernate (after 3h digestion)	0.39 [0.29]	4.9 [3.6]	-	-
SGF Supernate (after 22h digestion)	0.51 [0.47]	6.2 [5.8]	-	-
SIF Supernate (after 41h digestion)	0.21 [0.15]	2.6 [1.9]	-	-
Solids (undigested SGF/SIF residue)	5.52 [5.62]	68.2 [69.4]	-	-
Sequential enzyme hydrolyses			-	-
Protease	0.48	5.9	-	-
Amylase	0.07	0.9	-	-
Cellulase	0.14	1.7	-	-
Solids	5.56	68.7	-	-
Extraction with Dioxane and water				
Bjorkman lignin	0.14	1.7	-	-
Acidolysis lignin	0.25	3.0	-	-
Aqueous solution (unassigned)	0.89	11.0	-	-
Solids	4.59	56.7	-	-
Hydrolysis with 6N HCl			-	-
Total acid hydrolysate	1.08	13.3	-	-

Table 6.2.1-139: Extraction of the radioactive residues of glyphosate in canola seeds following foliar sequential post-emergence application of glyphosate

Fraction	Residues in seeds			
	mg/kg	% TRR	mg/kg	% TRR
	GT73 (2 × 0.9 kg as/ha)		GT73 (2 × 0.9 kg as/ha)	
	Extraction A		Extraction B	
	^{14}C treated		^{14}C treated	
DALT	79		79	
TRR	8.093	100	8.093	100
Derivatised with n-butanol	0.94	11.6	-	-
Solids	6.67	82.4	-	-
0.1N NaOH hydrolysis				-
Total basic hydrolysate	3.23	39.9	-	-
Unknown	0.28	3.5	-	-
Formate [6, 7]	1.34	16.5	-	-
AMPA [7]	0.47	5.8	-	-
Solids	*1.06*	*13.1*	-	-
2.5N NaOH hydrolysis				
Total basic hydrolysate	5.15	63.6	-	-
Filtered hydrolysate	3.56	43.9	-	-
Unknown	0.45	5.6	-	-
Formate [6, 7]	1.34	16.5	-	-
AMPA [7]	0.30	3.7	-	-
Natural products	1.21	14.9	-	-
Insoluble biopolymers	1.59	19.7	-	-
Solids	0.47	5.8	-	-
Identified[5]	***1.08***	***13.3***	-	-
Characterised[6]	***0.92 – 5.78***	***11.4 - 70.6***	-	-
ERR	***2.19 – 7.30***	***27.00 – 90.10***	***1.898***	***23.5***
Final residue	***0.47 – 6.32***	***5.8 – 78.10***	***6.364***	***78.8***
Total sum	***7.77***	***95.90***	***8.262***	***102.3***

All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
In round brackets % of RRR are given
In square brackets the residues after blank (no enzyme) SGF and SIF treatments are given
Total sum – Sum of radioactivity in extract and extracted RAC
DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue (considering combined extracts measured)
RRR – Residual radioactive residue
[1] After workup for HPLC analysis
[2] Total amount found in aqueous extract
[3] Only concentrated 1st aqueous extract was taken for Chelex chromatography in Extraction A
[4] The 22-hr SGF digestion was a continuation of the 3-hr digestion
[5] Identified: sum of compound identified in hexane and aqueous extracts
[6] Formate was shown to be a hydrolysis product of AMPA. Control experiments to determine the stability of AMPA showed that AMPA is gradually hydrolysed in base, and one of the hydrolysis products is formate. After 99 hours in 2.5 N NaOH at 110 C only 27.0 % remained as AMPA.
[7] AMPA and formate resulting after base hydrolysis were considered as characterised

C. Storage stability

Canola seed samples were stored frozen for maximum 495 days (16.5 months).
Storage stability was demonstrated in this study by comparing the HPLC analyses of an aqueous extract of the ^{14}C-treated test group GT73-90-T. The initial seed extraction was conducted 28 days after harvest. The aqueous extract was then analysed by HPLC. At the end of the study (501 days after harvest), a

portion of the seed that had been maintained in frozen storage (-20°C or lower) was again extracted and analysed in the same manner. In addition, the original extracts were re-analysed after frozen storage.
These results show that radioactive components in both the stored seed and the aqueous extract were stable over the course of the study.

Table 6.2.1-140: Extraction of the radioactive residues of glyphosate canola seeds – storage stability assessment

	Seeds	
Storage interval, days[1]	**28**	**501**
	% TRR	**% TRR**
TRR	**100**	**100**
Hexane extract	1.59 (1.53)	1.83 (2.00)
Aqueous extract	22.04 (21.22)	19.89 (21.78)
RRR	**80.20** **(77.25)**	**69.58** **(76.21)**
Total	**103.83** **(100)**	**91.29** **(100)**

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
RRR Residual radioactive residue
[1] Storage intervals were calculated using date of harvest and date of HPLC analysis (the latest event, reflecting the longest storage duration as the most critical scenario)
Values in parentheses are the % of total recovered dpm.

D. Degradation pathway

Please refer to the pathway of glyphosate crop group pulses and oilseeds presented at the end of the chapter.

III. Conclusions

The nature of the residues in glyphosate tolerant canola following the use of glyphosate was studied. Two different treatment regimens were utilised. Each line of Roundup® herbicide tolerant canola plants (GT73 and GT200) received two different treatment regimens. The first regimen involved a single broadcast application at a rate of 0.455 kg of glyphosate/ha at BBCH 12-14 (2-4 leaf growth stage, 14 days after planting). For the second regimen, canola plants received two sequential applications of Roundup® herbicide, each at a rate of approximately 0.90 kg a.s./ha. The first application was at BBCH 12-14 (2-4 leaf growth stage, 14 days after planting), and the second was at BBCH 16 (6-leaf growth stage, 22 days after planting).
The total radioactive residue (TRR) in canola seed samples of GT73 line taken 87 days after single early post-emergence application and 79 days after sequential post-emergence application were 0.483 and 8.093 mg/kg, respectively.
Extraction of canola seeds after early post-emergence application with hexane and water yielded 25.5 % TRR (0.123 mg/kg). In the aqueous fraction of canola seeds after early post-emergence application 7.7, 3.4, 0.9 and <2 % TRR (0.037, 0.017, 0.004 and <0.01 mg/kg) AMPA, N-glyceryl-AMPA, N-acetyl-AMPA and sucrose were identified, respectively. Moreover, 4.9 % TRR (0.024 mg/kg) was characterised as natural products.
Extraction of canola seeds after sequential post-emergence application with hexane and water yielded 26.5 % TRR (2.15 mg/kg). In the aqueous fraction 7.1, 3.9, 0.7 and 1.6 % TRR (0.58, 0.31, 0.06 and

0.13 mg/kg) AMPA, N-glyceryl-AMPA, N-acetyl-AMPA and sucrose were identified, respectively. Moreover, 3.7 % TRR (0.30 mg/kg) was characterised as natural products. In the hexane fraction 1.6 % TRR (0.13 mg/kg) were characterised as saponifiable fatty acids.

The non-extracted residues amounted to 78.8 % (6.38 mg/kg). The results of attempts to release non-extracted residues under mild conditions show that water, dilute acid (0.1 N HCl), DMF, an aqueous complexing agent (0.1 M EDTA) and an aqueous surfactant (1 % sodium lauryl sulfate with sonication) each released less than less than 5.8 % TRR (0.47 mg/kg).

Sequential enzymatic hydrolysis of the extracted meal with protease, amylase, and cellulase released 5.9, 0.9 and 1.7 % TRR (0.48, 0.07 and 0.14 mg/kg), respectively. Sequential digestion of the extracted meal with simulated gastric fluid (SGF) followed by simulated intestinal fluid (SIF) released 6.2 and 2.6 % TRR (0.51 and 0.21 mg/kg), respectively. Simulated gastric and intestinal fluid digestions, or sequential hydrolyses with protease, amylase, and cellulase, released only about 9 % TRR. These results suggest that only a small fraction of the ^{14}C-glyphosate-derived components in extracted meal would be biologically available if ingested by animals.

Extraction with dioxane and water was used to determine the amount of radioactivity associated with lignin in the extracted meal. The results indicated that less than 5 % TRR is associated with free or bound lignin.

Hydrolysis of extracted canola meal with 6 N HCl at 100 °C for 12 hours released 13.3 % TRR (1.08 mg/kg). Analysis of the acid hydrolysate by reverse phase HPLC showed that approximately 11.6 % TRR (0.94 mg/kg) eluted as a single peak, which was derivatised with n-butanol followed by trifluoroacetic anhydride and characterised to contain naturally occurring amino acids and organic acids. These results demonstrate that significant amounts of the acid extractable bound radioactivity are a result of incorporation of degraded one carbon units of ^{14}C-glyphosate into natural amino acids, sugars, and other biomolecules, and therefore are not of toxicological concern.

Rather harsh base hydrolysis was the only successful method for the release of significant amounts of bound radioactivity. Hydrolysis of extracted meal with 0.1 N NaOH at 100 °C released 39.9 % TRR (3.23 mg/kg). Hydrolysis of extracted canola meal with 2.5 N NaOH at 85 °C for 65 hours, followed by two extractions of the resulting hydrolysed meal with water at 85 °C, released 63.6 % TRR (5.15 mg/kg).

In the base hydrolysates 16.5 and 3.7 % TRR (1.34 and 0.30 mg/kg) formate and AMPA, respectively, was identified, 14.9 % TRR (1.21 mg/kg) was characterised as natural products and 5.6 % TRR (0.45 mg/kg) remained unknown. 19.7 % (1.59 mg/kg) was characterised as insoluble biopolymers.

Control experiments showed that AMPA is gradually hydrolysed in base, and one of the hydrolysis products is formate. Thus, the base hydrolysis results suggest that a significant amount of the bound residues in meal are due to bound AMPA; upon hydrolysis, the AMPA is released and partially converted to formate.

Thus, results of the numerous experiments to determine the nature of radioactivity in canola meal indicate there are two types of bound radioactivity. One type is the result of incorporation of one carbon ^{14}C fragments of glyphosate into numerous natural products in the seed. The other is postulated to be bound AMPA, which is the primary metabolite of glyphosate in canola.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in canola has been previously evaluated at EU level and was considered to be acceptable. It was performed under GLP and is considered to be scientifically valid. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501. Therefore, the study is considered reliable and supports the uses of the crop category pulses and oilseeds.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/022
Report author	[redacted] *et al.*
Report year	1994
Report title	Nature of Glyphosate Residues in Soybeans Tolerant to Roundup@ Herbicide
Report No	MSL-13520
Document No	M-650176-01-1
Guidelines followed in study	Pesticide Assessment Guideline Number 171-4(a) of Subdivision O: Nature of the Residues in Plants
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • For some matrices less than 90 % was identified and characterised due to high level of non-extracted radioactivity
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues after different treatments of soybean plants which contain the Roundup Ready gene (modified to express CP4 EPSPS protein) with Roundup® herbicide. The nature of residues resulting from soil uptake was investigated by a pre-emergence application of 5.38 kg glyphosate acid equivalents/ha to bare soil immediately before planting of soya beans. The nature of residues resulting from foliar uptake was investigated by two different post-emergence treatment regimens. The first regimen involved a single 0.84 kg glyphosate acid equivalents /ha early post-emergence application applied 21 days after planting (BBCH 23). The second treatment regimen consisted of two sequential post-emergence applications: 0.84 kg glyphosate acid equivalents /ha (21 days after planting, BBCH 23) followed by a 1.68 kg glyphosate acid equivalents /ha (43 days after planting, BBCH 51). Soya bean forage, hay and seeds were collected at normal harvest.

The test substance consisted of an isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled glyphosate with ^{13}C and ^{14}C located at the carbon atom between the nitrogen and phosphonate moieties. Carbon-^{13} was incorporated into the test substance in order to facilitate mass spectral identification of metabolites that were not totally free of biological matrix. For all applications, glyphosate was applied as the isopropylamine salt formulated as Roundup® herbicide, which is a water soluble commercial glyphosate formulation.

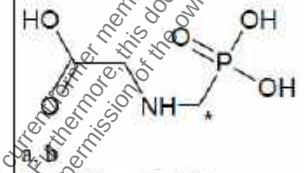
The total radioactive residue (TRR) in soybean forage, hay and seeds after sequential post-emergence treatment amounted to 23.651, 10.416 and 17.459 mg/kg in forage, hay and seeds, respectively. Early post-emergence treatment resulted in the TRRs of 0.863, 0.546 and 0.406 mg/kg in forage, hay and seeds respectively. After pre-emergence treatment only 0.239, 0.205 and 0.748 mg/kg were found in forage, hay and seeds respectively.

The radioactivity in forage, hay, and seeds treated pre-emergence is characterised as radiolabelled natural plant constituents derived by incorporation of $^{14}CO_2$ from the degradation of ^{14}C-glyphosate in the soil. Thus, in hay and seeds non-extracted radioactivity accounted for 74.4 % and 56.1 of the TRR, of which 38.1 % and 43.1 % of the TRR could be released by sequential hydrolysis with protease, amylase and cellulase.

After post-emergence treatment, glyphosate is slowly metabolised to AMPA, which is the primary plant metabolite. For plants that received the two sequential post-emergence applications, glyphosate accounted for 89.1, 53.6 and 25.2 % and AMPA accounted for 6.8, 12.8, and 49.1 % of the total radioactive residues in forage, hay, and seeds, respectively. Additional metabolites were identified as N-methyl-AMPA, N-glyceryl-AMPA, N-acetyl-AMPA, and N-malonyl-AMPA, all less than 2 % of the TRR. Moreover, 1.0 % (0.177 mg/kg) was attributed to AMPA conjugate AMPA conjugates are presumably formed via reaction with glyceric acid derivatives, acetyl-CoA, malonyl-CoA, and naturally occurring organic acid derivatives. Additionally, 2.7 % (0.468 mg/kg) was attributed to natural products. The radioactivity in hexane extracted oil from seeds was shown to be associated with naturally occurring fatty acids. In seeds that received the two post-emergence applications 5.1 % of the TRR was shown to be associated with naturally occurring organic and amino acids.

I. Materials and methods

A. Materials

1. Test material:	N-(phosphonomethyl)glycine; mixture of a) N-(phosphono-^{14}C-methyl)glycine (268.2 mg) b) N-(phosphono-^{13}C-methyl)glycine (307.1 mg) c) N-(phosphono-^{12}C-methyl)glycine (94.2 mg)
Chemical structure:	 a, b * Position of label
Radiochemical purity:	≥ 98 %
Specific activity:	1.62 MBq (7.42 mCi/mmol or 97412 dpm/µg)
CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate:	- 3.2, pH 7 at 25°C (glyphosate)

2. Test system

Soil:	Sable-91 soil: Clay loam (pH: 6.5; cation exchange capacity: 27.7 meq./100 g; bulk density: 1.08 g/cm^3; organic matter: 4.2 %; sand: 35 %; silt: 36 %; clay: 29 %; textural class (USDA): clay loam)
Crop:	Soybean plants, glyphosate tolerant (expressing glyphosate tolerant CP4 EPSPS, insertion event 40-3)
Botanical name:	*Glycine max (L.)* MERR.
Crop part(s):	Forage, mature stalk, seeds, cotton lint

B. Study design

1. In-life phase

For the investigation of the metabolism of glyphosate in CP4 EPSPS modified soya beans the plants were treated with a mixture of ^{12}C-, ^{13}C-, and ^{14}C glyphosate, labelled in the phosphonomethyl-moiety. Glyphosate was applied as the isopropylamine salt formulated as Roundup@ herbicide, which is a commercial glyphosate formulation. The study was conducted in controlled environment growth chambers.

The treatment consisted of three regimens. In pre-emergence a rate equivalent to 5.38 kg glyphosate acid equivalents/ha was applied to bare soil immediately before planting of soya beans. For post-emergence the first application was at a rate equivalent to 0.84 kg glyphosate acid equivalents/ha, applied 21 days after planting (BBCH 23). A second plot was conducted including also a second application at a rate equivalent to 1.68 kg glyphosate acid equivalents/ha 43 days after planting (BBCH 51 when the plants were at the flower bud initiation to early flower growth stage,). Approximately two weeks after each application, the foliage of the plants was washed to remove surface residues of non-absorbed ^{14}C-glyphosate. In parallel control plots were treated with ^{12}C-glyphosate. The plants were either kept in the same growth chamber with ^{14}C-treated plants to investigate the uptake of ^{14}C-CO_2 formed by degradation in soil or in separate chambers as control. To monitor the amount of carbon dioxide evolved by glyphosate degradation in the soil, the atmosphere of the growth chambers containing ^{14}C-treated plants was sampled for CO_2 throughout the in-life phase of the study.

2. Sampling

Crop samples (soya bean forage, hay and seeds) were collected to simulate agricultural practice. All samples were collected at normal harvest as described below. Forage and hay samples for all treatment groups were harvested 56 and 84 days after planting, respectively. This corresponds to 56, 35 and 13 DALT and 84, 63 and 41 DALT for forage and hay collected after pre-emergence, early post-emergence and sequential post-emergence, respectively. Soybean forage was harvested prior to pod formation (prior to BBCH 69), hay was collected after the pods are formed and before the leaves turn yellow and fall (approximately BBCH 80) and seeds were harvested at maturity (BBCH 89). For both forage and hay samples, randomly selected plants from each treatment group were cut at the base near the soil surface and the whole aerial portions of the harvested plants were composited in separate tared plastic bags for each treatment group. The bags were then sealed and placed in frozen storage until sample processing. Seeds samples for all treatment groups were harvested 104 days after planting. This corresponds to 104, 83 and 61 DALT for soybean seeds collected after pre-emergence, early post-emergence and sequential post-emergence, respectively. Mature pods were manually removed from all remaining plants for each test group. The pods were then broken open and the soybean seeds was manually removed. Harvested seeds for each test group was separately composited and placed in frozen storage until sample processing.
The samples of forage, hay and seeds were stored frozen at about -20°C until analysis.

3. Analytical procedures

Total radioactive residues (TRR) in all plant samples were determined by Liquid Scintillation Counting (LSC) following combustion. The limit of detection was for sequential post-emergence treatment group 0.004 mg/kg glyphosate acid equivalents (grain and hay), 0.002 mg/kg (forage) and 0.0004 mg/kg for samples for all other treatment groups.

Forage and hay samples were extracted four times with approximately a three-fold excess of water (w/w), with the exception of one forage sample which was extracted five times. The aqueous extract was concentrated. The aliquots were analysed by HPLC.

Seed samples were extracted first three times with hexane, then four times with acetonitrile:water (1:1, v/v) and finally with water. Aliquots of hexane extract, crude soybean oil was refluxed under nitrogen with 3 % methanolic KOH and afterwards extracted with diethyl ether. The combined aqueous extract was diluted with an equal amount of acetonitrile, centrifuged, and the supernatant was removed.

The extracted meal for each test group was analysed by combustion and LSC. Quantitative analysis by HPLC/LSC was carried out with each of the concentrated, whole aqueous extracts. No single HPLC

method was found that successfully separated all the components of the concentrates, so they were analyzed by both strong anion exchange (SAX HPLC, method #l) and cation exchange (CX HPLC, method #2) HPLC. On the strong anion exchange column, glyphosate, N-glyceryl-AMPA, N-acetyl-AMPA, and N-malonyl-AMPA were strongly retained and well resolved; however, AMPA and N-methyl-AMPA were weakly retained and co-eluted with a retention very close to that of neutral non-retained compounds. In contrast, on the cation exchange column, glyphosate, N-methyl-AMPA, and AMPA were well resolved, but N-glyceryl-AMPA, N-acetyl-AMPA, and N-malonyl-AMPA co-eluted near the compounds.

Non-extractable radioactivity in extracted control and ^{14}C-treated hay and seeds samples from soybeans treated pre-emergence with Roundup® herbicide was characterised by sequential enzyme hydrolysis with protease, amylase, and cellulase.

The extraction and fractionation procedure of the large scale experiment for seeds (after sequential post-emergence treatment) is summarised in the table below. Seeds from this test group were used since they contained the highest levels of ^{14}C-glyphosate-derived metabolites.

The ground soybean seeds were first extracted with hexane to remove the oil, and then were extracted with water (pH 4-4.5) to give three main samples: hexane extract, aqueous extract, and extracted soybean meal. The aqueous extracts were combined and profiled by HPLC. The aqueous extract was concentrated and acidified to pH 2 with HCl and then it was taken through a fractionation and clean-up scheme to provide material for identification of radioactive compounds. Clean-up of the extracts was carried out by passing the extract through a column containing Chelex® 100 resin. Phosphonate containing compounds are bound to the resin, and non-phosphonate containing materials are not retained. The retained phosphonate-containing compounds, in the form of their iron salts, were eluted from the column with HCl. Iron was removed from the eluate by passage through AG 1-X8 anion exchange resin (chloride form). The phosphonate-containing compounds were then separated on a cation exchange column with AG 50W-X8 resin (hydrogen form) into non-retained and retained fractions. Thus, the Chelex® column fractionation separated the initial aqueous extract into two fractions: Aqueous Fraction 1 (Chelex® non-retained) and the Chelex® retained fraction. The Chelex® retained fraction was further fractionated by cation exchange chromatography into four fractions: Aqueous Fraction 2 (cation non-retained), and Aqueous Fractions 3-5 (cation retained, in order of elution).

Aqueous fractions 2-4 (eluted with water) were used for isolation and identification of glyphosate, N-glyceryl-AMPA, N-acetyl-AMPA, and N-malonyl-AMPA. A part of aqueous fraction 2 (retained on Chelex column but not retained on cation exchange column) was hydrolysed with 1M HCl for 2 hours. The hydrolyzed solution contained one major hydrolysis product that coeluted with AMPA upon coinjection on CX HPLC/RAD.

Aqueous fraction 5 (eluted with 1 N HCl) was used for isolation of AMPA and N-methyl-AMPA.

For identification and quantification of glyphosate and metabolites several different High Performance Liquid Chromatography systems (HPLC) were employed using UV-detection and radioactive flow detector (RAD) equipped with either a liquid cell or a solid scintillant cell detection allowing direct measurements as well as isolating material for further identification.

The second method of detection, HPLC/LSC, consisted of fraction collection of the HPLC effluent employing a fraction collector with subsequent counting of the fractions by LSC.

Gas chromatography (positive ion chemical ionisation with MS detection (GC/PICI/MS) and gas chromatography (electron ionisation with MS detection (GC/EI/MS) was additionally used after derivatisation with trifluoroacetic anhydride/trifluoroethanol for identification of metabolites.

Thin layer chromatography (TLC) was used as second chromatographic method to confirm the identity of glyphosate and metabolites.

The chromatographic properties and mass spectra of radioactive metabolites were compared with reference standards of glyphosate, AMPA, N-glyceryl-AMPA, N-malonyl-AMPA, N-methyl-AMPA.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in soybean forage, hay and seeds are summarised in the table below. The TRRs were the highest after sequential post-emergence treatment and amounted to 23.651, 10.416 and 17.459 mg/kg in forage, hay and seeds, respectively. Early post-emergence treatment resulted in the TRRs of 0.863, 0.546 and 0.406 mg/kg in forage, hay and seeds respectively. After pre-emerge treatment only 0.239, 0.205 and 0.748 mg/kg were found in forage, hay and seeds, respectively. The residues in control were high after pre-emergence treatment, amounting to 0.135, 0.121 and 0.445 mg/kg, respectively. Since the ^{12}C-treated control plants were grown in soil treated with non-radiolabelled glyphosate, the radioactivity in the control plants is a result of incorporation of $^{14}CO_2$ derived from the degradation of ^{14}C-glyphosate in the soil of the ^{14}C-treated plants. The presence of high levels of $^{14}CO_2$ in the growth chamber atmosphere used for the pre-emergence plants was confirmed by sampling of the atmosphere for $^{14}CO_2$ and other ^{14}C-volatiles throughout the in-life phase of the study. The residues in control of both post-emergence plots were only 0.014 – 0.224 mg/kg. For plants treated with ^{12}C-glyphosate in separate growth chambers, no radioactivity above the LOQ of 0.001 mg/kg was observed.

Table 6.2.1-141: Total radioactive residues in glyphosate tolerant soybean forage, hay and seeds following foliar pre- or post-emergence application of glyphosate

Matrix	DALT	TRR, mg/kg		
		^{14}C treated	^{12}C treated (control, the same growth chamber as ^{14}C treated)	^{12}C treated (control, separate growth chamber)
Pre-emergence (5.38 kg glyphosate acid/ha)				
Forage	56	0.239	0.135	<0.001
Hay	84	0.205	0.121	<0.001
Seeds	104	0.748	0.445	<0.001
Early post-emergence (1 × 0.84 kg glyphosate acid /ha)				
Forage	35	0.863	0.014	<0.001
Hay	63	0.546	0.034	<0.001
Seeds	83	0.406	0.193	<0.001
Sequential post-emergence (1 × 0.84 kg glyphosate acid/ha + 1 × 1.68 kg glyphosate acid /ha)				
Forage	13	23.651	0.014	<0.001
Hay	41	10.416	0.033	<0.001
Seeds	61	17.459	0.224	<0.001

TRR: total radioactive residue, expressed as glyphosate acid equivalent
DALT: days after last treatment

B. Extraction and characterisation of residues

The ^{14}C-levels found in fractions of soybean forage, hay and seeds are shown in the tables below. For forage and hay aqueous extraction released maximum of about 100 % and 84.9 % of the TRR (corresponding to approximately 23.651 and 0.463 mg/kg) of the total radioactivity after post-emergence applications and only 26.6 and 32.9 % of the TRR (0.064 and 0.067 mg/kg) for pre-emergence application, respectively. In hay treated pre-emergence the radioactivity still bound after water extraction was subsequently released first by water extraction, then by enzyme treatment (protease, amylase and cellulase). Only 36.1 % TRR (0.074 mg/kg) remained non-extracted. Higher levels of non-extractable radioactivity are attributed to non-extractable radiolabelled natural plant constituents derived from incorporation of $^{14}CO_2$.

The aqueous fraction after early post-emergence and sequential post-emergence treatment was further analysed. In forage glyphosate was the main component amounting to 88.5 and 89.1 % of the TRR (0.764 and 21.078 mg/kg), followed by AMPA at 2.3 and 6.8 % of the TRR (0.020 and 1.619 mg/kg), respectively. After sequential post-emergence treatment, N-methyl-AMPA was detected at 0.6 % of the

TRR (0.140 mg/kg). A total of 1.5 to 2.6 % of TRR (0.013 to 0.618 mg/kg) was attributed to natural products.
Similarly, in hay glyphosate was the main component amounting to 64.7 and 53.6 % of the TRR (0.354 and 5.582 mg/kg), followed by AMPA at 5.3 and 12.8 % of the TRR (0.029 and 1.328 mg/kg) and N-methyl-AMPA at 0.6 and 1.3 % TRR (0.003 and 0.130 mg/kg), respectively. After sequential post-emergence treatment N-glyceryl-AMPA was detected at 0.8 % of the TRR (0.084 mg/kg) and one unknown at 0.6 %, 0.059 mg/kg. A total of 2.7 and 2.6 % of TRR (0.015 to 2.74 mg/kg) was attributed to natural products. The aqueous concentrate of ^{14}C early post-emergence treated plants was further analysed, glyphosate and AMPA being the main components (10.1 and 22.9 %, 0.041 and 0.093 mg/kg, respectively). N-glyceryl-AMPA, N-acetyl-AMPA and N-malonyl-AMPA were detected at max. 1.2 % (0.005 mg/kg).

Soybean seeds were first extracted with hexane, yielding up to 14.4 % TRR or up to 0.106 mg/kg (^{14}C treated samples). Acetonitrile/water and water extraction released the biggest portion of radioactivity: up to 83.3 %, corresponding to 14.545 mg/kg were found in aqueous concentrate.
In seeds treated pre-emergence the radioactivity still bound after water extraction was subsequently released first by water extraction, then by enzyme treatment (protease, amylase and cellulase). Only 13.0 % TRR (0.097 mg/kg) remained non-extracted. High levels of non-extractable radioactivity are attributed to non-extractable radiolabelled natural plant constituents derived from incorporation of $^{14}CO_2$.
Seeds after sequential post-emergence treatment were additionally extracted in a large scale experiment. The extracts and fractions obtained were used for compound isolation followed by identification.
After large scale extraction of seeds after sequential post-emergence treatment the hexane concentrate fraction amounted to 0.91 % of the TRR (corresponding to 0.159 mg/kg). The saponifiable fatty acids accounted for 0.8 % of the TRR (0.137 mg/kg) in this fraction. The radioactivity in hexane extracted oil from seeds was shown to be associated with naturally occurring fatty acids. The residues identified in seeds after sequential post-emergence treatment are summarised below.
Glyphosate and AMPA were the found to be the main components (25.2 and 49.1 %, 4.402 and 8.579 mg/kg, respectively). N-methyl-AMPA, N-glyceryl-AMPA, N-acetyl-AMPA and N-malonyl-AMPA were detected at max. 1.8 % (0.309 mg/kg). Additionally, an AMPA conjugate was found at 1 % of the TRR (0.177 mg/kg). A total of 5.1 % (0.897 mg/kg) was attributed to amino acids and natural organic acids natural products and 2.7 % (0.468 mg/kg) to other natural products.

Table 6.2.1-142: Extraction of the radioactive residues of glyphosate in soybean forage following foliar pre- or post-emergence application of glyphosate

Fraction	Residues in forage							
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	Pre-emergence		Pre-emergence		Early post-emergence		Sequential post-emergence	
Fraction	**^{14}C treated**		**^{12}C treated**		**^{14}C treated**		**^{14}C treated**	
DALT	56		56		35		13	
TRR	0.239	100	0.135	100.0	0.863	100.0	23.651	100.0
Aqueous extract	0.064	26.6	0.028	20.8	0.824	95.5	24.564	103.9
Aqueous concentrate	0.056	23.5	0.024	17.7	0.818	94.8	24.637	104.2
Glyphosate	n.a.	n.a.	n.a.	n.a.	0.764	88.5	21.078	89.1
AMPA	n.a.	n.a.	n.a.	n.a.	0.020	2.3	1.619	6.8
N-methyl-AMPA	n.a.	n.a.	n.a.	n.a.	-		0.140	0.6
Natural products	n.a.	n.a.	n.a.	n.a.	0.013	1.5	0.618	2.6
Identified	n.a.	n.a.	n.a.	n.a.	0.784	90.8	22.837	96.5
Characterised	n.a.	n.a.	n.a.	n.a.	0.013	1.5	0.618	2.6
ERR	0.064	26.6	0.028	20.8	0.824	95.5	24.564	103.9
RRR	0.175	73.2	0.113	83.7	0.040	4.7	0.908	3.8
Total	*0.239*	*99.8*	*0.141*	*104.5*	*0.864*	*100.2*	*25.472*	*107.7*
Total recovery	0.231	96.9	0.137	101.4	0.858	99.5	25.545	108

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate acid equivalents
n.a. not analysed
Values calculated upon dossier compilation are presented in italics.
Total represents sum of extracts and RRR
Total recovery, as calculated in the report represents sum of concentrated extracts and RRR

Table 6.2.1-143: Extraction of the radioactive residues of glyphosate in soybean hay following foliar, pre- or post-emergence application of glyphosate

	Residues in hay							
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	Pre-emergence		Pre-emergence		Early post-emergence		Sequential post-emergence	
Fraction	**^{14}C treated**		**^{12}C treated**		**^{14}C treated**		**^{14}C treated**	
DALT	84		84		63		41	
TRR	0.205	100.0	0.121	100.0	0.546	100.0	10.416	100.0
Aqueous extract	0.067	32.9	0.032	26.8	0.463	84.9	8.625	82.8
Aqueous concentrate	0.063	30.8	0.029	24.3	0.436	79.9	8.015	77.0
Glyphosate	n.a.	n.a.	n.a.	n.a.	0.354	64.7	5.582	53.6
AMPA	n.a.	n.a.	n.a.	n.a.	0.029	5.3	1.328	12.8
N-methyl-AMPA	n.a.	n.a.	n.a.	n.a.	0.003	0.6	0.130	1.3
N-glyceryl-AMPA	n.a.	n.a.	n.a.	n.a.	-		0.084	0.8
Unknown	n.a.	n.a.	n.a.	n.a.	-		0.059	0.6
Natural products	n.a.	n.a.	n.a.	n.a.	0.015	2.7	0.274	2.6
RRR	0.153	74.4	0.094	77.9	0.048	8.9	0.787	7.6
Water extract	0.009	4.2 (5.63)	0.005	4.3 (5.48)	n.a.	n.a.	n.a.	n.a.
Protease extract	0.037	18.2 (24.38)	0.016	13.2 (16.96)	n.a.	n.a.	n.a.	n.a.
Amylase extract	0.002	1.1 (1.47)	0.007	6.1 (7.80)	n.a.	n.a.	n.a.	n.a.
Cellulase extract	0.021	10.1 (13.50)	0.019	16.0 (20.59)	n.a.	n.a.	n.a.	n.a.
Non-extractable	0.074	36.3 (48.57)	0.038	31.2 (40.20)	n.a.	n.a.	n.a.	n.a.
Identified	n.a.	n.a.	n.a.	n.a.	0.386	70.6	7.124	68.5
Characterised	n.a.	n.a.	n.a.	n.a.	0.015	2.7	0.333	3.2
ERR	*0.136*	*66.4*	*0.080*	*66.4*	0.463	84.9	8.625	82.8
Final residue	0.074	36.3	0.038	31.2	0.048	8.9	0.787	7.6
Total	*0.210*	*102.5*	*0.118*	*97.7*	*0.512*	*93.8*	*9.416*	*90.4*
Total recovery	0.216	105.2	0.124	102.2	0.485	88.8	8.803	84.5

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate equivalents
n.a. not analysed
Values calculated upon dossier compilation are presented in italics.
In brackets percent of non-extractable radioactivity is given, as presented within the report
Total represents sum of extracts and RRR
Total recovery, as calculated in the report represents sum of concentrated extracts and RRR

Table 6.2.1-144: Extraction of the radioactive residues of glyphosate in soybean seeds following foliar pre- or post-emergence application of glyphosate

Fraction	Residues in seeds									
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	Pre-emergence		Pre-emergence		Early post-emergence		Early post-emergence		Sequential post-emergence	
Fraction	**^{14}C treated**		**^{12}C treated**		**^{14}C treated**		**^{12}C treated**		**^{14}C treated**	
DALT	104		104		83		83		61	
TRR	0.748	100.0	0.445	100.0	0.406	100.0	0.193	100.0	17.459	100.0
Hexane extract	0.108	14.4	0.080	18.0	0.041	10.0	0.044	22.8	0.096	0.55
Hexane concentrate	0.106	14.2	0.076	17.0	0.037	9.0	0.041	21.2	0.086	0.5
Acetonitrile/water extract	0.173	23.1	0.087	19.6	0.178	43.8	0.045	23.4	12.495	71.6
Aqueous extract	0.034	4.6	0.015	3.5	0.021	5.1	0.008	4.1	2.072	11.9
Aqueous concentrate	0.207	27.6	0.092	20.6	0.200	49.2	0.049	25.2	14.545	83.3
Glyphosate	n.a.	n.a.	n.a.	n.a.	0.041	10.1	n.a.	n.a.	n.a.	n.a.
AMPA	n.a.	n.a.	n.a.	n.a.	0.093	22.9	n.a.	n.a.	n.a.	n.a.
N-glyceryl-AMPA	n.a.	n.a.	n.a.	n.a.	0.005	1.2	n.a.	n.a.	n.a.	n.a.
N-acetyl-AMPA	n.a.	n.a.	n.a.	n.a.	0.004	1.0	n.a.	n.a.	n.a.	n.a.
N-malonyl-AMPA	n.a.	n.a.	n.a.	n.a.	0.003	0.9	n.a.	n.a.	n.a.	n.a.
RRR	0.420	56.1	0.269	60.5	0.148	36.5	0.102	52.8	2.020	11.6
Water extract	0.017	2.2 (3.98)	0.032	7.1 (11.72)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Protease extract	0.151	20.2 (35.92)	0.062	14.0 (23.14)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Amylase extract	0.013	1.8 (3.21)	0.014	3.3 (5.39)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Cellulase extract	0.024	3.2 (5.64)	0.028	6.2 (10.33)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Non-extractable	0.097	13.0 (23.15)	0.088	19.8 (32.67)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Identified	n.a.	n.a.	n.a.	n.a.	0.146	36.1	n.a.	n.a.	n.a.	n.a.
ERR	*0.485*	*64.8*	*0.303*	*68.2*	*0.240*	*58.9*	*0.097*	*50.3*	*14.67*	*84.05*
Final residue	0.097	13.0	0.088	19.8	0.148	36.5	0.102	52.8	2.020	11.6
Total	*0.582*	*77.8*	*0.391*	*88.0*	*0.388*	*95.4*	*0.199*	*103.1*	*16.670*	*95.65*
Total recovery	0.733	98.0	0.436	98.0	0.384	94.6	0.191	99.2	16.651	95.4

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
Final residue – residue remaining after final enzyme treatment
All residue data are expressed as mg/kg glyphosate equivalents
n.a. not analysed
Values calculated upon dossier compilation are presented in italics.
In brackets percent of non-extractable radioactivity is given, as presented within the report
Total represents sum of extracts and RRR
Total recovery, as calculated in the report represents sum of concentrated extracts and RRR

Table 6.2.1-145: Large-scale extraction followed by fractionation of the radioactive residues of glyphosate in soybean seeds after sequential post-emergence treatment

Fraction	Residues in seeds	
	mg/kg	**% TRR**
	Sequential post-emergence treatment Large scale extraction	
Fraction	**^{14}C treated**	
DALT	61	
TRR	17.459	100.0
Hexane extract	*0.155*	*0.89*
Hexane concentrate	0.159	0.91
Saponifiable fatty acids	0.137	0.8
Aqueous extract	14.480	82.94
Aqueous concentrate	12.392	70.98
Acidified concentrate	12.401	71.03
Chelex® chromatography of water extract		
Water eluate (Concentrated aqueous fraction 1)	0.470	2.69
0.1 N Eluate	0.119	0.68
6N Eluate	11.341	64.96
Anion exchange chromatography		
6 N HCl eluate	*11.274*	*64.58*
Cation exchange chromatography of 6N Eluate		
1st water eluate (aqueous fraction 2)	0.789	4.52
2nd water eluate (aqueous fraction 3)	3.324	19.04
3rd water eluate (aqueous fraction 4)	0.112	0.65
1st 1N HCl eluate (aqueous fraction 2)	6.772	38.79
ERR	*14.636*	*83.83*
RRR	1.863	10.67
Total	*16.499*	*94.50*

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate equivalents
n.a. not analysed
Values calculated upon dossier compilation are presented in italics.
In brackets percent of non-extractable radioactivity is given, as presented within the report
Total represents sum of extracts and RRR

Table 6.2.1-146: Distribution of radioactive residues of glyphosate in soybean forage and hay following early post-emergence or sequential post-emergence treatments

Fraction	Residues in forage				Residues in hay			
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
	Early post-emergence		**Sequential post-emergence**		**Early post-emergence**		**Sequential post-emergence**	
Fraction	**^{14}C treated**		**^{14}C treated**		**^{14}C treated**		**^{14}C treated**	
DALT	35		13		63		41	
TRR	0.863	100.0	23.651	100.0	0.546	100.0	10.416	100.0
Glyphosate	0.764	88.5	21.078	89.1	0.354	64.7	5.582	53.6
AMPA	0.020	2.3	1.619	6.8	0.029	5.3	1.328	12.8
N-methyl-AMPA	-	-	0.140	0.6	0.003	0.6	0.130	1.3
N-glyceryl-AMPA	-	-	-	-	-	-	0.084	0.8
Unknown	-	-	-	-	-	-	0.059	0.6
Natural	0.013	1.5	0.618	2.6	0.015	2.7	0.274	2.6

Table 6.2.1-146: Distribution of radioactive residues of glyphosate in soybean forage and hay following early post-emergence or sequential post-emergence treatments

Fraction	Residues in forage				Residues in hay			
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	Early post-emergence		Sequential post-emergence		Early post-emergence		Sequential post-emergence	
Fraction	^{14}C treated		^{14}C treated		^{14}C treated		^{14}C treated	
DALT	35		13		63		41	
TRR	0.863	100.0	23.651	100.0	0.546	100.0	10.416	100.0
products								
Identified	0.784	90.8	22.837	96.5	0.386	70.6	7.124	68.5
Characterised	0.013	1.5	0.618	2.6	0.015	2.7	0.333	3.2
ERR	0.824	95.5	24.564	103.9	0.463	84.9	8.625	82.8
RRR	0.040	4.7	0.908	3.8	0.048	8.9	0.787	7.6
Total	*0.864*	*100.2*	*25.472*	*107.7*	*0.512*	*93.8*	*9.416*	*90.4*

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate acid equivalents
n.a. not analysed
Values calculated upon dossier compilation are presented in italics.

Table 6.2.1-147: Distribution of radioactive residues of glyphosate in soybean seeds following early post-emergence or sequential post-emergence treatments

Fraction	Residues in seeds			
	mg/kg	% TRR	mg/kg	% TRR
	Early post-emergence		Sequential post-emergence[1]	
Fraction	^{14}C treated		^{14}C treated	
DALT	83		61	
TRR	0.406	100.0	17.459	100.0
Glyphosate	0.041	10.1	4.402	25.2
AMPA	0.093	22.9	8.579	49.1
N-methyl-AMPA	-	-	0.131	0.8
N-glyceryl-AMPA	0.005	1.2	0.278	1.6
N-acetyl-AMPA	0.004	1.0	0.235	1.4
N-malonyl-AMPA	0.003	0.9	0.309	1.8
AMPA conjugate	-	-	0.177	1.0
Natural products	-	-	0.468	2.7
Amino acids and natural organic acids	-	-	0.897	5.1
Saponifiable fatty acids			0.137	0.8
Identified	0.146	36.1	*13.934*	*79.9*
Characterised	-	-	*1.542*	*8.8*
ERR	*0.240*	*58.9*	14.636	83.83
RRR	0.148	36.5	1.183	10.67
Total	*0.388*	*95.4*	16.499	94.50

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate acid equivalents
n.a. not analysed
Values calculated upon dossier compilation are presented in italics
[1] after large scale extraction

C. Storage stability

Storage stability was demonstrated in this study by comparing the HPLC analyses of aqueous extracts of forage, hay and seeds. The initial aqueous extraction for each sample was conducted shortly after harvest. The aqueous extracts were then analysed by HPLC. All initial HPLC analyses were conducted within 34-49 days after harvest of each sample. These initial extractions and analyses were used for the definitive quantitation in the study. Towards the end of the study, aliquots of forage, hay and seed samples that had been maintained in frozen storage (-20 °C or lower) were again extracted and analysed in the same manner. In addition, aqueous extracts were re-analysed after frozen storage over periods of time for storage stability determination. Shortly after harvest and again following completion of the experimental phase of the study forage, hay and seed were extracted. The extracts and extracted samples were analysed to determine the distribution of radioactivity. Prior to extraction, aliquots of the samples were combusted to determine initial residues. The aqueous extracts were analysed by SAX HPLC. To determine the stability of the radioactive compounds in the aqueous extracts following frozen storage, extracts stored over long periods of time were analysed by SAX HPLC/LSC and compared with the HPLC profiles of fresh extracts.

The results show that there was no significant degradation in either the stored samples or the aqueous extracts over the course of the study. New metabolite fractions were not observed to form over the course of the study, nor did the distribution of radioactivity among metabolite fractions change significantly. Storage stability analyses thus demonstrated that glyphosate-derived residues were chemically stable during the course of the study in soybean tissues and extracts.

The samples of forage, hay and seeds were stored for a maximum of 266 days, this time period is covered by storage stability tested (Table 6.2.1-148).

Table 6.2.1-148: Extraction of the radioactive residues of glyphosate in forage, hay and seeds– storage stability assessment

	Forage (early post-emergence treatment)		**Hay (early post-emergence treatment)**		**Seeds (early post-emergence treatment)**	
Storage interval, days[1]	**34**	**370**	**36**	**343**	**49**	**323**
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
TRR	**100**	**100**	**100**	**100**	**100**	**100**
Hexane extract	-	-	-	-	0.55	1.08
Aqueous extract	103.86	104.30	82.81	79.77	83.44	81.27
RRR	**4.75**	**8.54**	**7.56**	**10.50**	**11.57**	**11.68**
Total	**108.61**	**112.83**	**90.37**	**90.28**	**95.56**	**94.03**

TRR Total radioactive residue (expressed as N-(phosphonomethyl) glycine (glyphosate) equivalents)
RRR Residual radioactive residue
[1] Storage intervals were calculated using date of harvest and date of HPLC analysis (the latest event, reflecting the longest storage duration as the most critical scenario)

D. Degradation pathway

Please refer to the pathway of glyphosate crop group pulses and oilseeds presented further below.

III. Conclusions

The nature and magnitude of glyphosate-derived residues after different treatments with Roundup® herbicide of soybean plants which contain the Roundup Ready gene (modified to express CP4 EPSPS protein) was studied. The nature of residues resulting from soil uptake was investigated after a pre-emergence application of 5.38 kg glyphosate acid equivalents/ha to bare soil immediately before planting

of soya beans. The nature of residues resulting from foliar uptake was investigated by two different post-emergence treatment regimens. The first regimen involved a single 0.84 kg glyphosate acid equivalents /ha early post-emergence application applied 21 days after planting (BBCH 23). The second treatment regimen consisted of two sequential post-emergence applications: 0.84 kg glyphosate acid equivalents /ha (21 days after planting, BBCH 23) followed by a 1.68 kg glyphosate acid equivalents /ha (43 days after planting, BBCH 51). Soya bean forage, hay and seeds were collected at normal harvest.
The total radioactive residue (TRR) in soybean forage, hay and seeds after sequential post-emergence treatment amounted to 23.651, 10.416 and 17.459 mg/kg in forage, hay and seeds, respectively. Early post-emergence treatment resulted in the TRRs of 0.863, 0.546 and 0.406 mg/kg in forage, hay and seeds respectively. After pre-emergence treatment only 0.239, 0.205 and 0.748 mg/kg were found in forage, hay and seeds respectively.
The radioactivity in forage, hay, and seeds treated pre-emergence is characterised as radiolabelled natural plant constituents derived by incorporation of $^{14}CO_2$ from the degradation of ^{14}C-glyphosate in the soil. Thus, in hay and seeds non-extracted radioactivity accounted for 74.4 % and 56.1 of the TRR, of which 38.1 % and 43.1 % of the TRR could be released by sequential hydrolysis with protease, amylase and cellulose, indicating on the incorporation of radioactive residues into natural compounds.
After post-emergence treatment, glyphosate is slowly metabolised to AMPA, which is the primary plant metabolite. For plants that received the two sequential post-emergence applications, glyphosate accounted for 89.1, 53.6 and 25.2 % and AMPA accounted for 6.8, 12.8, and 49.1 % of the total radioactive residues in forage, hay, and seeds, respectively. Additional metabolites were identified as N-methyl-AMPA, N-glyceryl-AMPA, N-acetyl-AMPA, and N-malonyl-AMPA, all less than 2 % of the TRR. Moreover, 1.0 % (0.177 mg/kg) was attributed to AMPA conjugate. AMPA conjugates are presumably formed *via* reaction with glyceric acid derivatives, acetyl-CoA, malonyl-CoA, and naturally occurring organic acid derivatives. Additionally, 2.7 % (0.468 mg/kg) was attributed to natural products. The radioactivity in hexane extracted oil from seeds was shown to be associated with naturally occurring fatty acids. In seeds that received the two post-emergence applications 5.1 % of the TRR was shown to be associated with naturally occurring organic and amino acids.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behavior of glyphosate in soybean has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with minor deficit: for some matrices less than 90 % has been identified and characterised due to high level of non-extractable radioactivity. The identification of metabolites was performed only after post-emergence and sequential post-emergence treatments. Thus, in forage, hay and seeds 92.3 – 99.1 %, 71.7 – 73.3 % and 36.1 – 88.7 % was identified and characterised. To reduce the non-extractable radioactivity and further characterize additional portions of the residue enzymatic hydrolysis has been performed for forage hay and seeds after pre-emergence treatment. In hay and seeds 38.1 and 43.1 % of TRR was extracted with sequential hydrolysis with protease, amylase and cellulase. This gives an indication, that a significant part of the non-extracted radioactivity could be attributed to natural plant constituents. Therefore, the study is considered reliable for the assessment of the metabolic behavior of glyphosate in soybean plants and in the whole group of pulses and oilseeds.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/023
Report author	[redacted]
Report year	1997
Report title	Nature of Glyphosate Residues in Cotton Plants (Genotype Line #1445) Tolerant to Roundup® Herbicide.
Report No	MSL-14113
Document No	Not available
Guidelines followed in study	Pesticide Assessment Guideline Number 171-4(a) of Subdivision O: Nature of the Residue - Plant Study
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • For seeds large portion of radioactivity remained unextracted (42-57 % TRR, 0.045-0.104 mg/kg) even after several attempts to release radioactivity.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The nature of the residues in glyphosate tolerant cotton (CP4 EPSPS modified) following the use of glyphosate was studied. Spray solution prepared from commercial Roundup®. ^{14}C-glyphosate was applied to the test plots at target rates of 0.93 kg a.s./ha for the first application and 1.27 kg a.s./ha for the second application. The control plots were treated at target rates of 0.85 and 1.28 kg a.s./ha for the first and second applications, respectively. The first application was made, when the plants were at the 3-4 leaf stage (BBCH 13-14); the second application was made 9 days thereafter, when the plants were at the 5-6 leaf stage (BBCH 15-16). Two different experiments were performed: In the treated protected plot the soil was protected during application to minimize contact of the radiolabelled substance with the soil. In the other treated plot (treated nonprotected), the soil was not protected during application, resulting in ^{14}C-glyphosate on both the plants and the soil.

Cotton plants were sampled from all control and treated plots approximately two hours after each application, and at the forage (27 days after last application) and mature boll (158 days after last application, BBCH 89) stages. The immature cotton samples consisted of the entire plant, and the mature crop was separated into cotton lint, seed, and stalks.

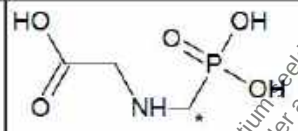
The total radioactive residue (TRR) in forage sample amounted to 15.2 - 30.4 mg/kg glyphosate equivalents, respectively. The control forage samples contained only 0.039 and 0.008 mg/kg. In contrast to the higher residues in the forage, the residues in the final harvest stalk, seed and lint samples were all <0.2 mg/kg.

In forage, 91.5 – 95.7 % (13.9 –29.1 mg/kg) of the radioactive residues were present as glyphosate; the most abundant metabolite, AMPA, accounted for less than 2 % of the TRR (0.201- 0.243 mg/kg). In seed, there was again very little AMPA relative to glyphosate, indicating that the metabolism of glyphosate to

AMPA occurs very slowly in cotton. In seeds 12.0 – 23.7 % of the TRR (0.022 – 0.025 mg/kg) was accounted to glyphosate, AMPA amounted to <1 – 1.38 % (<0.002 – 0.001 mg/kg). More than half the radioactive residues in the treated seed samples were either in the oil or remained in the extracted seed. The radioactivity in cotton seeds is characterised as radiolabelled natural plant constituents derived by incorporation of $^{14}CO_2$ from the degradation of ^{14}C-glyphosate in the soil. The largest part of the radioactivity remained unextracted, even after intensive extraction including acidic and basic solvents.

I. Materials and methods

A. Materials

Test Material:	N-(phosphonomethyl)glycine; mixture of a) N-(phosphono-^{14}C-methyl)glycine b) N-(phosphono-^{13}C-methyl)glycine c) N-(phosphono-^{12}C-methyl)glycine
Chemical structure:	 a, b * Position of label
Radiochemical purity:	> 98 %
Chemical purity:	> 95 %
Specific activity:	1.27 MBq/mg (5.81 mCi/mmol, 76272 dpm/µg)
CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate:	-3.2

Test system:

Soil:	Silt loam (pH: 6.3; cation exchange capacity: 16.2 meq./100 g; bulk density: 1.00 g/cm^3; organic matter: 3.0 %; sand: 24 %; silt: 55 %; clay: 21 %; textural class (USDA): silt loam)
Crop:	Cotton plants genotype 1445, glyphosate tolerant (CP4 EPSPS modified)
Botanical name:	*Gossipium sp.*
Crop part(s):	Forage, mature stalk, seed, cotton lint

B. Study design

1. In life phase

The test substance was formulated to simulate Roundup herbicide by combining the mixture of ^{12}C-, ^{13}C- and ^{14}C-glyphosate in water with isopropylamine and MON 0818 (an ethoxylated tallow-amine surfactant used in the commercial formulation of Roundup herbicide). The solution was then diluted with water to give the formulated test substance, with a final a concentration of 6.22 mg ^{14}C-glyphosate acid/g (7.9106 MBq/g).

Two different experiments were performed: In the treated protected plot the soil was protected during application to minimize contact of the radiolabelled substance with the soil. In the other treated plot (treated non-protected), the soil was not protected during application, resulting in ^{14}C-glyphosate on both the plants and the soil.
Two ^{14}C-treated test plots and two ^{12}C-treated control test plots were used for this study. Cotton seeds were planted in 4 plots with 60 seeds per plot for the two protected plots and 40 seeds per plot for the non-protected plots. The plots were irrigated to promote germination and emergence. All test plots were irrigated on an as-needed basis.
The ^{14}C-treated plots received two applications of spray solution prepared from the formulated test substance, and the control plots received two applications of spray solution prepared from commercial Roundup®. ^{14}C-glyphosate was applied to the test plots at target rates of 0.93 kg a.s./ha for the first application and 1.27 kg a.s./ha for the second application. The control plots were treated at target rates of 0.85 and 1.28 kg a.s./ha for the first and second applications, respectively. The first application was made, when the plants were at the 3-4 leaf stage (BBCH 13-14); the second application was made 9 days thereafter, when the plants were at the 5-6 leaf stage (BBCH 15-16). The soil surface of one of the ^{14}C-treated plots was protected with strips of plastic-lined absorbent paper during each application to minimize contact of the test substance with the soil, and the other ^{14}C-treated plot was left non-protected. Neither control plot was protected during application of the control solutions. One control plot was located adjacent to the each of the treated plots in order to monitor uptake of ^{14}C from the atmosphere.

2. Sampling

Cotton plants were sampled from all control and treated plots approximately two hours after each application, and at the forage (27 days after last application) and mature boll (158 days after last application, BBCH 89) stages. The immature cotton samples consisted of the entire plant, and the mature crop was separated into cotton lint, seed, and stalks. At the forage sampling, there were two replicate samples taken from the ^{14}C-treated plots; one replicate was rinsed with deionised water to remove surface residues, resulting in a rinsed forage sample and a water rinse.
Soil samples were taken in triplicate before and after each application of test substance, and at each plant sampling time point. The soil was sampled to 30 cm, but the core lengths were typically 20 - 25 cm in length. They were divided into two segments, 0 - 10 and 15 cm to the end, and pooled by time point.
The samples were stored frozen at about -20 °C until analysis.

3. Analytical procedures

Total radioactive residues (TRR) in all plant samples were determined by LSC following combustion.
The ^{14}C-treated forage and seed samples were extracted and analysed. In addition, the control seed sample from the non-protected plot was also extracted, but not analysed further due to very low levels of activity in the extract. The total radioactive residues in the other control forage and seed samples were less than 0.05 mg/kg, and therefore they were not extracted. The stalk and lint samples were collected only to determine the distribution of residues in the crop; they are not raw agricultural commodities, and thus were not extracted or analysed further.
The treated forage samples were extracted four times with water. The treated seed samples (protected and non-protected) and the control non-protected seed sample were first extracted three times with hexane, then four to six times with 50 % acetonitrile in water. The hexane-extracted seed was air-dried to remove the hexane, then analysed by combustion and LSC. Aliquots of hexane extract, crude cottonseed oil was refluxed under nitrogen with 3 % methanolic KOH and afterwards extracted with diethyl ether.
Residues in cotton seeds unextracted with hexane, and acetonitrile/water mixture were further characterised by extraction with one of the following solvents: water, acetonitrile, 0.1 N HCl, 0.1 N NaOH, 1 % sodium lauryl sulfate and acetone/water (7:3, v/v).
The extracted meal for each test group was analysed by combustion and LSC, and the aqueous extracts were analysed by LSC.
Quantitative analysis by HPLC/LSC was carried out with each of the aqueous extracts. Both strong anion exchange (SAX HPLC) and strong cation exchange (SCX HPLC) HPLC was used.

Additionally, HPLC systems were employed using UV-detection and radioactive flow detector (RAD) equipped with either a liquid cell or a solid scintillant cell detection.
The identification was done using co-injection of authentic reference standards with aqueous extracts. The limits of detection were typically 0.005 mg/kg for plant sample.
The chromatographic properties and mass spectra of radioactive metabolites were compared with reference standards of glyphosate and AMPA.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in forage sample from the non-protected and protected plots amounted to 15.2 and 30.4 mg/kg glyphosate equivalents, respectively. The control forage samples contained only 0.039 and 0.008 mg/kg, suggesting that $^{14}CO_2$ uptake did not make a significant contribution to the total residues in the forage samples in either the protected or non-protected plots.
Over half the total residues in forage were removed by rinsing with water (11.2 and 15.9 mg/kg in rinse water), only 6.28 and 6.98 mg/kg were found in the rinsed forage from non-protected and protected plots, respectively.
In contrast to the higher residues in the forage, the residues in the final harvest stalk, seed and lint samples were all < 0.2 mg/kg. In addition, a significant amount of the residues could be attributed to incorporation of $^{14}CO_2$, as indicated by the residues in the ^{12}C-control samples. For example, the residues in the final harvest samples from the ^{14}C-treated non-protected plot were 0.140 - 0.181 mg/kg. The residues in the samples from the corresponding control plot were 0.047 - 0.070 mg/kg, or 26 - 41 % of the activity in the ^{14}C-treated samples. Even in the samples from the protected plots, in which $^{14}CO_2$ formation was minimised by covering the soil during application, the controls contained 8 - 19 % of the radioactive residues in the ^{14}C-treated samples (0.008 – 0.018 mg/kg).

Table 6.2.1-149: Total radioactive residues in tolerant cotton matrices and soil following foliar application of ^{14}C-glyphosate

Matrix	DALT	mg/kg (^{14}C-glyphosate)		mg/kg (^{12}C-glyphosate, control)	
		unprotected soil	protected soil	unprotected soil	protected soil
Forage					
total	27	15.2	30.4	0.039	0.008
rinsed	27	6.28	6.98	-	-
rinse	27	11.2	15.9	-	-
Mature stalk	158	0.179	0.105	0.047	0.008
Seed	158	0.181	0.107	0.070	0.018
Cotton lint	158	0.140	0.083	0.057	0.016
Plant	After 1st application	289	180	0.005	0.006
Plant	After 2nd application	352	316	0.014	0.005
Soil	Before 1st application	0-15 cm: <0.001	0-15 cm: <0.001	not analysed	not analysed
		15-30 cm: <0.001	15-30 cm: <0.001		
	After 1st application	0-15 cm: 0.472	0-15 cm: 0.002	not analysed	not analysed
		15-30 cm: 0.01	15-30 cm: <0.001		
	Before 2nd application	0-15 cm: 0.569	0-15 cm: 0.001	not analysed	not analysed
		15-30 cm: -	15-30 cm: 0.003		
	After 2nd application	0-15 cm: 0.569	0-15 cm: 0.004	not analysed	not analysed
		15-30 cm: 0.002	15-30 cm: <0.001		
Soil, forage harvest	27	0-15 cm: 1.07	0-15 cm: 0.013	not analysed	not analysed
		15-30 cm: 0.018	15-30 cm: <0.001		
Soil, seed harvest	158	0-15 cm: 0.701	0-15 cm: 0.016	not analysed	not analysed
		15-30 cm: 0.009	15-30 cm: 0.023		

DALT days after last treatment
All residue data are expressed as mg/kg glyphosate equivalents

B. Extraction and characterisation of residues

The ^{14}C-levels found in fractions of cotton forage and seeds are shown in the table below. In cotton forage, a significant portion of the TRR (96.9 to 98.5 % TRR) was extracted with water.
The main component of the forage extract was glyphosate, accounting for 91.5 and 95.7 % of the TRR (13.9 and 29.1 mg/kg) in the samples non-protected and protected plots, respectively. AMPA accounted for 1.60 and 0.66 % TRR (0.243 and 0.201 mg/kg), glyphosate conjugates and natural products for maximum of 0.087 %, 0.54 mg/kg and 0.83 % TRR, 0.127 mg/kg, respectively.
The seed samples were extracted first with hexane to remove the oil and then with acetonitrile/water (aqueous extract). The oil extracted with hexane contained 14.7 and 11.3 % (0.027 and 0.012 mg/kg) in the samples of unprotected and protected plots, respectively. The saponifiable fatty acids accounted for 12.3 and 10.4 % of TRR (0.022 and 0.011 mg/kg) in the samples taken from non-protected and protected plots, respectively.
After hexane extraction the remaining solids were extracted with acetonitrile/water, containing 18.6 and 31.9 % TRR (each 0.034 mg/kg) in the samples of non-protected and protected plots, respectively. The main component of the seed aqueous fraction was glyphosate, accounting for 12.0 and 23.7 % (0.022 and 0.025 mg/kg), respectively. AMPA accounted for only 1.38 % (0.001 mg/kg) in the samples from protected plot and was not detected (<1 % TRR, <0.002 mg/kg) in the non-protected plot. Up to 6.93 % TRR (0.011 mg/kg) accounted for natural product.
Following the hexane and aqueous extractions of seed, a majority of the radioactivity was still unextracted. The unextracted residues accounted for 75.4 and 54.1 % TRR (0.136 and 0.058 mg/kg) in the seeds taken from non-protected and protected plots. In order to further characterize the unextracted residues in the seed, samples of hexane and aqueous extracted seeds were extracted at room temperature with one of six different solvents: water, acetonitrile, 0.1 N HCl, 0.1 N NaOH, 1 % sodium lauryl sulfate, and 70 % acetone/water. The residues remaining following these more effective extractions were 45.86-57.46 % TRR (0.083 - 0.104 mg/kg) in non-protected and 42.06 - 52.34 % TRR (0.045 - 0.056 mg/kg) in protected seed samples.
Analysis of the 0.1 N NaOH extract from seeds of non-protected plot showed that the bulk of the radioactivity eluted early in both chromatograms. There was no detectable (<0.001 mg/kg) glyphosate or AMPA present in the concentrate.

Table 6.2.1-150: Extraction of the radioactive residues of glyphosate in cotton forage following foliar application of glyphosate

Fraction	Residues in forage			
	mg/kg	% TRR	mg/kg	% TRR
	unprotected		protected	
	^{14}C treated		^{14}C treated	
DALT	27		27	
TRR	15.2	100	30.4	100
Aqueous extract	14.7	96.9	30.0	98.5
Glyphosate	13.9	91.5	29.1	95.7
AMPA	0.243	1.60	0.201	0.66
Glyphosate-conjugate	0.082	0.54	0.087	0.29
Natural products	0.127	0.83	0.123	0.40
Identified	**14.14**	**93.1**	**29.30**	**96.36**
Characterised	**0.209**	**1.37**	**0.21**	**0.69**
ERR	**14.7**	**96.9**	**30.0**	**98.5**
RRR	**0.708**	**4.7**	**0.447**	**1.47**
Total sum	**15.4**	**101.6**	**30.4**	**100.0**

DALT days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
Total sum Sum of radioactivity in extract and extracted RAC
All residue data are expressed as mg/kg glyphosate equivalents

Table 6.2.1-151: Extraction of the radioactive residues of glyphosate in cotton seeds following foliar application of glyphosate

Fraction	Residues in seeds					
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	unprotected		protected		unprotected	
	^{14}C treated		^{14}C treated		^{12}C treated	
DALT	158		158		158	
TRR	0.181	100	0.107	100	0.070	100
Hexane extracted oil	0.027	14.0	0.012	11.3	0.016	22.9
Basic ether	0.001	0.30	<0.001	0.03		-
Acidic ether	0.023	12.24	0.011	10.45	-	-
Saponifiable fatty acids	0.022	12.3	0.011	10.4	-	-
Aqueous	0.002	0.83	0.001	0.86	-	-
Aqueous extract	0.034	18.6	0.034	31.9	0.006	8.83
Glyphosate	0.022	12.0	0.025	23.7	-	-
AMPA	<0.002	<1 %	0.001	1.38	-	-
Glyphosate-conjugate	-	-	-		-	-
Natural products	0.011	5.83	0.007	6.93	-	-
Solids 1*	0.136	75.4	0.058	54.1	0.053	76.1
Solids 2**	**0.105**	**57.97**	**0.058**	**54.13**	**-**	**-**
Water	0.001	0.55	0.005	4.42	-	-
Solids 3	0.104	*57.46*	0.053	*49.53*	-	-
Solids 2**	**0.105**	**57.97**	**0.058**	**54.1**	**-**	**-**
Acetonitrile	0.002	1.28	0.002	1.61	-	-
Solids 4	0.103	*56.91*	0.056	*52.34*	-	-
Solids 2**	**0.105**	**57.97**	**0.058**	**54.1**	**-**	**-**
0.1N HCl	0.017	9.45	0.009	8.09	-	-
Solids 5	0.088	*48.62*	0.049	*45.79*	-	-
Solids 2**	**0.105**	**57.97**	**0.058**	**54.1**	**-**	**-**
0.1 N NaOH	0.019	10.67	0.011	10.72	-	-
Solids 6	0.086	*47.51*	0.047	*43.93*	-	-
Solids 2**	**0.105**	**57.97**	**0.058**	**54.1**	**-**	**-**
1 % Na-laurylsulfate	0.022	12.18	0.013	11.77	-	-
Solids 7	0.083	*45.86*	0.045	*42.06*	-	-
Solids 2**	**0.105**	**57.97**	**0.058**	**54.1**	**-**	**-**
70 % acetone/water	0.006	3.37	0.008	7.88	-	-
Solids 8	0.099	*54.67*	0.050	*46.73*		
Losses (solids were taken for storage stability analysis)	*0.031*	*17.43*	-	-	-	-
Identified	**0.024**	**13.0**	**0.026**	**25.08**	**-**	**-**
Characterised	***0.036 – 0.057***	***19.81 – 31.44***	***0.021 – 0.032***	***19.83 – 29.99***	**-**	**-**
ERR	***0.062 – 0.083***	***33.85 – 45.48***	***0.047 – 0.059***	***44.58 – 54.97***	**-**	**-**
Final residue	**0.083-0.104**	***45.86-57.46***	**0.045- 0.056**	***42.06 - 52.34***	**-**	**-**
Total sum	***0.165***	***91.3***	***0.104***	***97.0***	**0.075**	**107.8**

DALT days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate equivalents
Solids 1: solids after hexane and aqueous extraction (non-extracted radioactivity)
Solids 2: part of solids 1 was taken for extraction; solids2 are solids remaining after final storage stability extraction
Solids 3- solids 8: solids after water, acetonitrile, 0.1N HCl, 0.1 N NaOH, 1 % Na-Laurylsulfate and 70 % acetone/water extractions
Losses: pellet after hexane and aqueous extractions was taken for storage stability studies
Characterised: characterised by extraction and/or chromatographic behaviour
Values calculated upon dossier compilation are presented in italics

Table 6.2.1-152: Extraction of the radioactive residues of glyphosate in cotton forage and seeds following foliar application of glyphosate

Analyte	Residues in forage				Residues in seeds			
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
	unprotected		protected		unprotected		protected	
	^{14}C treated		^{14}C treated		^{14}C treated		^{14}C treated	
DALT	27		27		158		158	
TRR	15.2	100	30.4	100	0.181	100	0.107	100
Saponifiable fatty acids	NP	NP	NP	NP	0.022	12.3	0.011	10.4
Glyphosate	13.9	91.5	29.1	95.7	0.022	12.0	0.025	23.7
AMPA	0.243	1.60	0.201	0.66	<0.002	<1 %	0.001	1.38
Glyphosate-conjugate	0.082	0.54	0.087	0.29	-		-	
Natural products	0.127	0.83	0.123	0.40	0.011	5.83	0.007	6.93
Losses	-	-	-	-	*0.031*	*17.43*	-	-
Total identified	**14.14**	**93.1**	**29.3**	**96.36**	**0.024**	**13.0**	**0.026**	**25.08**
Total characterised	**0.209**	**1.37**	**0.21**	**0.69**	**0.033**	**18.3**	**0.018**	**17.33**
ERR	**14.7**	**96.9**	**30.0**	**98.5**	***0.092 – 0.114***	***51.28-62.78***	***0.048 – 0.059***	**44.81 - 54.97**
RRR	**0.708**	**4.7**	**0.447**	**1.47**	**0.083-0.104**	***45.86-57.46***	**0.045-0.056**	***42.06 - 52.34***
Total sum	**15.4**	**101.6**	**30.4**	**100**	**0.197**	**108.7**	**0.104**	**97.3**

DALT days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (considering combined extracts measured)
RRR Residual radioactive residue
All residue data are expressed as mg/kg glyphosate equivalents
Losses: pellet after hexane and aqueous extractions was taken for storage stability studies
Values calculated upon dossier compilation are presented in italics

C. Storage stability

The initial extractions and SAX HPLC/LSC analysis of the aqueous extracts were conducted within 10 weeks after harvest of each sample. At the end of the study, aliquots of forage and seed samples that had been maintained in frozen storage were again combusted, extracted and analysed in the same manner. The samples of forage and seed were extracted within 393 and 273 days of analysis, respectively. Storage stability analyses were conducted on the forage and the seed samples taken from non-protected plots. Samples were combusted and extracted, and the distribution of radioactivity in hexane phase and aqueous extracts were compared. The results show that the stored forage and seed samples were stable over the course of the study.

The samples of cotton forage and seeds were stored frozen for 393 and 260 days, which is proven stable by storage stability analysis.

In addition to the re-extraction and analysis of the stored samples, the aqueous extract of the forage from treated non-protected plot was re-analysed after frozen storage for 9 months. The extract, which originally contained primarily glyphosate (~ 95 %), contained two peaks of roughly equal size after storage. In addition to glyphosate, there was an extra peak. No further characterisation of the new peak was done. This apparent lack of stability of the extract did not affect the study.

The extracts of forage and seed were stored for a maximum of 10 days after extraction, with exception of one forage sample, which was stored for approximately one month after extraction. The definitive analysis of forage of treated protected plot was done about 1 month after extraction showed that the extract contained 96.57 % glyphosate (consistent with the earlier results of 97.76 % glyphosate), with no significant amounts of the new peak present.

Table 6.2.1-153: Extraction of the radioactive residues of glyphosate in forage, hay and seeds– storage stability assessment

	Forage (non-protected)		Seeds (non-protected)	
Storage interval, days[1]	**46**	**393**	**69**	**273**
	% TRR	**% TRR**	**% TRR**	**% TRR**
TRR	**100**	**100**	**100**	**100**
Hexane extract	-	-	13.83	12.38
Aqueous extract	85.76	96.86	17.66	18.11
RRR	**4.53**	**4.66**	**62.89[2]**	**57.97**
Total	**90.29**	**101.52**	**94.38**	**88.46**

TRR Total radioactive residue (expressed as N-(phosphonomethyl)glycine (glyphosate) equivalents)
RRR Residual radioactive residue
[1] Storage intervals were calculated using date of harvest and date of HPLC analysis (the latest event, reflecting the longest storage duration as the most critical scenario)
[2] includes initial extracted seed and pellet from aqueous extract.

D. Degradation pathway

Please refer to the pathway of glyphosate crop group pulses and oilseeds presented further below.

III. Conclusions

The nature of the residues in glyphosate tolerant cotton following the use of glyphosate was studied. Spray solution prepared from commercial Roundup®. ^{14}C-glyphosate was applied to the test plots at target rates of 0.93 kg a.s./ha for the first application and 1.27 kg a.s./ha for the second application. The control plots were treated at target rates of 0.85 and 1.28 kg a.s./ha for the first and second applications, respectively. The first application was made, when the plants were at the 3-4 leaf stage (BBCH 13-14); the second application was made 9 days thereafter, when the plants were at the 5-6 leaf stage (BBCH 15-16). Cotton plants were sampled from all control and treated plots approximately two hours after each application, and at the forage (27 days after last application) and mature boll (158 days after last application, BBCH 89) stages. The immature cotton samples consisted of the entire plant, and the mature crop was separated into cotton lint, seed, and stalks.

The total radioactive residue (TRR) in forage sample amounted to 15.2 - 30.4 mg/kg glyphosate equivalents, respectively. The control forage samples contained only 0.039 and 0.008 mg/kg, suggesting that $^{14}CO_2$ uptake did not make a significant contribution to the total residues in the forage samples in either the protected or non-protected plots. In contrast to the higher residues in the forage, the residues in the final harvest stalk, seed and lint samples were all <0.2 mg/kg.

In forage, 91.5 – 95.7 % (13.9-29.1 mg/kg) of the radioactive residues were present as glyphosate; the most abundant metabolite, AMPA, accounted for less than 2 % of the TRR (0.201- 0.243 mg/kg). In seed, there was again very little AMPA relative to glyphosate, indicating that the metabolism of glyphosate to AMPA occurs very slowly in cotton. A glyphosate-conjugate accounted for up to 0.54 % of the TRR. Natural products accounted for up to 0.83 % of the TRR.

In seeds 12.0 – 23.7 % of the TRR (0.022 – 0.025 mg/kg) was accounted to glyphosate, AMPA amounted to <1 - 1.38 % (<0.002 – 0.001 mg/kg). Saponifiable fatty acids accounted for up to 12.3 % of the TRR in seeds. Natural products accounted for up to 6.93 % of the TRR. More than half the radioactive residues in the treated seed samples remained in the extracted seed. The radioactivity in cotton seeds is characterised as radiolabelled natural plant constituents derived by incorporation of $^{14}CO_2$ from the degradation of ^{14}C-glyphosate in the soil. The largest part of the radioactivity remained unextracted, even after intensive extraction including acidic basic or organic solvents.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in cotton has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with minor deficit: in cotton seeds less than 90 % has been identified and characterised due to high level of non-extractable radioactivity. Only 32.6 to 43.2 % of TRR could be initially extracted in cotton seeds from unprotected and protected plots, respectively. Additional treatments with water, acetonitrile, 0.1N HCl, 0.1N NaOH, 1 % Na-laurylsulfate and 70 % acetonitrile/water were done in parallel to reduce the non-extractable radioactivity and further characterize additional portions of the residue. Up to 12.18 and 11.77 % TRR could be additionally extracted in seeds from protected and unprotected plots. Thus, 44.81 – 62.78 % (0.048 – 0.114 mg/kg) remained non-extracted in seeds. It must be pointed out, that in case of sequential extractions the extraction rate would be probably higher.

The high levels of TRRs in control seeds from non-protected plot indicated that incorporation of $^{14}CO_2$ into natural components was a major contributor to the non-extracted residues. Similar in other metabolism studies e.g. in canola seeds ([redacted] 1994) it was shown after parallel treatments that 8.5 % TRR were released enzymatic digestion with protease, amylase and cellulase, 15.7 % were released with dioxane 13.3 % and 63.6 % were released after acid (6N HCl) and basic (2.5 N NaOH) hydrolysis. This gives a strong indication that the initially non-extracted radioactivity could be attributed to natural plant constituents.

The study is considered reliable for the assessment of the metabolic behaviour of glyphosate in cotton.

Assessment and conclusion by RMS:

GAT modification

Cereal/grass crops

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/024
Report author	[redacted]
Report year	2007
Report title	The Metabolism of [^{14}C]Glyphosate in Optimum™ *GAT*™ (Event DP-Ø9814Ø-6) Field Corn
Report No	807194
Document No	DuPont-19529
Guidelines followed in study	OPPTS 860.1300, Nature of the Residue - Plants; Canadian PMRA Residue Chemistry Test Guidelines Dir 98-02, Section 2, Nature of the Residue Plants; and the recommendations of EU Commission Directive 96/68/EC Annex II, Section 8.1 (21 October 1996).

Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Certificate of analysis for test material was not provided, but the purity of the used batch is given in chapter 3.1.1 of the report and was re-analysed in the treatment solutions after each application • Certificates of analyses for reference substances were not provided, but the purities of the used batches are given in chapter 3.1.2 of the report • Identification was done by HPLC retention time comparison with authenticated standards and TLC (including admixed and co-spotted samples) with radiolabelled standards • Storage stability not discussed in the report, but dates of sampling and analyses are given in App. 6 and Quality Assurance Statement
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues after treatments to maize plants modified to express the glyphosate *N*-acetyltransferase (*gat*) gene of event DP-Ø9814Ø-6. The nature of residues resulting from soil uptake was investigated by a pre-emergence application of 4.37 kg glyphosate acid equivalents/ha to bare soil immediately prior to emergence. The nature of residues resulting from foliar uptake was investigated following three foliar applications of 1.10 to 1.13 kg glyphosate acid equivalents/ha made at V6, V8, and R5 growth stages. The test substance consisted of ^{14}C labelled glyphosate formulated with Touchdown Total™ inert ingredients and 2 % ammonium sulphate (AMS).

Maize plants were harvested as immature foliage (growth stage V6; 48 days after soil treatment), immediately prior to the first foliar application; then as forage (growth stage V19, R5; 59 days after the second foliar application) and finally at maturity (growth stage R6; 7 days after the third foliar application) whereupon plants were separated into stover, cob, and grain fractions.

At each sample point tissues were homogenised and extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v) followed by enzyme (α-amylase then amyloglucosidase and cellulase), alkaline, then acid digestion. The total radioactive residue (TRR) was determined as the sum of the total dpm in extractable and unextracted residues expressed as mg/kg equivalents of glyphosate. Extracts containing ≥0.01 mg/kg were analysed by high-performance liquid chromatography (HPLC). The identification of residues was accomplished by HPLC retention time comparison with authenticated radiolabelled standards and TLC with these standards (including admixed and co-spotted samples).

TRR in the immature foliage was low (0.022 mg/kg) with 31.0 % extracted with 0.1 % formic acid:methanol (96:4 v/v). Residues in the immature foliage resulted from root uptake of radioactive glyphosate and/or its soil degradates from the pre-emergent soil application. The low levels of extractable and unextracted radioactivity in the immature foliage were not investigated further.

The TRR in forage was 3.652 mg/kg with the majority of the residues extracted with 0.1 % formic acid:methanol (96:4 v/v) and α-amylase (87.0 % and 9.1 %, respectively). A small amount (≤1.3 % TRR) was extracted using a mixture of amyloglucosidase and cellulase, NaOH, then HCl, leaving 0.9 % TRR as unextracted residues. The major component in forage was glyphosate (58.0 % TRR), with *N*-acetylglyphosate present at 27.0 % TRR. AMPA and *N*-acetyl-AMPA comprised 4.0 % and 1.7 % TRR, respectively.

At maturity, the majority of the TRR was present in stover (12.255 mg/kg), with 0.686 mg/kg in cobs, and 0.275 mg/kg in grain. The majority of the radioactivity in the mature maize fractions was extracted using 0.1 % formic acid:methanol (96:4 v/v) and α-amylase (69.3-85.0 % and 11.1-20.5 % TRR, respectively). Extraction with amyloglucosidase and cellulase followed by NaOH released a maximum of 3.3 % TRR in the individual extracts of stover, cobs, and grain. Extraction of stover with HCl released a further 0.7 % TRR. Unextracted residues comprised 0.9-7.9 % TRR.

The major component of stover was glyphosate (74.9 % TRR), and *N*-acetylglyphosate was the most abundant metabolite (17.8 % TRR). The metabolites AMPA and *N*-acetyl-AMPA were also detected but at much lower levels (4.4 % and 1.3 % TRR, respectively).

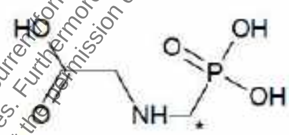
The major component of cobs and grain was *N*-acetylglyphosate, which comprised 63.8 % and 51.2 % TRR, respectively. *N*-acetyl-AMPA was the second most prominent metabolite, present at 5.0 % and 9.4 % TRR respectively. AMPA and glyphosate were detected in grain at lower concentrations, 6.1 % and 0.1 % TRR, respectively.

I. Materials and Methods

A. Materials

Test Material: N-(phosphono-^{14}C-methyl)glycine

Chemical structure:



* Position of label

Radiochemical purity: ≥97.5 %

Specific activity: 10.59 µCi/mg (0.39 MBq/mg)

CAS No: 1071-83-6

Log $P_{o/w}$ for glyphosate: Not reported

Test system

Soil: Sandy loam [textural class (UK)] (pH: 6.4; cation exchange capacity: 15.4 mg/L; organic carbon: 3.2 %; particle size 0.063-2 mm: 65.93 %; particle size 0.002-0.063 mm: 18.71 %; particle size <0.002 mm: 15.36 %)

Crop: Field corn plants, glyphosate tolerant, Optimum™ *GAT*™ (Event DP-Ø9814Ø-6)

Botanical name: *Zea mays*

Crop part(s): Immature foliage, forage, mature stover, cobs, grain

B. Study design

1. In-life phase

For the investigation of the metabolism of glyphosate in Optimum™ *GAT*™ (Event DP-Ø9814Ø-6) corn plants were treated with ^{14}C glyphosate labelled in the phosphonomethyl-moiety. Glyphosate was co-formulated with Touchdown Total™ inert ingredients and 2 % ammonium sulphate (w/v). The study was conducted in a single glasshouse compartment. Corn seeds (Optimum™ *GAT*™ Event DP-Ø9814Ø-6) were sown into 6 pots at a depth of approximately 2 cm, with 3 seeds sown per pot.

The treatment consisted of a pre-emergence application at a target rate equivalent to 4.26 kg glyphosate acid equivalents/ha applied to bare soil immediately prior to emergence, followed by three foliar applications at a target rate of 1.12 kg glyphosate acid equivalents/ha each, made at the V6 (10 days prior to V8), V8, and R5 (7 days prior to maturity) growth stages.

The formulation was applied to the soil surface or to the foliage using a hand-held sprayer system with a single flat-fan nozzle at a pressure of approx. 1 bar. Following each application, the sprayer was rinsed with ≤10 mL of Milli-Q water and the rinse was also applied to the soil or plant surface. Polythene sheeting was erected around pots prior to application to avoid contamination during application and removed afterwards. After application, the amount of residual radioactivity associated with the sprayer, each spray container and the operator's gloves were determined to calculate the actual amount of radioactivity applied.

The radiochemical purity of each treatment solution was determined before and after each application. Aliquots of the treatment solutions were removed for analysis by LSC and HPLC to determine the total amount of glyphosate applied.

The plants were watered as required, and fertilizers and biological pest controls were applied when necessary. The plants were observed weekly for evaluation of growth stages. In parallel, control plants were grown and treated with Touchdown Total™ inert ingredients and ammonium sulphate.

2. Sampling

A foliage harvest of control and treated plants was conducted at growth stage V6, immediately prior to the first foliar application and 48 days after soil treatment, by removing two plants from each pot. Two plants were removed from two separate pots as a forage harvest at growth stage V19, R5 (59 days after the second foliar application). The remaining four plants were harvested at maturity, growth stage R6 (7 days after the third foliar application). At foliage and forage harvests, tissues were weighed and manually chopped into small sections prior to storage. At maturity, plants were separated into stover, cobs, and grain. The stover fraction was shredded and cobs manually cut into small sections. Samples were stored frozen at -20 °C immediately after sampling and within two days were homogenised using a food processor or, in the case of cobs and grain, a blender.

The samples from the foliage harvest, forage harvest, and mature harvest were stored frozen at approximately -20 °C until extraction, no longer than 8, 4, and 4 days, respectively. HPLC analyses were completed 21 days after extraction for forage samples and 17 days after extraction for mature harvest samples; foliage samples were not analysed by HPLC.

3. Analytical procedures

Portions of each homogenised tissue were extracted three times with 0.1 % formic acid (aqueous):methanol (96:4 v/v). Centrifugation was applied to separate each extract (supernatant) from the unextracted residue (pellet). The three supernatants were combined into one extract and the pellet of the last extraction used for consecutive further extraction steps. The pellet was then enzyme digested twice with α-amylase followed by amyloglucosidase and cellulase. The remaining pellet was then hydrolysed in 0.1 N NaOH (60 ºC, 6 hours). A further extraction with 1.0 N HCl (60 ºC, 6 hours) was conducted where

necessary. Extracts were concentrated and reconstituted in 0.1 % (v/v) aqueous formic acid prior to LSC and High Performance Liquid Chromatography (HPLC).

The concentrates from the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extraction derived from forage, stover, cobs, and grain were centrifuged, and the sediments added to the pellets prior to enzyme digestion while the supernatants were analysed by LSC and HPLC.

The α-amylase digest of grain was subjected to solid phase extraction (SPE) using a Waters Oasis HLB 1 g LP extraction cartridge. After the flow-through was collected, retained material was then eluted with formic acid and methanol. The formic acid eluates were combined, concentrated, and reconstituted 0.1 % (v/v) aqueous formic acid prior to LSC and HPLC.

The total radioactive residue (TRR) was determined as the sum of the total dpm in extractable and unextracted residues expressed as mg/kg equivalents of glyphosate. Levels of radioactivity were determined in each extract by Liquid Scintillation Counting (LSC) and in the unextracted residues by oxidative combustion and LSC. The limits of detection for quantification of radioactive peaks on chromatograms were assessed as <0.1 % TRR (<0.001 ppm.).

For identification and quantification of glyphosate and metabolites, a HPLC system was employed using on-line UV detection and a radiodetector equipped with an yttrium silicate solid cell. Following on-line radiodetection, effluent fractions were collected for quantification of radioactivity via LSC. The HPLC method to determine stock radiochemical purity did not include fraction collection. The identification of residues was accomplished by HPLC retention time comparison with authenticated radiolabelled standards.

Thin layer chromatography (TLC) with authenticated radiolabelled standards (including admixed and co-spotted samples) followed by phosphor imaging was used as second chromatographic method to confirm the identity of glyphosate and metabolites.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in maize was calculated as the sum of extractable and unextracted residues. The extractable radioactive residue (ERR), the residual radioactive residue (RRR) and the TRR are expressed as glyphosate equivalents in the tables below.

The TRR in maize foliage and forage is summarised in in the table below.

The TRR in immature maize foliage was 0.022 mg/kg following a single application of ^{14}C-glyphosate to soil immediately prior to emergence. The TRR in maize harvested as forage was 3.652 mg/kg following a single soil application and two foliar applications of ^{14}C-glyphosate.

The distribution of TRR from maize harvested at maturity is presented. Plants harvested at maturity, following a total of one soil application and three foliar applications of ^{14}C-glyphosate, were separated into stover, cobs, and grain. The TRR was greatest in stover, 12.255 mg/kg. The TRR in cobs and grain were lower, 0.686 mg/kg and 0.275 mg/kg, respectively.

Table 6.2.1-154: Total radioactive residues in glyphosate-tolerant maize foliage and forage

Application		4.37 kg glyphosate acid/ha pre-emergent application	4.37 kg glyphosate acid/ha pre-emergent application + two foliar applications (1.12 and 1.11 kg glyphosate acid/ha)
Matrix		**Maize foliage, 48 DALT**	**Maize forage, 59 DALT**
0.1 % Formic acid:methanol extract	% TRR	31.0	87.0
	mg/kg	0.007	3.204
α-Amylase extract	% TRR	-	9.1
	mg/kg	-	0.316
Amyloglucosidase and cellulase extract	% TRR	-	1.3
	mg/kg	-	0.045
NaOH extract	% TRR	-	1.0
	mg/kg	-	0.035
HCl extract	% TRR	-	0.6
	mg/kg	-	0.021
Unextracted Residue	% TRR	69.0	0.9
	mg/kg	0.015	0.031
Total	mg/kg	0.022	*3.652*

TRR: total radioactive residue
DALT: days after last treatment
Italic: recalculated value (faulty in report)

Table 6.2.1-155: Total radioactive residues in glyphosate-tolerant maize stover, cobs, and grain following one pre-emergent application at 4.37 kg glyphosate acid equivalents/ha + three foliar applications at 1.12, 1.11 and 1.10 kg glyphosate acid equivalents/ha

Matrix		Stover, 7 DALT	Cobs, 7 DALT	Grain, 7 DALT
0.1 % Formic acid:methanol extract	% TRR	85.0	69.3	71.0
	mg/kg	10.406	0.475	0.195
α-Amylase extract	% TRR	11.1	20.5	17.6
	mg/kg	1.359	0.141	0.048
Amyloglucosidase and cellulase extract	% TRR	1.3	3.3	2.4
	mg/kg	0.159	0.023	0.007
NaOH extract	% TRR	1.1	2.7	1.0
	mg/kg	0.135	0.019	0.003
HCl extract	% TRR	0.7	-	-
	mg/kg	0.086	-	-
Unextracted Residue	% TRR	0.9	4.2	7.9
	mg/kg	0.110	0.029	0.022
Total	mg/kg	*12.255*	0.686	0.275

TRR: total radioactive residue
DALT: days after last treatment
Italic: recalculated value (faulty in report)

B. Extraction and characterisation of residues

Characterisation and Identification of Residues in Maize Foliage

Residues in the immature foliage resulted from the root uptake of radioactive glyphosate and/or its soil degradates from the pre-emergent soil application. Maize foliage was extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v). This released 31.0 % TRR (0.007 mg/kg) with 69.0 % TRR (0.015 mg/kg) remaining in the unextracted residue. The extracted residue was too low (0.007 mg/kg) to warrant further analysis by HPLC. Immature maize foliage is not considered a raw agricultural commodity (RAC) therefore no further extractions were conducted.

Characterisation of Residues in Maize Forage

The distribution of TRR in maize forage is presented above. The majority of the TRR (87.0 %, 3.204 mg/kg) in maize forage was extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v). A further 9.1 % TRR, 0.316 mg/kg, was released by extraction with α-amylase. Extraction with the amyloglucosidase and cellulase mixture released 1.3 % TRR, 0.045 mg/kg, while extraction with NaOH and HCl released 1.0 % TRR (0.035 mg/kg) and 0.6 % TRR (0.021 mg/kg), respectively. A small proportion of the TRR (0.9 % TRR, 0.031 mg/kg) remained associated with the unextracted residue.

Extracts of maize forage were separately concentrated and analysed by HPLC. Glyphosate was the most abundant component, with 53.3 % TRR (1.852 mg/kg) extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v), 4.1 % TRR (0.143 mg/kg) extracted with α-amylase, and 0.6 % TRR (0.021 mg/kg) extracted with amyloglucosidase and cellulase mixture. *N*-acetylglyphosate was present as 24.3 % TRR (0.845 mg/kg) in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extraction, 2.1 % TRR (0.072 mg/kg) in the α-amylase extraction, 0.4 % TRR (0.015 mg/kg) in the amyloglucosidase and cellulase extraction, and 0.2 % TRR (0.005 mg/kg) in the NaOH extraction. Other metabolites in maize forage included AMPA, present at 3.4 % TRR (0.118 mg/kg) in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extraction and 0.6 % TRR (0.022 mg/kg) in the α-amylase extraction. *N*-acetyl-AMPA was also detected in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extraction (1.6 % TRR, 0.056 mg/kg) and in the α-amylase extraction (0.1 % TRR, 0.004 mg/kg). Multiple low-level components remained unidentified although none of these exceeded 0.4 % TRR (0.013 mg/kg).

Characterisation and Identification of Radioactive Residues in Maize Harvested at Maturity

The distributions of TRR from maize stover, cobs, and grain are presented above. The majority of the TRR in stover (85.0 %, 10.406 mg/kg) was released in 0.1 % formic acid (aqueous):methanol (96:4 v/v). A further 11.1 % TRR (1.359 mg/kg) was released by extraction with α-amylase. Extraction with the amyloglucosidase and cellulase mixture, NaOH, and HCl released 1.3 % (0.159 mg/kg), 1.1 % (0.135 mg/kg), and 0.7 % TRR (0.086 mg/kg), respectively. The remainder, 0.9 % TRR (0.110 mg/kg), was associated with unextracted residues.

The distribution of TRR in maize cobs and grain was similar to that in maize stover. In cobs, the majority of the TRR was released in 0.1 % formic acid (aqueous):methanol (96:4 v/v) (69.3 %, 0.475 mg/kg) and extraction with α-amlyase (20.5 % TRR, 0.141 mg/kg). A further 3.3 % (0.023 mg/kg) and 2.7 % (0.019 mg/kg) of TRR was present in extracts from amyloglucosidase/cellulase and NaOH extractions. The remaining 4.2 % TRR (0.029 mg/kg) was associated with unextracted residues.

In maize grain, again the majority of the TRR was released in 0.1 % formic acid (aqueous):methanol (96:4 v/v) (71.0 %, 0.195 mg/kg), with 17.6 % TRR (0.048 mg/kg) present in the α-amylase extract. A further 2.4 % (0.007 mg/kg) and 1.0 % TRR (0.003 mg/kg) were present in extracts from amyloglucosidase/cellulase and NaOH. The unextracted residues contained 7.9 % TRR (0.022 mg/kg).

The distribution of glyphosate and its metabolites in maize stover, cobs, and grain extracts is presented in a table below. The most abundant component in all stover extracts was glyphosate, comprising 66.8 % TRR (8.174 mg/kg) in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extract and 6.8 % TRR (0.836 mg/kg) in in the α-amylase extract. Glyphosate was also detected in stover at lower levels in the amyloglucosidase and cellulase mixture extract and in the NaOH extract at 0.7 % TRR (0.090 mg/kg) and 0.6 % TRR (0.066 mg/kg), respectively. *N*-acetylglyphosate comprised 15.5 % (1.899 mg/kg), 2.0 % (0.249 mg/kg), 0.2 % (0.026 mg/kg), and 0.1 % TRR (0.014 mg/kg) in the 0.1 % formic acid (aqueous):methanol (96:4 v/v), α-amylase, amyloglucosidase/cellulase, and NaOH extracts, respectively. AMPA was detected in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) and α-amylase extractions at 3.0 % TRR (0.368 mg/kg) and 0.4 % TRR (0.054 mg/kg), respectively. *N*-acetyl-AMPA was detected in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) and α-amylase extractions at concentrations not exceeding 1.1 % TRR (0.134 mg/kg) in either extract. Several unidentified components were present in each stover extract, the greatest was present in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extract and comprised 0.2 % TRR (0.025 mg/kg).

The most abundant component in maize cobs was *N*-acetylglyphosate, present in all extracts. *N*-acetylglyphosate comprised 57.5 % TRR (0.394 mg/kg) and 4.6 % TRR (0.030 mg/kg) in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) and α-amylase extractions, however, its concentration did not exceed 1.3 % TRR (0.009 mg/kg) in the amyloglucosidase/cellulase and NaOH extractions. Glyphosate was not detected in any cob extract. *N*-acetyl-AMPA was detected in cobs in the 0.1 % formic acid (aqueous):methanol (96:4 v/v) extraction (4.0 % TRR, 0.028 mg/kg) and in the α-amylase extraction (<0.01 mg/kg). Several unidentified components were present in cob extracts, none exceeding 2.0 % TRR (0.014 mg/kg).

The distribution of glyphosate and its metabolites in the 0.1 % formic acid (aqueous) methanol (96:4 v/v) extract of maize grain was similar to that in maize cobs. The most abundant metabolites present in the 0.1 % formic acid (aqueous) methanol (96:4 v/v) extraction were *N*-acetylglyphosate (50.1 % TRR, 0.138 mg/kg) and *N*-acetyl-AMPA (9.1 % TRR, 0.026 mg/kg). AMPA was detected at 2.0 % TRR (0.005 mg/kg). A single, low-level unidentified component was present in grain at 2.6 % TRR (0.007 mg/kg). AMPA was the main component in the α-amylase extraction (4.1 % TRR, 0.011 mg/kg). *N*-Acetylglyphosate, *N*-acetyl-AMPA, and glyphosate were also detected in the α-amylase grain extract at 1.1 %, 0.3 %, and 0.1 % TRR, respectively. A total of 14 unidentified components were present in the various grain extracts, none exceeded 1.3 % TRR (0.006 mg/kg).

Table 6.2.1-156: Extraction of the radioactive residues of glyphosate-tolerant maize forage following one pre-emergent application at 4.37 kg glyphosate acid equivalents/ha and two foliar applications at 1.12 and 1.11 kg glyphosate acid equivalents/ha

	Forage Residues, 59 days after last treatment	
Fraction	**mg/kg**	**% TRR**
0.1 % Formic acid:methanol extract*		
AMPA	0.118	3.4
Glyphosate	1.852	53.3
N-acetyl-AMPA	0.056	1.6
N-acetylglyphosate	0.845	24.3
Unidentified[1]	0.034	1.0
α-Amylase extract*		
AMPA	0.022	0.6
Glyphosate	0.143	4.1
N-acetyl-AMPA	0.004	0.1
N-acetylglyphosate	0.072	2.1
Unidentified[2]	0.017	0.3
Amyloglucosidase and cellulase extract*		
Glyphosate	0.021	0.6
N-acetylglyphosate	0.015	0.4
NaOH extract*		
N-acetylglyphosate	0.005	0.2
Unidentified[3]	0.006	0.3
Total characterised/identified	3.210	92.3
HCl extract	0.021	0.6
Differences during processing[4]	*0.390*	*6.2*
ERR	*3.621*	*99.1*
RRR	0.031	0.9
Total	*3.652*	100.0

Table 6.2.1-156: Extraction of the radioactive residues of glyphosate-tolerant maize forage following one pre-emergent application at 4.37 kg glyphosate acid equivalents/ha and two foliar applications at 1.12 and 1.11 kg glyphosate acid equivalents/ha

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue
Italic: recalculated value (faulty in report)
*: Extracts processed for HPLC
1 Comprised of 6 components, one 0.4 % TRR, 0.013 mg/kg, all others less than 0.2 % TRR, 0.007 mg/kg
2 Comprised of 7 components, none greater than 0.2 % TRR, 0.009 mg/kg
3 Comprised of 18 components, none greater than 0.1 % TRR, 0.004 mg/kg
4 Differences during processing reflect losses incurred during processing of samples for HPLC

Table 6.2.1-157: Extraction of the radioactive residues of glyphosate tolerant maize stover, cobs, and grain following one pre-emergent application at 4.37 kg glyphosate acid equivalents/ha and three foliar applications at 1.12, 1.11 and 1.10 kg glyphosate acid equivalents/ha

	Residues, 7 days after last treatment					
	Stover		Cobs		Grain	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
0.1 % Formic acid:methanol extract*						
AMPA	0.368	3.0	-	-	0.005	2.0
Glyphosate	8.174	66.8	-	-	-	-
N-acetyl-AMPA	0.134	1.1	0.028	4.0	0.026	9.1
N-acetylglyphosate	1.899	15.5	0.394	57.5	0.138	50.1
Unidentified	0.061[1]	0.4	0.003[5]	0.5	0.007[9]	2.6
α-Amylase extract*						
AMPA	0.054	0.4	-	-	0.011	4.1
Glyphosate	0.836	6.8	-	-	<0.001	0.1
N-acetyl-AMPA	0.018	0.2	0.006	0.9	<0.001	0.3
N-acetylglyphosate	0.249	2.0	0.030	4.6	0.003	1.1
Unidentified	0.027[2]	0.2	0.069[6]	9.9	0.034[10]	8.3
Amyloglucosidase and cellulase extract*						
Glyphosate	0.090	0.7	-	-	-	-
N-acetyl-AMPA		-	<0.001	0.1	-	-
N-acetylglyphosate	0.026	0.2	0.002	0.4	-	-
Unidentified	0.002[3]	<0.1	<0.001[7]	2.3	-	-
NaOH extract*						
Glyphosate	0.066	0.6	-	-	-	-
N-acetylglyphosate	0.014	0.1	0.009	1.3	-	-
Unidentified	0.002[4]	<0.1	<0.001[8]	<0.1	-	-
Total characterised/identified	12.020	98.0	0.541	81.5	0.224	77.7
HCl extract	0.086	0.7	-	-	-	-
Differences during processing[11]	*0.039*	*0.4*	*0.116*	*14.3*	*0.029*	*14.4*
ERR	*12.145*	*99.1*	*0.657*	*95.8*	*0.253*	*92.1*
RRR	0.110	0.9	0.029	4.2	0.022	7.9
Total	*12.255*	100.0	0.686	100.0	0.275	100.0

Table 6.2.1-157: Extraction of the radioactive residues of glyphosate tolerant maize stover, cobs, and grain following one pre-emergent application at 4.37 kg glyphosate acid equivalents/ha and three foliar applications at 1.12, 1.11 and 1.10 kg glyphosate acid equivalents/ha

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue
Italic: recalculated value (faulty in report)
*: Extracts processed for HPLC
1 Comprised of 5 components, one 0.2 % TRR, 0.025 mg/kg, all others less than 0.1 % TRR, 0.016 mg/kg
2 Comprised of 4 components, none greater than 0.1 % TRR, 0.009 mg/kg
3 Comprised of a single component
4 Comprised of 2 components, both <0.1 % TRR, 0.001 mg/kg
5 Comprised of a single component
6 Comprised of 12 components, one at 2.0 % TRR, 0.014 mg/kg, the rest <1.4 % TRR, 0.009 mg/kg
7 Comprised of 13 components, none greater than 0.3 % TRR, <0.001 mg/kg
8 Comprised of two components, both <0.1 % TRR, <0.001 mg/kg
9 Comprised of a single component
10 Comprised of 14 components, none greater than 1.3 % TRR, 0.006 mg/kg
11 Differences during processing reflect losses incurred during processing of samples for HPLC.

Table 6.2.1-158: Distribution of radioactive residues of glyphosate and its metabolites in glyphosate tolerant maize forage, stover, cobs, and grain

	Forage		Stover		Cobs		Grain	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
AMPA	0.140	4.0	0.422	3.4	-	-	0.016	6.1
Glyphosate	2.016	58.0	9.166	74.9	-	-	<0.001	0.1
N-acetyl-AMPA	0.060	1.7	0.152	1.3	0.034	5.0	0.026	9.4
N-acetylglyphosate	0.937	27.0	2.188	17.8	0.435	63.8	0.141	51.2
Unidentified[1]	0.057	1.6	0.092	0.6	0.072	12.7	0.041	10.9
Total identified	3.153	90.7	11.928	97.4	0.469	68.8	0.183	66.8
Total characterised	0.057	1.6	0.092	0.6	0.072	12.7	0.041	10.9
Losses during processing/ extracts not analysed[2]	0.411	6.8	0.125	1.1	0.116	14.3	0.029	14.4
ERR	3.621	99.1	12.145	99.1	0.657	95.8	0.253	92.1
RRR	0.031	0.9	0.110	0.9	0.029	4.2	0.022	7.9
Total	3.652	100.0	12.255	100.0	0.686	100.0	0.275	100.0

[1]: Sum of 2 - 18 components, none greater than 2.0 % TRR or 0.025 mg/kg
[2]: Losses during processing reflect losses incurred during processing of samples for HPLC. HCl extracts were not analysed.

E. Storage stability

The samples remained frozen prior to analysis. An analysis of storage stability was not conducted as part of this study since samples were analysed within 6 months of collection.

F. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III Conclusions

The nature of the residue in Optimum™ GAT™ maize following one pre-emergent (soil) application and three foliar applications of [^{14}C]glyphosate is adequately understood.

The TRR in the immature foliage was low (0.022 mg/kg indicating that only low levels of radioactive soil residues were taken into the developing maize plants.

The TRR in forage was 3.652 mg/kg with the majority extractable; 92.3 % TRR (3.210 mg/kg) of the forage residues were characterised/identified. The major component in forage was glyphosate (58.0 % TRR, 2.016 mg/kg) while *N*-acetylglyphosate was present at 27.0 % TRR (0.937 mg/kg). AMPA and *N*-acetyl-AMPA comprised 4.0 % TRR (0.140 mg/kg) and 1.7 % TRR (0.060 mg/kg), respectively.

TRR in stover was 12.255 mg/kg; 98.0 % TRR (12.020 mg/kg) was characterised/identified. The major extractable components in stover were glyphosate (74.9 % TRR, 9.166 mg/kg) and *N*-acetylglyphosate (17.8 % TRR, 2.188 mg/kg). The metabolites AMPA and *N*-acetyl-AMPA were also detected but at lower levels, 3.4 % TRR (0.422 mg/kg) and 1.3 % TRR (0.152 mg/kg) respectively.

TRR in cobs and grain were 0.686 mg/kg and 0.275 mg/kg respectively. The majority of the cob (81.5 % TRR, 0.541 mg/kg) and grain (77.7 % TRR, 0.224 mg/kg) residues were characterised/identified. *N*-acetylglyphosate was the major extractable component in cobs (63.8 % TRR, 0.435 mg/kg) and grain (51.2 % TRR, 0.141 mg/kg). *N*-acetyl-AMPA was the second most prominent metabolite in cobs and grain, present at 5.0 % TRR (0.034 mg/kg) and 9.4 % TRR (0.026 mg/kg) respectively. AMPA and glyphosate were detected in grain at 6.1 % TRR (0.016 mg/kg) and 0.1 % TRR (<0.001 mg/kg), respectively.

Unextractable residues accounted for 0.9 % TRR in forage and stover, 4.2 % TRR in cobs and 7.9 % TRR in grain.

The metabolic pathway of glyphosate in Optimum™ GAT™ maize is consistent with that in Optimum™ GAT™ soybean plants.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in field maize has been previously evaluated at EU level (RAR (2015). It was performed under GLP. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501, with minor deficits (certificate of analysis for test materials was not provided, but the purity of the used batch is given in chapter 3.1.1 of the report and was re-analysed in the treatment solutions before each application; certificates of analyses for reference substances were not provided, but the purities of the used batches are given in chapter 3.1.2 of the report; identification was done by HPLC retention time comparison with authenticated standards and TLC (including admixed and co-spotted samples) with radiolabelled standards).
The study is considered reliable for the assessment of the metabolic behaviour of glyphosate in maize.

Assessment and conclusion by RMS:

Pulses and oilseeds

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/025
Report author	[redacted]
Report year	2010
Report title	The Metabolism of [^{14}C]Glyphosate in 0827 Canola
Report No	808685

Document No	DuPont-26109
Guidelines followed in study	OPPTS 860.1300, Nature of the Residue - Plants; Canadian PMRA Residue Chemistry Test Guidelines Dir 98-02, Section 2, Nature of the Residue Plants; and the recommendations of EU Commission Directive 96/68/EC Annex II, Section 8.1 (21 October 1996).
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Certificate of analysis for test materials was not provided, but the purity of the used batch is given in chapter 3.1.1 of the report and was re-analysed in the treatment solutions before each application • Certificates of analyses for reference substances were not provided, but the purities of the used batches are given in chapter 3.1.2 of the report • Identification was done by HPLC retention time comparison with authenticated standards and TLC (including admixed and co-spotted samples) with radiolabelled standards • Storage stability not discussed in the report. Dates of sampling and analyses are given in App. 5 and Quality Assurance Statement but refer to initial dates
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues after treatments to 0827 canola plants modified to express the glyphosate *N*-acetyltransferase (*gat*) gene. The nature of residues resulting from foliar uptake was investigated a pre-emergence application of 4.50 kg glyphosate acid equivalents/ha to bare soil on the day of sowing and three foliar applications of 0.94 to 1.03 kg glyphosate acid equivalents/ha made at BBCH 12, BBCH 15, and BBCH 87 growth stages. The test substance consisted of ^{14}C-labelled glyphosate formulated with Touchdown Total® formulation blank and 2 % ammonium sulphate (AMS).

Canola plants were taken as immature foliage (growth stage BBCH 69; 38 days after the second foliar application). A pre-harvest sample was taken prior to the final foliar application (growth stage BBCH 87; 90 days after the second foliar application; immediately prior to the last foliar application) and separated into foliage and pods (with seed). At maturity (growth stage BBCH 89; 7 days after the third foliar application) whereupon plants were separated into seed and foliage (including pods, retained without analysis).

At each sampling point, tissues were homogenised and extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v) (hereafter referred to as aqueous medium) followed by enzyme (α-amylase then amyloglucosidase and cellulase), alkaline, then acid digestion. The total radioactive residue (TRR) was determined as the sum of the total dpm in extractable and unextracted residues expressed as mg/kg equivalents of glyphosate. Extracts containing ≥0.01 mg/kg were analysed by high-performance liquid

chromatography (HPLC) and the identification of residues accomplished with reference to authenticated reference standards.

TRR in the immature foliage from the first harvest was 5.979 mg/kg and the majority of the radioactivity was extracted using aqueous medium (97.3 % of the TRR, 5.818 mg/kg). *N*-Acetylglyphosate was the major extractable radioactive component accounting for 89.5 % of the TRR (5.351 mg/kg). Glyphosate, *N*-acetyl AMPA, and AMPA were also detected at low levels accounting for 3.0 % of the TRR (0.179 mg/kg), 3.4 % of the TRR (0.203 mg/kg), and 1.4 % of the TRR (0.084 mg/kg), respectively. The unextracted residue contained 2.7 % of the TRR (0.161 mg/kg).

TRR in immature pods from the pre-harvest sampling immediately prior to the final application was 1.273 mg/kg and the majority of the radioactivity was recovered in aqueous medium (79.6 % of the TRR, 1.013 mg/kg). *N*-Acetylglyphosate was the only radioactive component detected accounting for 79.6 % of the TRR (1.013 mg/kg). The unextracted residue contained 20.4 % of the TRR, 0.260 mg/kg. TRR in the immature foliage from the second harvest was 1.550 mg/kg and the majority of the radioactivity in foliage was recovered with aqueous medium (93.0 % of the TRR, 1.442 mg/kg). *N*-Acetylglyphosate was the major radioactive component detected in this extract (93.0 % of the TRR, 1.442 mg/kg). Additional residues were extracted in α-amylase (1.7 % of the TRR, 0.026 mg/kg), amyloglucosidase and cellulose (0.5 % of the TRR, 0.008 mg/kg), NaOH (0.8 % of the TRR, 0.012 mg/kg), and HCl (0.4 % of the TRR, 0.006 mg/kg) digests. Chromatographic analysis of these digests was not conducted due to the low TRR. The terminal unextracted residue contained 3.6 % of the TRR, 0.056 mg/kg.

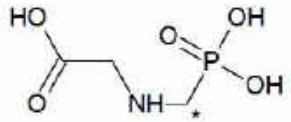
At final harvest, TRR in mature seed was 2.155 mg/kg, and the majority of the radioactivity was recovered in aqueous medium (78.4 % of the TRR, 1.690 mg/kg). Additional residues were extracted in α-amylase (11.7 % of the TRR, 0.252 mg/kg), amyloglucosidase and cellulose (2.3 % of the TRR, 0.050 mg/kg), NaOH (3.2 % of the TRR, 0.069 mg/kg) and HCl (0.9 % of the TRR, 0.019 mg/kg) digests. *N*-Acetylglyphosate was the major radioactive component in the seed accounting for 51.1 % of the TRR, 1.101 mg/kg. Glyphosate (20.8 % of the TRR, 0.448 mg/kg), *N*-acetyl AMPA (14.7 % of the TRR, 0.316 mg/kg) and AMPA (1.9 % of the TRR, 0.041 mg/kg) were also detected. Numerous other metabolites which did not correspond to known reference standards were also present; none individually exceeded 1.0 % of the TRR (0.022 mg/kg). The terminal unextracted residue was 3.5 % of the TRR, 0.075 mg/kg.

I. Materials and methods

A. Materials

Test Material: N-(phosphono-^{14}C-methyl)glycine

Chemical structure:



* Position of label

Radiochemical purity:	≥ 98.4 %
Specific activity:	10.59 µCi/mg (0.39 MBq/mg)
CAS No:	1071-83-6
Log $P_{o/w}$:	- 3.2

Test system

Soil:	Sandy loam [textural class (UK)] (pH: 6.3; cation exchange capacity: 0.154 mol/kg; organic carbon: 3.2 %; particle size 0.063-2 mm: 65.93 %; 0.002-0.063 mm: 18.71 %; <0.002 mm: 15.36 %)
Crop:	0827 Canola plants, glyphosate tolerant
Botanical name:	*Brassica napus* L
Crop part(s):	Immature foliage, immature pods (with seed), seed

B. Study design

1. In-life phase

For the investigation of the metabolism of glyphosate in 0827 canola plants were treated with ^{14}C glyphosate labelled in the phosphonomethyl-moiety. Glyphosate was co-formulated with Touchdown Total® formulation blank and 2 % ammonium sulphate (w/v). The study was conducted in a single glasshouse compartment. Canola seeds were sown into three crates filled with sandy loam soil.

The application plan consisted of a pre-emergence application at a target rate equivalent to 4.5 kg glyphosate acid equivalents/ha to bare soil and three foliar applications of 1.0 kg glyphosate acid equivalents/ha targeted at BBCH 11-13, BBCH 14-16, and BBCH 87 growth stages. The actual treatments were 4.50 kg glyphosate acid equivalents/ha to bare soil on the day of sowing and three foliar applications of 0.94 to 1.03 kg glyphosate acid equivalents/ha made at BBCH 12, BBCH 15, and 7 days prior to maturity at BBCH 87 growth stages.

Applications were made using a hand-held sprayer system comprising a brass header with trigger valve and a single polyacetal flat-fan nozzle with 100 mesh sieve. Following each application, the sprayer was rinsed with water equivalent to *ca* 10 % of the original spray volume. This rinsate was also applied to the treatment area. Polythene sheeting was erected around crates prior to application to avoid contamination during application and removed afterwards. After application, the amount of residual radioactivity associated with each spray container was determined. Spray containers were immersed together in detergent (10 %, v/v), soaked at least overnight and aliquots removed for LSC. Results were used to calculate the amount of radioactivity applied. The radiochemical purity of each treatment solution was determined before and after each application.

The plants were watered as required and fertilizers were applied when necessary. The plants were observed for evaluation of growth stages. In parallel, control plants were grown and treated with Touchdown Total® formulation blank and ammonium sulphate.

2. Sampling

Whole aerial portions of 0827 canola plants were taken at each sampling. Canola plants were taken as immature foliage (growth stage BBCH 69; 38 days after the second foliar application). A pre-harvest sample was taken prior to the final foliar application (growth stage BBCH 87; 90 days after the second foliar application; immediately prior to the last foliar application) and separated into foliage and pods (with seed). At maturity (growth stage BBCH 89; 7 days after the third foliar application) whereupon plants were separated into seed and foliage (including pods, retained without analysis).

The samples were stored frozen at least overnight (*ca.* -20 °C) prior to pulverizing. Frozen plant tissue was pulverised with excess solid carbon dioxide chips using a food processor or commercial blender. If further homogenisation was required prior to extraction, then subsamples of the homogenised tissue were

freezer-milled in liquid nitrogen. For each sample, the carbon dioxide was allowed to sublime while frozen prior to removal of subsamples for combustion and extraction.

The samples from the foliage harvest, pre-harvest, and mature harvest were stored frozen at approximately -20 °C until initial extraction, no longer than 7, 14, and 7 days, respectively. Initial HPLC analyses were completed 2 days after extraction for foliage samples, 6 days after extraction for pre-harvest samples, and 6 days after extraction for mature harvest samples.

3. Analytical procedures

Portions of each homogenised tissue were extracted three times with 0.1 % formic acid (aqueous):methanol (96:4 v/v) (aqueous medium). Select unextracted residues were then enzyme digested twice with α-amylase. The unextracted residues remaining after α-amylase digestion were incubated twice with a mix of amyloglucosidase and cellulose enzymes in sodium acetate buffer. The remaining unextracted residues after enzyme hydrolysis were then incubated twice in 0.1 N NaOH (60 ºC, 6 hours). The remaining unextracted residues after alkaline digestion were incubated twice with 1.0 N HCl (60 ºC, 6 hours) where necessary. Extracts were concentrated and reconstituted in a suitable solvent prior to liquid scintillation counting (LSC) and High Performance Liquid Chromatography (HPLC).

For identification and quantification of glyphosate and metabolites, a HPLC system was employed using on-line UV detection and a radiodetector. Following on-line radiodetection, effluent fractions were collected for quantification of radioactivity via LSC. Authenticated analytical reference standards of glyphosate, AMPA, *N*-acetyl AMPA, and *N*-acetylglyphosate were analysed by HPLC to verify column and instrument operation and to determine the retention times of these compounds.

The total radioactive residue (TRR) was determined as the sum of the total dpm in extractable and unextracted residues expressed as mg/kg equivalents of glyphosate. Levels of radioactivity were determined in each extract by Liquid Scintillation Counting (LSC) and in the unextracted residues by oxidative combustion and LSC. The limits of detection for quantification of radioactive peaks on chromatograms were assessed as <0.1 % of the TRR (<0.001 mg/kg).

Thin layer chromatography (TLC) was conducted to confirm the identity of metabolites detected using HPLC. Radioactive areas on the developed plates were located using a phosphor imager. The sample was applied to a TLC plate, co-spotted and/or, admixed with the appropriate radiolabelled reference standards. The radioactive components were then compared with standard reference compounds for identification. The identity of AMPA in canola fractions was not confirmed using a secondary chromatographic method as AMPA was present (3 – 5 %) in the treatment solution and was only detected at low levels (< 2 % of the TRR) in the canola fractions.

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in canola foliage is summarised below. The TRR in the immature foliage from the first harvest was 5.979 mg/kg following a single soil application and two foliar applications of N-(phosphono-^{14}C-methyl)glycine. The TRR in immature pods was 1.273 mg/kg and the TRR in the immature foliage was 1.550 mg/kg.

The distribution of TRR from the final harvest sampling is presented. The TRR in mature seed was 2.155 mg/kg following a single soil application and three foliar applications of N-(phosphono-^{14}C-methyl)glycine.

Table 6.2.1-159: Total radioactive residues in glyphosate-tolerant canola foliage and pods following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and two foliar applications at 0.98 and 1.03 kg glyphosate acid equivalents/ha

		Harvest 1	Pre-maturity Harvest 2	
Matrix		Foliage, 38 DAT 3	Foliage, 90 DAT 3	Pods (with seed), 90 DAT 3
0.1 % Formic acid:methanol extract	% TRR	97.3	93.0	79.6
	mg/kg	5.818	1.442	1.013
α-Amylase extract	% TRR	-	1.7	-
	mg/kg	-	0.026	-
Amyloglucosidase and cellulase extract	% TRR	-	0.5	-
	mg/kg	-	0.008	-
NaOH extract	% TRR	-	0.8	-
	mg/kg	-	0.012	-
HCl extract	% TRR	-	0.4	-
	mg/kg	-	0.006	-
RRR	% TRR	2.7	3.6	20.4
	mg/kg	0.161	0.056	0.260
Total	mg/kg	5.979	1.550	1.273

TRR: total radioactive residue, expressed as glyphosate equivalent
RRR: residual radioactive residues (after conventional and exhaustive extractions)
DAT #: days after the numbered treatments specified for each harvest below
Harvest 1 = immature foliage sampling (growth stage BBCH 69); 38 days after the second foliar application
Harvest 2 = a pre-harvest sample was taken prior to the final foliar application (growth stage BBCH 87; 90 days after the second foliar application; immediately prior to the last foliar application) and separated into foliage and pods (with seed).

Table 6.2.1-160: Total radioactive residues in glyphosate-tolerant canola seed following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and three foliar applications at 0.94 to 1.03 kg glyphosate acid equivalents/ha

Matrix		Harvest 3 Seed, 7 DAT 4
0.1 % Formic acid:methanol extract	% TRR	78.4
	mg/kg	1.690
α-Amylase extract	% TRR	11.7
	mg/kg	0.252
Amyloglucosidase and cellulase extract	% TRR	2.3
	mg/kg	0.050
NaOH extract	% TRR	3.2
	mg/kg	0.069
HCl extract	% TRR	0.9
	mg/kg	0.019
RRR	% TRR	3.5
	mg/kg	0.075
Total	mg/kg	2.155

TRR: Total radioactive residue, expressed as glyphosate equivalent
RRR: Residual radioactive residue (after conventional and exhaustive extractions)
DAT #: days after the numbered treatments specified for each harvest below.
Harvest 3 = at maturity (growth stage BBCH 89; 7 days after the third foliar application) whereupon plants were separated into seed and foliage (including pods, retained without analysis).

B. Extraction and characterisation of residues

Characterisation and identification of residues in canola immature foliage

The distribution of TRR in canola immature foliage is shown above. TRR in the immature foliage from the first harvest was 5.979 mg/kg and the majority of the radioactivity was extracted using aqueous medium (97.3 % of the TRR, 5.818 mg/kg). The residual radioactive residue (RRR) contained 2.7 % of the TRR (0.161 mg/kg).

The extraction and distribution of glyphosate and its metabolites in immature foliage is shown below. *N*-Acetylglyphosate was the major extractable radioactive component accounting for 89.5 % of the TRR (5.351 mg/kg). Glyphosate, *N*-acetyl AMPA, and AMPA were also detected at low levels accounting for 3.0 % of the TRR (0.179 mg/kg), 3.4 % of the TRR (0.203 mg/kg), and 1.4 % of the TRR (0.084 mg/kg), respectively.

Table 6.2.1-161: Extraction of the radioactive residues of glyphosate-tolerant canola immature foliage following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and two foliar applications at 0.98 to 1.03 kg glyphosate acid equivalents/ha

Matrix	Harvest 1 Immature foliage, 38 DAT 3	
Fraction	**mg/kg**	**% TRR**
TRR	**5.979**	**100**
0.1 % Formic acid:methanol extract	5.818	97.3
AMPA	0.084	1.4
Glyphosate	0.179	3.0
N-acetyl AMPA	0.203	3.4
N-acetylglyphosate	5.351	89.5
Total characterised/identified	**5.818**	**97.3**
ERR	**5.818**	**97.3**
RRR	**0.161**	**2.7**

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extractions)
DAT #: days after the numbered treatments specified for each harvest below.
Harvest 1 = immature foliage sampling (growth stage BBCH 69); 38 days after the second foliar application
All residue data are expressed as mg/kg glyphosate equivalents

Characterisation and identification of residues in pre-harvest canola pods and foliage

The distribution of TRR in pre-harvest canola foliage and pods (with seed) is presented above. The majority of TRR in immature pods was recovered in aqueous medium (79.6 % of the TRR, 1.013 mg/kg). The unextracted residue contained 20.4 % of the TRR, 0.260 mg/kg. The majority of TRR in the immature foliage was recovered with 0.1 % formic acid (aqueous):methanol (96:4 v/v) (93.0 % of the TRR, 1.442 mg/kg). Additional residues were extracted in α-amylase (1.7 % of the TRR, 0.026 mg/kg), amyloglucosidase and cellulose (0.5 % of the TRR, 0.008 mg/kg), NaOH (0.8 % of the TRR, 0.012 mg/kg), and HCl (0.4 % of the TRR, 0.006 mg/kg) digests. Chromatographic analysis of these digests was not conducted. The terminal unextracted residue contained 3.6 % of the TRR, 0.056 mg/kg.

The extraction and distribution of glyphosate and its metabolites in pre-harvest canola pods and foliage is shown in the tables below. *N*-Acetylglyphosate was the only radioactive component detected accounting for 79.6 % of the TRR (1.013 mg/kg) in immature pods and 93.0 % of the TRR (1.442 mg/kg) in foliage.

Table 6.2.1-162: Extraction of the radioactive residues of glyphosate-tolerant canola foliage and pods following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and two foliar applications at 0.98 to 1.03 kg glyphosate acid equivalents/ha

	Pre-maturity Harvest 2			
Matrix	**Pods (with seed) 90 DAT 3**		**Foliage 90 DAT 3**	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.273**	**100**	**1.550**	**100**
0.1 % Formic acid:methanol extract	1.013	79.6	1.442	93.0
N-acetylglyphosate	1.013	79.6	1.442	93.0
α-Amylase extract	-	-	0.026	1.7
Amyloglucosidase and cellulase extract	-	-	0.008	0.5
NaOH extract	-	-	0.012	0.8
HCl extract	-	-	0.006	0.4
Total identified	**1.013**	**79.6**	**1.442**	**93.0**
ERR	**1.013**	**79.6**	**1.494**	**96.4**
RRR	**0.260**	**20.4**	**0.056**	**3.6**

TRR: Total radioactive residue
RRR: Residual radioactive residues (after conventional and exhaustive extractions)
ERR: Extractable radioactive residue (considering combined extracts measured)
DAT #: days after the numbered treatments specified for each harvest below.
Harvest 2 = a pre-harvest sample was taken prior to the final foliar application (growth stage BBCH 87; 90 days after the second foliar application; immediately prior to the last foliar application) and separated into foliage and pods (with seed).
All residue data are expressed as mg/kg glyphosate equivalents

Characterisation and identification of radioactive residues in canola seed harvested at maturity

The distribution of TRR from canola seed is presented above. At final harvest, the majority of the radioactivity in mature seed was recovered in aqueous medium (78.4 % of the TRR, 1.690 mg/kg). Additional residues were extracted in α-amylase (11.7 % of the TRR, 0.252 mg/kg), amyloglucosidase and cellulose (2.3 % of the TRR, 0.050 mg/kg), NaOH (3.2 % of the TRR, 0.069 mg/kg) and HCl (0.9 % of the TRR, 0.019 mg/kg) digests. The terminal unextracted residue was 3.5 % of the TRR, 0.075 mg/kg.

The extraction and distribution of glyphosate and its metabolites in canola seed at maturity is shown in the tables below. *N*-Acetylglyphosate was the major radioactive component in the seed accounting for 51.1 % of the TRR, 1.101 mg/kg. Glyphosate (20.8 % of the TRR, 0.448 mg/kg), *N*-acetyl AMPA (14.7 % of the TRR, 0.316 mg/kg) and AMPA (1.9 % of the TRR, 0.041 mg/kg) were also detected. Numerous other metabolites which did not correspond to known reference standards were also present; none individually exceeded 1.0 % of the TRR (0.022 mg/kg).

Table 6.2.1-163: Extraction of the radioactive residues of glyphosate-tolerant canola seed following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and three foliar applications at 0.94 to 1.03 kg glyphosate acid equivalents/ha

Matrix	**Maturity, Harvest 3 Seed, 7 DAT 4**	
Fraction	**mg/kg**	**% TRR**
TRR	**2.155**	**100**
0.1 % Formic acid:methanol extract	1.690	78.4
Glyphosate	0.414	19.2
N-acetyl AMPA	0.308	14.3
N-acetylglyphosate	0.966	44.8
α-Amylase extract	0.252	11.7
AMPA	0.028	1.3
Glyphosate	0.032	1.5
N-acetyl AMPA	0.004	0.2
N-acetylglyphosate	0.114	5.3
Unidentified[1]	0.076	3.3
Amyloglucosidase and cellulase extract	0.050	2.3
AMPA	0.005	0.2
N-acetylglyphosate	0.015	0.7
Unidentified[2]	0.031	1.3
NaOH extract	0.069	3.2
AMPA	0.008	0.4
Glyphosate	0.002	0.1
N-acetyl AMPA	0.004	0.2
N-acetylglyphosate	0.006	0.3
Unidentified[3]	0.045	2.5
HCl extract	0.019	0.9
Total identified	***1.906***	***88.5***
Total characterised	***0.171***	***8.0***
ERR	**2.080**	**96.5**
RRR	**0.075**	**3.5**

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extractions)
DAT #: days after the numbered treatments specified for each harvest below.
Values in *italics* were recalculated during dossier compilation.
Harvest 3 = at maturity (growth stage BBCH 89; 7 days after the third foliar application) whereupon plants were separated into seed and foliage (including pods, retained without analysis).
All residue data are expressed as mg/kg glyphosate equivalents
1 Comprised of 17 components, no single component greater than 1.0 % TRR, 0.022 mg/kg.
2 Comprised of 15 components, no single component greater than 0.2 % TRR, 0.005 mg/kg.
3 Comprised of 28 components, no single components greater than 0.2 % TRR, 0.004 mg/kg.

Table 6.2.1-164: Distribution of the radioactive residues of glyphosate-tolerant canola seed following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and thee foliar applications at 0.94 to 1.03 kg glyphosate acid equivalents/ha

Matrix	**Harvest 1**		**Pre-maturity Harvest 2**				**Maturity Harvest 3**	
	Immature foliage, 38 DAT 2		**Pods (with seed) 90 DAT 3**		**Foliage 90 DAT 3**		**Seed, 7 DAT 4**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**5.979**	**100**	**1.273**	**100**	**1.550**	**100**	**2.155**	**100**
Glyphosate	0.179	3.0	n.d	n.d	n.d	n.d	0.448	20.8
N-acetyl	0.203	3.4	n.d	n.d	n.d	n.d	0.316	14.7

Table 6.2.1-164: Distribution of the radioactive residues of glyphosate-tolerant canola seed following one pre-emergent application at 4.50 kg glyphosate acid equivalents/ha and thee foliar applications at 0.94 to 1.03 kg glyphosate acid equivalents/ha

Matrix	Harvest 1		Pre-maturity Harvest 2				Maturity Harvest 3	
	Immature foliage, 38 DAT 2		Pods (with seed) 90 DAT 3		Foliage 90 DAT 3		Seed, 7 DAT 4	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
AMPA								
N-acetyl-glyphosate	5.351	89.5	1.013	79.6	1.442	93.0	1.101	51.1
AMPA	0.084	1.4	n.d	n.d	n.d	n.d	0.041	1.9
Unidentified	n.d.	n.d	n.d	n.d	n.d	n.d	0.152[1]	7.1
Total identified	**5.818**	**97.3**	**1.013**	**79.6**	**1.442**	**93.0**	***1.906***	***88.5***
Total characterised	**n.d.**	**n.d**	**n.d**	**n.d**	**n.d**	**n.d**	***0.171***	***8.0***
ERR	**5.818**	**97.3**	**1.013**	**79.6**	**1.494**	**96.4**	**2.080**	**96.5**
RRR	**0.161**	**2.7**	**0.260**	**20.4**	**0.056**	**3.6**	**0.075**	**3.5**

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extractions)
DAT #: days after the numbered treatments specified for each harvest below.
Harvest 1 = immature foliage sampling (growth stage BBCH 69); 38 days after the second foliar application
Harvest 2 = a pre-harvest sample was taken prior to the final foliar application (growth stage BBCH 87; 90 days after the second foliar application; immediately prior to the last foliar application) and separated into foliage and pods (with seed).
Harvest 3 = at maturity (growth stage BBCH 89; 7 days after the third foliar application) whereupon plants were separated into seed and foliage (including pods, retained without analysis).
n.d. = not detected
Values in *italics* were recalculated during dossier compilation.
All residue data are expressed as mg/kg glyphosate equivalents
[1] multiple components, no single component greater than 1.0 % of the TRR, 0.022 mg/kg

E. Storage stability

Samples of foliage harvest, pre-harvest and mature harvest were stored frozen (-20 °C) prior to extraction. An analysis of storage stability was not conducted as part of this study. Initial samples were analysed within 7 to 14 days of collection. Final extraction dates are not stated within the study report. However dates of sampling and analyses are given in Appendix 5 and quality assurance statement. The latest date of extraction given in the quality assurance statement is March 30, 2009. The first sampling was done on January 19, 2009. Therefore, the maximum storage duration can be estimated to be 70 days and therefore no storage stability investigations were necessary.

F. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusions

This study investigated the metabolism of N-(phosphono-^{14}C-methyl)glycine in 0827 canola (*Brassica napus* L*)* modified to express the glyphosate N-acetyltransferase (gat) gene following a single pre-emergent soil application at 4.5 kg glyphosate acid equivalents/ha and three foliar applications (0.98, 1.03, and 0.94 kg glyphosate acid equivalents/ha).

The TRR measured in mature seed was 2.155 mg/kg. TRR levels in foliage and immature pods (with seed) were 1.550 to 5.979 mg/kg and 1.273 mg/kg, respectively. The increases and/or decreases in TRR measured in foliage were attributable to multiple foliar applications and to growth and development of the crop.

A total of 95.6 % of the TRR (2.058 mg/kg) was identified in the mature seed and 79.6 to 97 % of the TRR (1.013 - 5.817 mg/kg) was identified in foliage and pod. *N*-Acetylglyphosate was the principal extractable component accounting for 51.1 % of the TRR (1.101 mg/kg) in seed and 79.6 to 93.0 % of the TRR (1.013-5.351 mg/kg) in the foliage and pod samples. At final harvest 7 days after the last application increased levels of glyphosate were found at 20.8 % of the TRR, 0.448 mg/kg compared to levels seen in earlier harvests (3.0 % of the TRR, 0.179 mg/kg in foliage). The other major metabolite was *N*-acetyl AMPA, accounting for 14.7 % of the TRR (0.316 mg/kg) in seed and 3.4 % of the TRR (0.203 mg/kg) in foliage sample. Low levels of AMPA were detected in the seed (1.9 % of the TRR, 0.041 mg/kg) and foliage (1.4 % of the TRR, 0.084 mg/kg), however [^{14}C]AMPA was also present in the treatment solutions at low concentrations (*ca* 3-5 %).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in canola has been previously evaluated at EU level. It was performed under GLP. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with minor deficits (certificate of analysis for test materials was not provided, but the purity of the used batch is given in chapter 3.1.1 of the report and was re-analysed in the treatment solutions before each application; certificates of analyses for reference substances were not provided, but the purities of the used batches are given in chapter 3.1.2 of the report; identification was done by HPLC retention time comparison with authenticated standards and TLC (including admixed and co-spotted samples) with radiolabelled standards; storage stability not discussed in the report. Dates of sampling and analyses are given in App. 5 and Quality Assurance Statement but refer to initial dates).

No information on storage duration of frozen plant samples and plant extracts is given in the study report. However, based on the date of first sampling (January 19, 2009) and the latest date for extraction (March 30, 2009, available in the quality assurance statement) the maximum duration can be estimated to be ~70 days (~ 2 months) and therefore no storage stability investigations were necessary. Still, a high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies.

No degradation of glyphosate and its metabolites was found in matrices with high water content comparable to the present study, like corn forage, fodder, cotton forage, soybean forage. Over an investigated storage duration of 215-393 days no degradation was observed ([redacted] 1995, CA 6.2.1/020; [redacted] 1997, CA 6.2.1/023 and [redacted] et al, 1994, CA 6.2.1/022). In matrices with high oil content like cotton, soybean and canola seeds glyphosate-derived residues were stable for 273 to 501 days ([redacted] 1997, CA 6.2.1/023, [redacted] et al, 1994, CA 6.2.1/022 and [redacted] *et al.*, 1994, CA 6.2.1/021). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1).

The characterisation/identification performed in canola commodities after pre-emergent soil application followed by three foliar applications gave comprehensive information on the metabolite pattern present. The study is therefore considered to be reliable for the assessment of the metabolic behaviour of glyphosate in glyphosate-tolerant GAT 0827 canola.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.1/026
Report author	[redacted]
Report year	2007
Report title	The Metabolism of [^{14}C]Glyphosate in *gat/gm-hra* (DP-356Ø43-5, PHP20163a) Soybeans
Report No	806960
Document No	DuPont-19530
Guidelines followed in study	OPPTS 860.1300, Nature of the Residue - Plants; Canadian PMRA Residue Chemistry Test Guidelines Dir 98-02, Section 2, Nature of the Residue Plants; and the recommendations of EU Commission Directive 96/68/EC Annex II, Section 8.1 (21 October 1996).
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Certificate of analysis for test materials was not provided, but the purity of the used batch is given in chapter 3.1.1 of the report and was re-analysed in the treatment solutions before each application • Certificates of analyses for reference substances were not provided, but the purities of the used batches are given in chapter 3.1.2 of the report • Identification was done by HPLC retention time comparison with authenticated standards and TLC (including admixed and co-spotted samples) with radiolabelled standards • Storage stability not discussed in the report. Dates of sampling and analyses are given in App. 5 and Quality Assurance Statement but refer to initial dates
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues after treatments to Optimum™ *GAT*™ soybean plants genetically modified to express the glyphosate *N*-acetyltransferase (*gat)* gene of event DP 356Ø43-5. The nature of residues resulting from soil uptake was investigated by a pre-emergence application of an actual rate of 3.290 kg glyphosate acid equivalents/ha to bare soil immediately prior to emergence. The nature of residues resulting from foliar uptake was investigated after a pre-emergence actual application of 3.290 kg glyphosate acid equivalents/ha to bare soil immediately prior to emergence and foliar applications of 1.410 kg glyphosate acid equivalents/ha made at the V7 growth stage (unifoliolate and seven trifoliolate leaves are fully developed), 2.284 kg glyphosate acid equivalents/ha made at the R2 growth stage (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf), and 0.880 kg glyphosate acid

equivalents/ha made at the R7 growth stage (one normal pod on the main stem that has reached its mature pod colour). The test substance consisted of N-(phosphono-^{14}C-methyl)glycine (^{14}C-labelled glyphosate) formulated with Touchdown Total™ inert ingredients and 2 % ammonium sulphate (AMS).

Soybean plants were taken as forage (growth stage V6, unifoliolate and six trifoliolate leaves are fully developed, 36 days after the pre-emergence application). Hay samples were taken at growth stage R1-R2 (open flower at any node on the main stem or open flower at one of the two uppermost nodes on the main stem with a fully developed leaf, 4 days after the first foliar application). A pre-harvest sampling was taken at growth stage R7 (one normal pod on the main stem that has reached its mature pod colour, 82 days after the second foliar application and immediately before the third foliar application) whereupon plants were separated into foliage (with pods) and grain. At maturity, samples were taken at growth stage R8 (95 % of the pods have reached their mature pod colour, 14 days after the third foliar application) whereupon plants were separated into foliage, pods, and grain.

At each sampling point, tissues were homogenised and extracted with 0.1 % formic acid (aqueous):methanol (96:4 v/v) (hereafter referred to as aqueous medium) followed by enzyme (α-amylase then amyloglucosidase and cellulase), alkaline (NaOH), then acid (HCl) digestion. The total radioactive residue (TRR) was determined as the sum of the total dpm in extractable and unextracted residues expressed as mg/kg equivalents of glyphosate. Extracts containing ≥0.01 mg/kg were analysed by high-performance liquid chromatography (HPLC) and the identification of residues accomplished with reference to authenticated reference standards or metabolite isolates. Thin layer chromatography (TLC) was conducted to confirm the identity of metabolites.

The majority of the radioactivity in all sample was recovered in the aqueous medium extract (28.7 – 95.9 % of the TRR, 0.123 – 15.639 mg/kg). Additional amounts of radioactivity were recovered in enzyme [α-amylase, amyloglucosidase and cellulase], NaOH and HCl digests. Final extractabilities for all samples ranged between 98.3 to 99.2 % of the TRR for all sample materials with the exception of forage after the first harvest where extractability accounted for only 57.1 % of the TRR. However, these were found to be associated with cellulose and lignin.

TRR in soybean forage collected 36 days after the pre-emergent soil application contained 0.428 mg/kg. AMPA was the major extractable radioactive component in the forage sample accounting for 39.3 % of the TRR (0.166 mg/kg). Glyphosate and *N*-acetylglyphosate were also detected accounting for 9.1 % of the TRR (0.039 mg/kg) and 1.9 % of the TRR (0.009 mg/kg), respectively. Two other metabolites were detected that did not correspond to known reference standards, neither of these metabolites exceeded 0.4 % of the TRR (0.002 mg/kg). The unextracted residue contained 42.9 % of the TRR (0.184 mg/kg).

TRR in hay collected 4 days after the first foliar application contained 13.444 mg/kg. Glyphosate was the major radioactive component detected in the hay sample accounting for 72.5 % of the TRR, 9.740 mg/kg. *N*-Acetylglyphosate (19.2 % of the TRR, 2.581 mg/kg), AMPA (5.3 % of the TRR, 0.704 mg/kg), and *N*-acetyl AMPA (0.7 % of the TRR, 0.096 mg/kg) were also detected. Thirteen other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.3 % of the TRR (0.040 mg/kg). The unextracted residue contained 0.9 % of the TRR (0.121 mg/kg).

TRR in pre-harvest foliage (including pods) and grain collected 82 days after application 3 (immediately prior to application 4) contained 11.225 mg/kg and 1.905 mg/kg, respectively. *N*-Acetylglyphosate was the major radioactive component detected in the pre-harvest grain accounting for 60.6 % of the TRR (1.156 mg/kg). Glyphosate (22.7 % of the TRR, 0.434 mg/kg) and AMPA (5.3 % of the TRR, 0.103 mg/kg) were also detected. Seven other metabolites which did not correspond to known reference standards were also detected, no single unidentified metabolite exceeded 0.5 % of the TRR (0.009 mg/kg). The unextracted residue contained 1.7 % of the TRR, 0.032 mg/kg.
Glyphosate and *N*-acetylglyphosate were the major radioactive components detected in foliage accounting for 43.6 % of the TRR (4.895 mg/kg) and 41.5 % of the TRR (4.659 mg/kg), respectively. AMPA (7.3 % of the TRR, 0.822 mg/kg) and *N*-acetyl AMPA (2.3 % of the TRR, 0.256 mg/kg) were also detected.

Twenty-three other metabolites which did not correspond to known reference standards were also detected, no single unidentified metabolite exceeded 0.4 % of the TRR (0.040 mg/kg). The unextracted residue contained 1.3 % of the TRR, 0.146 mg/kg.

TRR in grain, pods, and foliage collected at maturity (14 days PHI) contained 3.142 mg/kg, 17.751 mg/kg, and 22.087 mg/kg, respectively. *N*-Acetylglyphosate was the major radioactive component detected in the mature grain accounting for 56.9 % of the TRR, 1.788 mg/kg. Glyphosate (3.2 % of the TRR, 0.102 mg/kg), AMPA (11.2 % of the TRR, 0.351 mg/kg) and *N*-acetyl AMPA (23.5 % of the TRR, 0.738 mg/kg) were also detected. Eleven other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.9 % of the TRR (0.029 mg/kg). The unextracted residue contained 1.5 % of the TRR, 0.047 mg/kg.
Glyphosate was the major radioactive component detected in the mature pods accounting for 56.9 % of the TRR, 10.101 mg/kg. *N*-Acetylglyphosate (27.7 % of the TRR, 4.906 mg/kg), AMPA (10.2 % of the TRR, 1.794 mg/kg) and *N*-acetyl AMPA (3.3 % of the TRR, 0.574 mg/kg) were also detected. Twenty-seven other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.1 % of the TRR (0.021 mg/kg). The unextracted residue contained 0.7 % of the TRR (0.124 mg/kg).
Glyphosate was the major radioactive component detected in the mature foliage accounting for 53.4 % of the TRR, 11.791 mg/kg. *N*-Acetylglyphosate (31.9 % of the TRR, 7.039 mg/kg), AMPA (10.3 % of the TRR, 2.250 mg/kg) and N-acetyl AMPA (1.4 % of the TRR, 0.308 mg/kg) were also detected. Thirty-five other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.1 % of the TRR (0.021 mg/kg). The unextracted residue contained 1.1 % of the TRR (0.243 mg/kg).

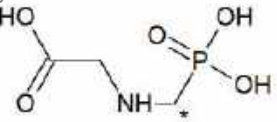
Permanganate oxidation of terminal unextractable residues demonstrated that the majority of the residues in the post extraction solids (PES) was associated with the plant's cellulose and lignin fractions.

I. Materials and methods

A. Materials

Test Material: *N*-(phosphono-^{14}C-methyl)glycine

Chemical structure:



* Position of label

Radiochemical purity:	≥ 98.4 %
Specific activity:	10.59 µCi/mg (0.39 MBq/mg)
CAS No:	1071-83-6
Log $P_{o/w}$:	- 3.2

Test system

Soil:	Sandy loam [textural class (UK)] (pH: 6.2; cation exchange capacity: 9.3 mg/L; organic carbon: 1.4 %; particle size 0.050-2 mm: 70 %; 0.002-0.050 mm: 15 %; <0.002 mm: 15 %)
Crop:	Soybean plants, glyphosate tolerant, Optimum™ *GAT*™ (*gat/gm-hra*)

Botanical name:	*Glycine max*
Crop part(s):	Forage, hay, foliage, grain, pods

B. Study design

1. In-life phase

For the investigation of the metabolism of glyphosate in Optimum™ *GAT*™ (*gat/gm-hra*) soybean plants were treated with N-(phosphono-^{14}C-methyl)glycine (^{14}C glyphosate, labelled in the phosphonomethyl-moiety). Glyphosate was co-formulated with Touchdown Total™ inert ingredients and 2 % ammonium sulphate (w/v). The study was conducted in a single glasshouse compartment. Soybean seeds (Optimum™ *GAT*™ event DP 356Ø43-5) were sown into pots (28 cm diameter) filled with sandy loam soil.

The application plan consisted of a pre-emergence application at a target rate equivalent to 3.363 kg glyphosate acid equivalents/ha to bare soil and foliar applications of 1.458 kg glyphosate acid equivalents/ha made at the V7 growth stage (unifoliolate and first seven trifoliolate leaves are fully developed), 2.353 kg glyphosate acid equivalents/ha made at the R2 growth stage (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf), and 0.897 kg glyphosate acid equivalents/ha made at the R7 growth stage (one normal pod on the main stem that has reached its mature pod colour). The actual treatments were 3.290 kg glyphosate acid equivalents/ha to bare soil on the day of sowing and foliar applications of 1.410 kg glyphosate acid equivalents/ha made at the V7 growth stage (unifoliolate and first seven trifoliolate leaves are fully developed), 2.284 kg glyphosate acid equivalents/ha made at the R2 growth stage (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf), and 0.880 kg glyphosate acid equivalents/ha made at the R7 growth stage (one normal pod on the main stem that has reached its mature pod colour).

Applications were made using a hand-held sprayer system comprising a brass header with trigger valve and a single polyacetal flat-fan nozzle with 100 mesh sieve. The sprayer was operated at a pressure of *ca* 1 bar and delivered all the contents of each spray container as a band of even width. Following each application, the sprayer was rinsed with water equivalent to approximately 10 % of the original spray volume. This was also applied to the soil or plant surface. Polythene sheeting was erected around pots prior to application to avoid contamination during application and removed afterwards. After application, the amount of residual radioactivity associated with each spray container and the operator's gloves was determined. These were immersed together in detergent solution (1 %, v/v) and aliquots removed for liquid scintillation counting (LSC). Results were used to calculate the amount of radioactivity applied. The radiochemical purity of each treatment solution was determined before and after each application.

The plants were watered as required and fertilizers were applied when necessary. The plants were observed for evaluation of growth stages. In parallel, control plants were grown and treated with Touchdown Total™ inert ingredients and ammonium sulphate.

2. Sampling

Whole aerial portions of soybean plants were taken at each sampling. Soybean plants were taken as forage (growth stage V6, unifoliolate and first six trifoliolate leaves are fully developed 36 days after the pre-emergence application). Hay samples were taken at growth stage R1–R2 (open flower at any node on the main stem or open flower at one of the two uppermost nodes on the main stem with a fully developed leaf, 4 days after the first foliar application). A pre-harvest sampling was taken at growth stage R7 (one normal pod on the main stem that has reached its mature pod colour, 82 days after the second foliar application and immediately before the third foliar application) whereupon plants were separated into foliage (with pods) and grain. At maturity, samples were taken at growth stage R8 (95 % of the pods have reached their mature pod colour, 14 days after the third foliar application) whereupon plants were separated into foliage, pods, and grain.

The samples were stored frozen at least overnight (*ca.* -20 °C) prior to pulverizing. Frozen plant tissue was pulverised with excess solid carbon dioxide chips using a food processor. For each sample, the carbon dioxide was allowed to sublime while frozen prior to removal of subsamples for combustion and extraction.

The samples from each of the four harvests were stored frozen at approximately -20 °C until initial extraction, no longer than 7, 11, 8, and 7 days, respectively. Initial HPLC analyses were completed 5 days after extraction for forage samples, on the same day for the hay samples, 5 days after extraction for pre-harvest samples, and 2 days after extraction for mature harvest samples.

3. Analytical procedures

Portions of each homogenised tissue were extracted three times with 0.1 % formic acid (aqueous):methanol (96:4 v/v) (aqueous medium). Select unextracted residues were then enzyme digested twice with α-amylase. The unextracted residues remaining after α-amylase digestion were incubated twice with a mix of amyloglucosidase and cellulose. The remaining unextracted residues after enzyme hydrolysis were then incubated in 0.1 N NaOH (60 ºC, 6 hours). The remaining unextracted residues after alkaline digestion were incubated with 1.0 N HCl (60 ºC, 6 hours) where necessary. Extracts were concentrated and reconstituted in a suitable solvent prior to Liquid Scintillation Counting (LSC) and High Performance Liquid Chromatography (HPLC).

To investigate the incorporation of TRR into lignin and cellulose, a potassium permanganate oxidation method was utilised. Permanganate solution was added to the remaining residues (after extraction mentioned above) until the solution remained purple (indicating that oxidation was complete). The remaining fibre was washed with the demineralising solution (to remove permanganate remains) and ethanol. The filtrates (permanganate spent solution and washes) were combined and filtered. Levels of radioactivity were determined in the filtrate by LSC and in the precipitate and remaining fibre by oxidative combustion followed by LSC.

For identification and quantification of glyphosate and metabolites, a HPLC system was employed using on-line UV detection. Following UV detection, effluent was analysed by on-line radiodetection or fractions were collected for quantification of radioactivity via LSC. Authenticated analytical reference standards of glyphosate, AMPA, *N*-acetyl AMPA, and *N*-acetylglyphosate were analysed by HPLC to determine the retention times of these compounds.

The total radioactive residue (TRR) was determined as the sum of the total dpm in extractable and unextracted residues expressed as mg/kg equivalents of glyphosate. Levels of radioactivity were determined in each extract by LSC and in the unextracted residues by oxidative combustion and LSC. The limits of detection for quantification of radioactive peaks on chromatograms were assessed as <0.1 % of the TRR (<0.001 ppm.).

A portion mature soybean pods aqueous medium extract and corn stover ([redacted] 2007, CA 6.2.1/024) was subjected to solid phase extraction (SPE). SPE cartridges were eluted with aqueous formic acid, concentrated, and applied to HPLC. Fractions that eluted with retention times that corresponded to AMPA, glyphosate, *N*-acetyl AMPA, and *N*-acetylglyphosate were combined to form an isolate of each metabolite.

Thin layer chromatography (TLC) was conducted to confirm the identity of metabolites detected using HPLC. Radioactive areas on the developed plates were located using a phosphor imager. Where possible, non-labelled standards were visualised by staining with a 0.5 % ninhydrin solution in ethanol.
Isolates of parent and metabolites (described above) were applied to TLC plates, co-spotted and, in the case of AMPA, admixed with the appropriate radiolabelled reference standard. Confirmation was obtained when a more intense radioactive spot was detected (having the same retention time as the isolated metabolite).

II. Results and discussion

A. Total radioactive residues (TRRs)

The total radioactive residue (TRR) in soybean forage, hay, foliage, grain, and pods is summarised below. The TRR in the forage from the first harvest was 0.428 mg/kg following a single soil application of N-(phosphono-^{14}C-methyl)glycine. The TRR in hay from the second harvest was 13.444 mg/kg following a single soil and a single foliar application of ^{14}C-glyphosate. The TRR in grain and foliage (with pods) from the third harvest was 1.905 and 11.225 mg/kg, respectively, following a single soil and two foliar applications of ^{14}C-glyphosate. The TRR in grain, pods, and foliage from the fourth harvest was 3.142, 17.751, and 22.087 mg/kg, respectively, following a single soil and three foliar applications of N-(phosphono-^{14}C-methyl)glycine.

Table 6.2.1-165: Total radioactive residues in glyphosate-tolerant soybean following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha and three foliar applications at 1.458, 2.284 and 0.880 kg glyphosate acid equivalents/ha

Matrix		Harvest 1 Forage 36 DAT1	Harvest 2 Hay 4 DAT2	Harvest 3 Grain 82 DAT3	Harvest 3 Foliage/ pods 82 DAT3	Harvest 4 Grain 14 DAT4	Harvest 4 Pods 14 DAT4	Harvest 4 Foliage 14 DAT4
0.1 % Formic acid:methanol extract	% TRR	28.7	95.9	88.9	86.2	88.0	88.1	88.2
	mg/kg	0.123	12.893	1.694	9.676	2.765	15.639	19.481
α-Amylase extract	% TRR	14.8	2.3	6.7	10.0	7.6	7.8	8.3
	mg/kg	0.063	0.309	0.128	1.123	0.239	1.385	1.833
Amyloglucosidase and cellulase extract	% TRR	6.0	0.6	2.0	2.1	2.4	2.5	2.0
	mg/kg	0.026	0.081	0.038	0.236	0.075	0.444	0.442
NaOH extract	% TRR	4.4	0.2	0.4	0.2	0.4	0.6	0.2
	mg/kg	0.019	0.027	0.008	0.022	0.013	0.107	0.044
HCl extract	% TRR	3.2	0.1	0.3	0.2	0.3	0.2	0.2
	mg/kg	0.014	0.013	0.006	0.022	0.009	0.036	0.044
Unextracted Residue	% TRR	42.9	0.9	1.7	1.3	1.5	0.7	1.1
	mg/kg	0.184	0.121	0.032	0.146	0.047	0.124	0.243
Total	mg/kg	0.428	13.444	1.905	11.225	3.142	17.751	22.087

TRR: total radioactive residue, expressed as glyphosate equivalent
DAT#: days after the numbered treatment, treatments specified for each harvest below.
Harvest 1 = forage harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha.
Harvest 2 = hay harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and a single foliar application of 1.410 kg glyphosate acid equivalents/ha.
Harvest 3 = pre-application 4 harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and two foliar applications of 1.410 and 2.284 kg glyphosate acid equivalents/ha.
Harvest 4 = maturity harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and three foliar applications of 1.410, 2.284, and 0.880 kg glyphosate acid equivalents/ha.

B. Extraction and characterisation of residues

<u>Characterisation and identification of residues in soybean forage</u>

The distribution of TRR in soybean forage collected 36 days after the pre-emergent soil application is shown above. Of the 57.1 % of the TRR (0.245 mg/kg) extractable from the soybean forage sample, the majority was found in the aqueous medium (28.7 % of the TRR, 0.123 mg/kg). Additional amounts of radioactivity were recovered in enzyme [α-amylase: 14.8 % of the TRR, 0.063 mg/kg; amyloglucosidase and cellulase: 6.0 % of the TRR, 0.026 mg/kg)], NaOH (4.4 % of the TRR, 0.019 mg/kg), and HCl (3.2 % of the TRR, 0.014 mg/kg) digests. In total 57.1 % of the TRR (0.245 mg/kg) were extractable.
The unextracted residue contained 42.9 % of the TRR (0.184 mg/kg).

AMPA was the major extractable radioactive component in the forage sample accounting for 39.3 % of the TRR (0.166 mg/kg). Glyphosate and *N*-acetylglyphosate were also detected accounting for 9.1 % of the TRR (0.039 mg/kg) and 1.9 % of the TRR (0.009 mg/kg), respectively. Two other metabolites were detected that did not correspond to known reference standards, neither of these metabolites exceeded 0.4 % of the TRR (0.002 mg/kg). The unextracted residue contained 42.9 % of the TRR (0.184 mg/kg).

Table 6.2.1-166: Extraction of the radioactive residues of glyphosate-tolerant soybean forage following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha

Matrix	**Harvest 1 Forage, 36 DAT1**	
Fraction	**mg/kg**	**% TRR**
TRR	**0.428**	**100**
0.1 % Formic acid:methanol extract	0.123	28.7
AMPA	0.081	19.3
Glyphosate	0.033	7.6
N-acetylglyphosate	0.003	0.6
Unidentified[1]	0.002	0.4
α-Amylase extract	0.063	14.8
AMPA	0.054	12.9
Glyphosate	0.006	1.5
Amyloglucosidase and cellulase extract	0.026	6.0
AMPA	0.017	4.0
NaOH extract	0.019	4.4
AMPA	0.002	0.6
N-acetylglyphosate	0.006	1.3
Unidentified[2]	0.001	0.2
HCl extract	0.014	3.2
AMPA	0.012	2.5
Total identified	***0.214***	***50.3***
Total characterised	**0.003**	**0.6**
ERR	**0.245**	**57.1**
RRR	**0.184**	**42.9**

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extraction)
DAT#: days after the numbered treatment, treatments specified for each harvest below.
Harvest 1 = forage harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha.
All residue data are expressed as mg/kg glyphosate equivalents
Values in *italics* were recalculated during dossier compilation.
1 Comprised of 1 component.
2 Comprised of 1 component.

Characterisation and identification of residues in soybean hay

The distribution of TRR in soybean hay collected 4 days after the first foliar application is presented above. The majority of the radioactivity in the soybean hay sample was recovered in the aqueous medium extract (95.9 % of the TRR, 12.893 mg/kg). Additional amounts of radioactivity were recovered in the α-amylase (2.3 % of the TRR, 0.309 mg/kg), amyloglucosidase and cellulase (0.6 % of the TRR, 0.081 mg/kg), NaOH (0.2 % of the TRR, 0.027 mg/kg), and HCl (0.1 % of the TRR, 0.013 mg/kg) hydrolysates. In total 99.1 % of the TRR (13.323 mg/kg) were extractable. The unextracted residue contained 0.9 % of the TRR (0.121 mg/kg).

Glyphosate was the major radioactive component detected in the hay sample accounting for 72.5 % of the TRR, 9.740 mg/kg. *N*-Acetylglyphosate (19.2 % of the TRR, 2.581 mg/kg), AMPA (5.3 % of the TRR, 0.704 mg/kg), and *N*-acetyl AMPA (0.7 % of the TRR, 0.096 mg/kg) were also detected. Thirteen other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.3 % of the TRR (0.040 mg/kg).

Table 6.2.1-167: Extraction of the radioactive residues of glyphosate-tolerant soybean hay following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha and one foliar application at 1.410 kg glyphosate acid equivalents/ha

Matrix	Harvest 2, Hay, 4 DAT2	
Fraction	**mg/kg**	**% TRR**
TRR	**13.444**	**100**
0.1 % Formic acid:methanol extract	12.893	95.9
AMPA	0.408	3.0
Glyphosate	9.678	72.0
N-acetyl AMPA	0.096	0.7
N-acetylglyphosate	2.574	19.2
Unidentified[1]	0.064	0.4
α-Amylase extract	0.309	2.3
AMPA	0.230	1.7
Glyphosate	0.045	0.4
Unidentified[2]	0.015	<0.1
Amyloglucosidase and cellulase extract	0.081	0.6
AMPA	0.043	0.4
Glyphosate	0.017	0.1
N-acetylglyphosate	0.003	<0.1
NaOH extract	0.027	0.2
AMPA	0.011	0.1
N-acetylglyphosate	0.004	<0.1
HCl extract	0.013	0.1
AMPA	0.012	0.1
Total identified	***13.121***	***97.7***
Total characterised	**0.079**	**0.4**
ERR	**13.323**	**99.1**
RRR	**0.121**	**0.9**

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extraction)
DAT#: days after the numbered treatment, treatments specified for each harvest below.
Harvest 2 = hay harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and a single foliar application of 1.410 kg glyphosate acid equivalents/ha.
All residue data are expressed as mg/kg glyphosate equivalents
Values in *italics* were recalculated during dossier compilation.
1 Comprised of 5 components, no single component greater than 0.3 % of the TRR, 0.040 mg/kg.
2 Comprised of 8 components, no single component greater than 0.1 % of the TRR, 0.005 mg/kg.

Characterisation and identification of radioactive residues in soybean grain and foliage (with pods)

The distribution of TRR from soybean grain and foliage (with pods) collected 82 days after the second foliar application is presented above. The majority of the radioactivity in the soybean grain sample was recovered in the aqueous medium (88.9 % of the TRR, 1.694 mg/kg). Additional amounts of radioactivity were recovered in α-amylase (6.7 % of the TRR, 0.128 mg/kg), amyloglucosidase and cellulase (2.0 % of the TRR, 0.038 mg/kg), NaOH (0.4 % of the TRR, 0.008 mg/kg), and HCl (0.3 % of the TRR, 0.006 mg/kg) digests. In total 98.3 – 98.7 % of the TRR (1.874 – 11.079 mg/kg) were extractable. The

unextracted residue in grain contained 1.7 % of the TRR, 0.032 mg/kg, while the unextracted residue in foliage contained 1.3 % of the TRR, 0.146 mg/kg.

N-Acetylglyphosate was the major radioactive component detected in the pre-harvest grain accounting for 60.6 % of the TRR (1.156 mg/kg). Glyphosate (22.7 % of the TRR, 0.434 mg/kg) and AMPA (5.3 % of the TRR, 0.103 mg/kg) were also detected. Seven other metabolites which did not correspond to known reference standards were also detected, no single unidentified metabolite exceeded 0.5 % of the TRR (0.009 mg/kg).

The majority of the radioactivity in the pre-harvest foliage sample was recovered in the aqueous medium extract (86.2 % of the TRR, 9.676 mg/kg). Additional amounts of radioactivity were recovered in the α-amylase (10.0 % of the TRR, 1.123 mg/kg), amyloglucosidase and cellulase (2.1 % of the TRR, 0.236 mg/kg), NaOH (0.2 % of the TRR, 0.022 mg/kg), and HCl (0.2 % of the TRR, 0.022 mg/kg) digests.

Glyphosate and *N*-acetylglyphosate were the major radioactive components detected in foliage accounting for 43.6 % of the TRR (4.894 mg/kg) and 42.0 % of the TRR (4.659 mg/kg), respectively. AMPA (7.4 % of the TRR, 0.819 mg/kg) and *N*-acetyl AMPA (2.2 % of the TRR, 0.255 mg/kg) were also detected. Twenty-three other metabolites which did not correspond to known reference standards were also detected, no single unidentified metabolite exceeded 0.4 % of the TRR (0.040 mg/kg).

Table 6.2.1-168: Extraction of the radioactive residues of glyphosate-tolerant soybean grain and foliage (with pods) following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha and two foliar applications at 1.410 and 2.284 kg glyphosate acid equivalents/ha

Matrix	**Harvest 3**			
Harvest 3	**Grain, 82 DAT3**		**Foliage (with pods), 82 DAT3**	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**1.905**	**100**	**11.225**	**100**
0.1 % Formic acid:methanol extract	1.694	88.9	9.676	86.2
AMPA	0.071	3.7	0.596	5.3
Glyphosate	0.433	22.7	4.402	39.2
N-acetyl AMPA	-	-	0.229	2.0
N-acetylglyphosate	1.089	57.2	4.262	38.0
Unidentified	0.022[1]	1.2	0.148[4]	1.3
α-Amylase extract	0.128	6.7	1.123	10.0
AMPA	0.028	1.4	0.107	1.0
Glyphosate	-	-	0.456	4.1
N-acetylglyphosate	0.060	3.1	0.397	3.6
Unidentified	0.009[2]	0.5	-	-
Amyloglucosidase and cellulase extract	0.038	2.0	0.236	2.1
AMPA	0.004	0.2	0.090	0.8
Glyphosate	0.001	<0.1	0.028	0.2
N-acetyl AMPA	-	-	0.026	0.2
N-acetylglyphosate	0.007	0.3	0.040	0.4
Unidentified	0.001[3]	<0.1	0.030[5]	0.1
NaOH extract	0.008	0.4	0.022	0.2
AMPA	n.a.	n.a.	0.005	0.1
Glyphosate	n.a.	n.a.	0.008	0.1
N-acetyl AMPA	n.a.	n.a.	<0.001	<0.1
N-acetylglyphosate	n.a.	n.a.	<0.001	<0.1
Unidentified	n.a.	n.a.	<0.001[6]	<0.1
HCl extract	0.006	0.3	0.022	0.2
AMPA	n.a.	n.a.	0.021	0.2
Total identified	***1.693***	***88.6***	***10.667***	***95.2***
Total characterised	**0.032**	**1.7**	**0.179**	**1.6**
ERR	**1.874**	**98.3**	**11.079**	**98.7**

Table 6.2.1-168: Extraction of the radioactive residues of glyphosate-tolerant soybean grain and foliage (with pods) following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha and two foliar applications at 1.410 and 2.284 kg glyphosate acid equivalents/ha

Matrix	Harvest 3			
Harvest 3	Grain, 82 DAT3		Foliage (with pods), 82 DAT3	
Fraction	mg/kg	% TRR	mg/kg	% TRR
TRR	1.905	100	11.225	100
RRR	0.032	1.7	0.146	1.3

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extraction)
DAT#: days after the numbered treatment, treatments specified for each harvest below.
Harvest 3 = pre-application 4 harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and two foliar applications of 1.410 and 2.284 kg glyphosate acid equivalents/ha.
n.a.: not analysed
All residue data are expressed as mg/kg glyphosate equivalents
Values in *italics* were recalculated during dossier compilation.
1 Comprised of 5 components, no single component greater than 0.4 % of the TRR, 0.007 mg/kg.
2 Comprised of 1 single component.
3 Comprised of 1 single component.
4 Comprised of 9 components, no single component greater than 0.4 % of the TRR, 0.040 mg/kg.
5 Comprised of 8 components, no single component greater than 0.1 % of the TRR, 0.007 mg/kg.
6 Comprised of 6 components, all components <0.1 % of the TRR, <0.001 mg/kg.

Characterisation and identification of radioactive residues in soybean grain, pods, and foliage harvested at maturity

The distribution of TRR from soybean grain, pods, and foliage collected at maturity (14 days PHI) is presented above. The majority of the radioactivity in the mature soybean grain sample was recovered in the aqueous medium extract (88.0 % of the TRR, 2.765 mg/kg). Additional amounts of radioactivity were recovered in the α-amylase (7.6 % of the TRR, 0.239 mg/kg), amyloglucosidase and cellulase (2.4 % of the TRR, 0.075 mg/kg), NaOH (0.4 % of the TRR, 0.013 mg/kg), and HCl (0.3 % of the TRR, 0.009 mg/kg) digests. The unextracted residue contained 1.5 % of the TRR, 0.047 mg/kg.
N-Acetylglyphosate was the major radioactive component detected in the mature grain accounting for 56.9 % of the TRR, 1.788 mg/kg. Glyphosate (3.2 % of the TRR, 0.102 mg/kg), AMPA (11.2 % of the TRR, 0.351 mg/kg) and *N*-acetyl AMPA (23.5 % of the TRR, 0.738 mg/kg) were also detected. Eleven other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.9 % of the TRR (0.029 mg/kg).

The majority of the radioactivity in the mature soybean pods was recovered in aqueous medium extract (88.1 % of the TRR, 15.639 mg/kg). Additional amounts of radioactivity were recovered in enzyme [α-amylase (7.8 % of the TRR, 1.385 mg/kg) and amyloglucosidase and cellulase (2.5 % of the TRR, 0.444 mg/kg)], NaOH (0.6 % of the TRR, 0.107 mg/kg), and HCl (0.2 % of the TRR, 0.036 mg/kg) digests. The unextracted residue contained 0.7 % of the TRR (0.124 mg/kg).
Glyphosate was the major radioactive component detected in the mature pods accounting for 56.9 % of the TRR, 10.101 mg/kg. *N*-Acetylglyphosate (27.7 % of the TRR, 4.906 mg/kg), AMPA (10.2 % of the TRR, 1.794 mg/kg) and *N*-acetyl AMPA (3.3 % of the TRR, 0.574 mg/kg) were also detected. Twenty-seven other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.1 % of the TRR (0.021 mg/kg).

The majority of the radioactivity in the mature soybean foliage (straw) was recovered in the aqueous medium extract (88.2 % of the TRR, 19.481 mg/kg). Additional amounts of radioactivity were recovered in enzyme [α-amylase (8.3 % of the TRR, 1.833 mg/kg) and amyloglucosidase and cellulase (2.0 % of the TRR, 0.442 mg/kg)], NaOH (0.2 % of the TRR, 0.044 mg/kg), and HCl (0.2 % of the TRR, 0.044 mg/kg) digests. The unextracted residue contained 1.1 % of the TRR (0.243 mg/kg).

Glyphosate was the major radioactive component detected in the mature foliage accounting for 53.4 % of the TRR, 11.791 mg/kg. *N*-Acetylglyphosate (31.9 % of the TRR, 7.039 mg/kg), AMPA (10.3 % of the TRR, 2.250 mg/kg) and *N*-acetyl AMPA (1.4 % of the TRR, 0.308 mg/kg) were also detected. Thirty-five other metabolites which did not correspond to known reference standards were also detected, no single metabolite exceeded 0.5 % of the TRR (0.108 mg/kg).

Table 6.2.1-169: Extraction of the radioactive residues of glyphosate-tolerant soybean grain, pods, and foliage following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha and three foliar applications at 1.458, 2.284, and 0.880 kg glyphosate acid equivalents/ha

	Harvest 4					
Matrix	**Grain, 14 DAT4**		**Pods, 14 DAT4**		**Foliage, 14 DAT4**	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**3.142**	**100**	**17.751**	**100**	**22.087**	**100**
0.1 % Formic acid:methanol extract	2.765	88.0	15.639	88.1	19.481	88.2
AMPA	0.191	6.1	0.641	3.6	1.177	5.3
Glyphosate	0.094	3.0	9.821	55.3	11.195	50.7
N-acetyl AMPA	0.738	23.5	0.532	3.0	0.236	1.1
N-acetylglyphosate	1.742	55.4	4.645	26.2	6.629	30.0
Unidentified	-	-	-	-	0.180[7]	0.9
α-Amylase extract	0.239	7.6	1.385	7.8	1.833	8.3
AMPA	0.120	3.8	0.845	4.8	0.743	3.4
Glyphosate	0.008	0.2	0.190	1.1	0.545	2.5
N-acetyl AMPA	-	-	0.017	0.1	0.022	0.1
N-acetylglyphosate	0.037	1.2	0.206	1.2	0.368	1.7
Unidentified	0.032[1]	1.0	0.108[4]	0.6	0.123[8]	0.6
Amyloglucosidase and cellulase extract	0.075	2.4	0.444	2.5	0.442	2.0
AMPA	0.030	1.0	0.215	1.2	0.256	1.2
Glyphosate	-	-	0.082	0.5	0.046	0.2
N-acetyl AMPA	-	-	0.017	0.1	0.050	0.2
N-acetylglyphosate	0.008	0.2	0.051	0.3	0.042	0.2
Unidentified	0.003[2]	0.1	0.051[5]	0.1	0.031[9]	<0.1
NaOH extract	0.013	0.4	0.107	0.6	0.044	0.2
AMPA	0.005	0.2	0.066	0.4	0.032	0.2
Glyphosate	-	-	0.008	<0.1	0.005	<0.1
N-acetyl AMPA	-	-	0.008	0.1	<0.001	<0.1
N-acetylglyphosate	0.001	0.1	0.004	<0.1	<0.001	<0.1
Unidentified	<0.001[3]	<0.1	0.006[6]	<0.1	<0.001[10]	<0.1
HCl extract	0.009	0.3	0.036	0.2	0.044	0.2
AMPA	0.005	0.1	0.027	0.2	0.042	0.2
Total identified	***2.979***	***94.8***	***17.375***	***98.1***	***21.388***	***97.0***
Total characterised	**0.035**	**1.1**	**0.165**	**0.7**	**0.334**	**1.5**
ERR	**3.101**	**98.7**	**17.611**	**99.2**	**21.844**	**98.9**
RRR	**0.047**	**1.5**	**0.124**	**0.7**	**0.243**	**1.1**

Table 6.2.1-169: Extraction of the radioactive residues of glyphosate-tolerant soybean grain, pods, and foliage following one pre-emergent application at 3.290 kg glyphosate acid equivalents/ha and three foliar applications at 1.458, 2.284, and 0.880 kg glyphosate acid equivalents/ha

TRR: Total radioactive residue
ERR: Extractable radioactive residue (considering combined extracts measured)
RRR: Residual radioactive residue (after conventional and exhaustive extraction)
DAT#: days after the numbered treatment, treatments specified for each harvest below.
Harvest 4 = maturity harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and three foliar applications of 1.410, 2.284, and 0.880 kg glyphosate acid equivalents/ha.
All residue data are expressed as mg/kg glyphosate equivalents
Values in *italics* were recalculated during dossier compilation.
1 Comprised of 2 components, no single component greater than 0.9 % of the TRR, 0.029 mg/kg.
2 Comprised of 1 single component.
3 Comprised of 8 components, all components <0.1 % of the TRR, <0.001 mg/kg.
4 Comprised of 9 components, no single component greater than 0.1 % of the TRR, 0.021 mg/kg.
5 Comprised of 14 components, no single component greater than 0.1 % of the TRR, 0.008 mg/kg.
6 Comprised of 4 components, all components <0.1 % of the TRR, no single component greater than 0.003 mg/kg.
7 Comprised of 4 components, no single component greater than 0.5 % of the TRR, 0.108 mg/kg.
8 Comprised of 9 components, no single component greater than 0.1 % of the TRR, 0.024 mg/kg.
9 Comprised of 8 components, all components <0.1 % of the TRR, no single component greater than 0.007 mg/kg.
10 Comprised of 14 components, all components <0.1 % of the TRR, <0.001 mg/kg.

Table 6.2.1-170: Distribution of radioactive residues of glyphosate and its metabolites in glyphosate-tolerant soybean forage, hay, grain, foliage, and pod

	Harvest 1		Harvest 2		Harvest 3				Harvest 4					
Matrix	**Forage**		**Hay**		**Grain**		**Foliage**		**Grain**		**Pod**		**Foliage**	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	**0.428**	**100**	**13.444**	**100**	**1.905**	**100**	**11.225**	**100**	**3.142**	**100**	**17.751**	**100**	**22.087**	**100**
AMPA	0.166	39.3	0.704	5.3	0.103	5.3	0.819	7.4	0.351	11.2	1.794	10.2	2.250	10.3
Glyphosate	0.039	9.1	9.740	72.5	0.434	22.7	4.894	43.6	0.102	3.2	10.101	56.9	11.791	53.4
N-acetyl AMPA	n.d.	n.d.	0.096	0.7	n.d.	n.d.	0.255	2.2	0.738	23.5	0.574	3.3	0.308	1.4
N-acetyl-glyphosate	0.009	1.9	2.581	19.2	1.156	60.6	4.699	42.0	1.788	56.9	4.906	27.7	7.039	31.9
Unidentified[1]	0.003	0.6	0.079	0.4	0.032	1.7	0.179	1.6	0.035	1.1	0.165	0.7	0.334	1.5
Total identified	***0.214***	***50.3***	***13.121***	***97.7***	***1.693***	***88.6***	***10.667***	***95.2***	***2.979***	***94.8***	***17.375***	***98.1***	***21.388***	***97.0***
Total characterised	**0.003**	**0.6**	**0.079**	**0.4**	**0.032**	**1.7**	**0.179**	**1.6**	**0.035**	**1.1**	**0.165**	**0.7**	**0.334**	**1.5**
ERR	**0.245**	**57.1**	**13.323**	**99.1**	**1.874**	**98.3**	**11.079**	**98.7**	**3.101**	**98.7**	**17.611**	**99.2**	**21.844**	**98.9**
RRR	**0.184**	**42.9**	**0.121**	**0.9**	**0.032**	**1.7**	**0.146**	**1.3**	**0.047**	**1.5**	**0.124**	**0.7**	**0.243**	**1.1**

Harvest 1 = forage harvest, received a single pre-emergent soil application of [^{14}C]glyphosate.
Harvest 2 = hay harvest, received a single pre-emergent soil application and a single foliar application of [^{14}C]glyphosate.
Harvest 3 = pre-application 4 harvest, received a single pre-emergent soil application and two foliar applications of [^{14}C]glyphosate.
Harvest 4 = maturity harvest, received a single pre-emergent soil application and three foliar applications of [^{14}C]glyphosate.
1 = 2 - 35 components, none greater than 0.9 %TRR
n.d. = not detected
Values in *italics* were recalculated during dossier compilation.

Incorporation of TRR into Lignin and Cellulose using Potassium Permanganate

Terminal unextractable soybean residues were subjected to permanganate oxidation to investigate incorporation of glyphosate residues into the plant's lignin and cellulose fractions. Unextractable forage residues (42.9 % of the TRR, 0.184 mg/kg) were associated with cellulose (19.4 % of the TRR, 0.082 mg/kg) and lignin (15.3 % of the TRR, 0.065 mg/kg) fractions. A precipitate that formed in the extract contained 3.4 % of the TRR, 0.015 mg/kg. At all other sample points the terminal unextracted residue (RRR) in the various crop fractions represented 0.7 – 1.7 % of the TRR (0.032–0.243 mg/kg). Of

these residues, 0.4 – 1.5 % of the TRR (0.019 – 0.133 mg/kg) was found to be associated with the cellulose fraction and 0.2 – 0.9 % of the TRR (0.006 – 0.056 mg/kg) was associated with a lignin fraction. A precipitate that formed in the extracts accounted for < 0.1 – 0.3 % of the TRR (< 0.001 –0.009 mg/kg).

Table 6.2.1-171: Total radioactive residues in lignin and cellulose using potassium permanganate

Matrix		Harvest 1 Forage 36 DAT1	Harvest 2 Hay 4 DAT2	Harvest 3 Grain 82 DAT3	Harvest 3 Foliage/ pods 82 DAT3	Harvest 4 Grain 14 DAT4	Harvest 4 Pods 14 DAT4	Harvest 4 Foliage 14 DAT4
Initial sample	% TRR	42.9	0.9	1.7	1.3	1.5	0.7	1.1
	mg/kg	0.184	0.121	0.032	0.146	0.047	0.124	0.243
Extract (soluble lignin)	% TRR	15.3	0.4	0.3	0.5	0.9	0.2	0.2
	mg/kg	0.065	0.054	0.006	0.056	0.028	0.036	0.044
Precipitate	% TRR	3.4	<0.1	0.1	<0.1	0.3	<0.1	<0.1
	mg/kg	0.015	<0.001	0.002	<0.001	0.009	<0.001	<0.001
Fibre (cellulose)	% TRR	19.4	0.5	1.3	0.6	0.6	0.4	0.6
	mg/kg	0.083	0.067	0.025	0.067	0.019	0.071	0.133
Total	% TRR	38.1	0.9	1.7	1.1	1.8	0.6	0.8
	mg/kg	0.163	0.121	0.033	0.123	0.056	0.107	0.177

TRR: total radioactive residue, expressed as glyphosate equivalent
DAT#: days after the numbered treatment, treatments specified for each harvest below.
Harvest 1 = forage harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha.
Harvest 2 = hay harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and a single foliar application of 1.410 kg glyphosate acid equivalents/ha.
Harvest 3 = pre-application 4 harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and two foliar applications of 1.410 and 2.284 kg glyphosate acid equivalents/ha.
Harvest 4 = maturity harvest, received a single pre-emergent soil application of 3.290 kg glyphosate acid equivalents/ha and three foliar applications of 1.410, 2.284, and 0.880 kg glyphosate acid equivalents/ha.

C. Storage stability

The samples remained frozen (-20°C) prior to extraction. An analysis of storage stability was not conducted as part of this study. Initial samples were analysed within 7 to 11 days of collection. Final extraction dates are not stated within the study report. However, dates of sampling and analyses are given in Appendix 5 and quality assurance statement. The first sampling was done on June 30, 2006. The latest date of chromatography given in the quality assurance statement is March 8, 2007. Therefore, the maximum storage duration can be estimated to be 312 days (~10.4 months) at latest. A high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies showing no degradation of glyphosate and its metabolites for up to 215 – 393 days and 273 – 501 days in commodities with high water and high oil content, respectively.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. CONCLUSIONS

This study investigated the metabolism of N-(phosphono-^{14}C-methyl)glycine in Optimum™ *GAT*™ (*gat/gm-hra)* soybeans following a single pre-emergent soil application at 3.290 kg glyphosate acid equivalents/ha and three foliar applications (1.410, 2.284, and 0.880 kg glyphosate acid equivalents/ha).

TRRs in the soybean commodities of forage after a single pre-emergent soil application were found at 0.428 mg/kg. After soil application followed by a single foliar application the TRR in hay accounted for

13.444 mg/kg. After soil application followed by two foliar applications radioactive residues in grain and foliage accounted for 1.905 and 11.225 mg/kg, respectively.
In grain, pods and foliage the radioactive residues accounted for 3.142, 17.751 and 22.087 mg/kg after soil followed by three foliar applications respectively.

The major radioactive component detected in grain was *N*-acetylglyphosate accounting for 56.9 % of the TRR (1.788 mg/kg) at maturity. Glyphosate, AMPA, and *N*-acetyl AMPA levels in mature soybean grain were 3.2 % of the TRR (0.102 mg/kg), 11.2 % of the TRR (0.351 mg/kg) and 23.5 % of the TRR (0.738 mg/kg), respectively.

TRR detected in soybean forage was consistent with uptake of radioactive residues from soil. Glyphosate (9.1 % of the TRR, 0.039 mg/kg) and AMPA (39.3 % of the TRR, 0.166 mg/kg) were the principal extractable components in forage. A significant portion of the forage residues were incorporated into naturally occurring compounds associated with the cellulose or lignin fraction of the plant. The subsequent increase and/or decrease in TRR detected in soybean foliage was attributable to multiple foliar applications and to growth and development of the crop.
Glyphosate (72.5 % of the TRR, 9.740 mg/kg) and *N*-acetylglyphosate 19.2 % of the TRR, 2.581 mg/kg) were the major radioactive residues detected in soybean hay. *N*-Acetyl AMPA (0.7 % of the TRR, 0.096 mg/kg) and AMPA (5.3 % of the TRR, 0.704 mg/kg) were also observed, to a lesser extent, in the hay.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in soybean (forage, foliage, hay, grain and pod) has been previously evaluated at EU level. It was performed under GLP. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with minor deficits (certificate of analysis for test materials was not provided, but the purity of the used batch is given in chapter 3.1.1 of the report and was re-analysed in the treatment solutions before each application; certificates of analyses for reference substances were not provided, but the purities of the used batches are given in chapter 3.1.2 of the report; identification was done by HPLC retention time comparison with authenticated standards and TLC (including admixed and co-spotted samples) with radiolabelled standards; storage stability not discussed in the report. Dates of sampling and analyses are given in App. 5 and Quality Assurance Statement but refer to initial dates).
No information on storage duration of frozen plant samples and plant extracts is given in the study report. However, based on the date of first sampling (30th of June 2006) and the latest date for chromatography (available in the quality assurance statement, 8th March 2007) the maximum duration can be estimated to be ~312 days (10.4 months). A high number of storage stability investigations are available in different metabolism studies as well as in special storage stability studies.
No degradation of glyphosate and its metabolites was found in matrices with high water content comparable to the present study, like corn forage, fodder, cotton forage, soybean forage. Over an investigated storage duration of 215 - 393 days no degradation was observed ([redacted] 1995, CA 6.2.1/20; [redacted] 1997, CA 6.2.1/23 and [redacted] et al, 1994, CA 6.2.1/22). In matrices with high oil content like cotton, soybean and canola seeds glyphosate-derived residues were stable for 273 - 501 days ([redacted] 1997, CA 6.2.1/23, [redacted] et al, 1994, CA 6.2.1/22 and [redacted] *et al.*, 1994, CA 6.2.1/21). Additional detailed information on storage stability of glyphosate and its metabolites is available under CA 6.1).
The characterisation/identification performed in soybean commodities after pre-emergent soil application followed by three foliar applications gave comprehensive information on the metabolite pattern present. The study is therefore considered to be reliable for the assessment of the metabolic behaviour of glyphosate in glyphosate-tolerant GAT/GM-HRA soybeans.

Assessment and conclusion by RMS:

Overall assessment and conclusion on metabolism studies

The metabolism and distribution of ^{14}C-glyphosate in more than 25 varieties of conventional and modified (CP4 EPSPS, CP4 EPSPS and GOX modifications) crops was reviewed in the 2001 EU glyphosate evaluation and is summarised in the glyphosate monograph. Application methods that were investigated include application to soil and hydroponic solutions, applications to stems and trunks, and foliar applications of glyphosate to non-tolerant/conventional crops and pre-and post-emergence application of glyphosate to tolerant crops.
In addition to primary crop metabolism studies, confined rotational crop studies are available which also include investigations of primary crops (for details please refer to CA 6.6.1.)
For GAT modified crops the metabolism and distribution of ^{14}C-glyphosate was reviewed in the 2015 EU glyphosate and is summarised in the RAR for glyphosate. Application methods that were investigated include pre-and post-emergence application followed by foliar applications of glyphosate.

Overall summaries per crop category are given in the following. Data are summarised also in Appendix G, and those studies where the nature of residues was investigated are considered for the definition of residues (see CA 6.7.1).

Non-tolerant plants

Fruit crops

Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **fruits.** An overview on the studies, the application scenarios and application rates used is given in the following table:

Table 6.2.1-172: Overview over available plant metabolism studies for crop category fruits

Plant	Application	Application rate	Reference
Citrus (calamondin citrus, lemon)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine or amino-^{14}C-methyl- phosphonic acid at 2.24 kg a.s./ha	CA 6.2.1/001: [redacted] 1975, The metabolism of CP 67573 by citrus, Report No. 328
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine or amino-^{14}C-methyl- phosphonic acid at 10 mg/kg hydroponic solution	
	Foliar application	4 mg *N*-(phosphono-^{14}C-methyl)glycine per leaf surface	
	Soil application	*N*-(phosphono-^{14}C-methyl)glycine trimesium salt at 3.9 kg a.s./ha (expressed in glyphosate equiv.)	CA 6.2.1/002: [redacted] 1987, The nature of the residue of SC-0224 in citrus, Report No. PMS-158 MRC-86-08
Tree nuts (walnut, almond, and pecan)	Soil application	*N*-(phosphonomethyl- ^{14}C)-glycine at 5.07 kg/ha for pecan and walnut at 2.43 kg/ha for almonds	CA 6.2.1/003: [redacted] 1976, Absorption, translocation, and metabolism of Roundup® herbicide in walnut, almond, and pecan trees, Report No. 403

Table 6.2.1-172: Overview over available plant metabolism studies for crop category fruits

Plant	Application	Application rate	Reference
	Foliar application	*N*-(phosphonomethyl- ^{14}C)-glycine at 100 µg per leaf surface	
Apple	Soil application	*N*-(phosphono-^{14}C-methyl)glycine at 3.36 kg a.s./ha or amino-^{14}C-methyl-phosphonic acid at 1.68 kg/ha as AMPA (corresponding to 2.56 kg a.s./ha expressed in glyphosate equiv.)	CA 6.2.1/004: [redacted] 1974, CP 67573 residue and metabolism Part 23: The metabolism of CP 67573 in apple trees, Report No. 342
	Trunk application	*N*-(phosphono-^{14}C-methyl)glycine 92.4 µg/tree	
	Foliar application	*N*-(phosphono-^{14}C-methyl)glycine 10 µg/leaf or 10.7 mg/leaf	
Grapes	Soil application	*N*-(phosphono-methyl)glycine trimesium salt 8.1 (PMG-label) and 7.8 kg a.s./ha (TMS-label) corresponding to 5.6 or 5.4 kg glyphosate equiv./ha, respectively	CA 6.2.1/005: [redacted] 1991, Glyphosate – Trimesium. Uptake and metabolism in USA grape vines, Report No. RJ1002B
	Overspray on bunches	*N*-(phosphono-methyl)glycine trimesium salt 14.3 mg per 10 bunches (PMG-label) and 13.2 mg per 10 bunches (TMS-label) corresponding to 5.6 or 5.4 kg glyphosate equiv./ha, respectively	
	Soil application (drench)	*N*-(phosphono-methyl)glycine trimesium salt 8.3 kg a.s./ha (PMG-label) (corresponding to 5.7 kg glyphosate equiv./ha) or 7.1 kg/ha (TMS label) (corresponding to 4.9 kg glyphosate equiv./ha)	CA 6.2.1/006: [redacted] 1990, ICIA0224: Uptake and metabolism in grape-vines, Report No. RJ 0815B
	Soil application	*N*-(phosphono-^{14}C-methyl)glycine 3.36 kg a.s./ha or amino-^{14}C-methylphosphonic acid: 1.68 kg/ha (corresponding to 2.56 kg glyphosate equiv./ha)	CA 6.2.1/007: [redacted] 1974, CP 67573 residue and metabolism Part 20: The metabolism of CP 67573 in grape plants, Report No. 335
	Trunk application	*N*-(phosphono-^{14}C-methyl)glycine 40 µg per tree (corresponding to 0.17 kg glyphosate/ha)	

Table 6.2.1-172: Overview over available plant metabolism studies for crop category fruits

Plant	Application	Application rate	Reference
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine 5, 10, 20 or 40 mg/kg	
	Foliar application	*N*-(phosphono-^{14}C-methyl)glycine 20 µg per leaf (120 µg per plant)	

Citrus

Different application scenarios were investigated on calamondin citrus (mandarin) such as soil, hydroponic and foliar application ([redacted] **1975**).

Less than 0.1 % of the applied radioactivity of *N*-(phosphono-^{14}C-methyl)glycine (^{14}C glyphosate) was absorbed from treated soil or translocated into the leaves, stems, immature fruit and mature fruit. Comparable low rates of absorption were observed after soil application of ^{14}C-AMPA. During hydroponic treatment the percentage of radioactivity recovered was 1.3 % or 1.8 % in the leaves, 0.3 % in the stems both for ^{14}C-glyphosate or ^{14}C-AMPA treatment, and 4.2 % and 5.5 % in the roots, for ^{14}C-glyphosate or ^{14}C-AMPA, respectively. After foliar application treated leaves contained 76.6 % of the applied radioactivity after one week. The radioactivity in treated leaves declined to 10.3 % after 6 weeks. In fruit, <0.1 to 1.4 % were recovered at 1 - 6 weeks, while 9.8 % of the applied activity were found after 8 weeks.

Calamondin citrus extracts after foliar treatment investigated by ^{13}C-NMR and GC-MS indicated the presence of **glyphosate**. No indications of **AMPA** could be found.

An additional metabolism study on lemon trees was designed to determine the nature and magnitude of glyphosate-derived residues, resulting from soil uptake after spraying of glyphosate trimesium to bare soil ([redacted] **1987**).

Lemons, leaves of the trees, and soil around the trees were sampled directly after application, and two months and four months after treatment. The total radioactive residue (TRR) in full-grown lemons harvested four months after treatment was very low (<0.01 mg/kg), although some residue was found in the leaves. The average level of residue found in the lemons from treated trees ranged between 0.001 - 0.010 mg/kg expressed as glyphosate equivalents. Due to the low level of residue in mature lemons, characterisation of metabolites was not pursued.

Tree nuts

In the metabolism study on tree nuts the uptake and metabolism of N-(phosphonomethyl- ^{14}C)-glycine following soil treatment or foliar application to tree nut seedlings (almond, pecan and walnut) was investigated [redacted] **1976**).

Soil application experiment yielded low residues in comparison to radioactivity applied, demonstrating low plant uptake of ^{14}C-glyhosate from soil.

After foliar treatment of single plants most of the radioactivity applied was located on the treated leaves. The translocation into untreated plant parts (roots, other tops) was minor. The results of the first foliar experiment with walnut, almond and pecan were difficult to interpret because of the residues in controls (artefact during the simultaneous conduction of the foliar and soil experiments in one cubicle). For walnut a second foliar experiment was performed in a cubicle that was not being used with any other ^{14}C-experiment, leading to no significant residues in controls. This experiment showed that walnut trees only slowly metabolise **glyphosate** (81.6 to 94.8 % of the TRR), and the only recognisable product was **AMPA** (<3 % of the TRR) in all tree commodities tested (treated leaves, other tops, roots). No relevant food or feed items were investigated.

Apple

The uptake and metabolism of ^{14}C-labelled *N*-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) and ^{14}C-labelled aminomethylphosphonic acid (^{14}C-AMPA) in apple trees were investigated following different treatment scenarios such as soil, foliar or trunk application ([redacted] **1974**).

The uptake of ^{14}C-glyphosate or ^{14}C-AMPA was very low after soil treatment (maximum of 0.134 % of the applied radioactivity at 12 weeks after treatment). After trunk treatment, uptake and translocation was also minimal with 0.08 % of the applied activity recovered in leaves and stems and untreated trunk. 0.1 % of the applied radioactivity was recovered in roots, while 72.05 % of the applied radioactivity were found in treated trunk.

After foliar treatment, TRRs in treated leaves ranged between 98.06 and 144.3 mg/kg during the course of the study. Foliar applied ^{14}C-glyphosate in formulation was rapidly and efficiently transported throughout the apple tree from the treated leaves. The highest amount was observed in the growing stem and leaves immediately above the treatment. Significant amounts of compound could also be found in other new growth, trunk and roots.

Within samples taken after foliar application the major residue in treated leaves, new growth above the treatment, other new growth, roots, and trunk of the apple trees was parent **glyphosate** (64.43 – 101.3 % of the TRR). A maximum of 6.45 % of the TRR (7.95 mg/kg) behaved in a manner chromatographically identical to **AMPA/*N*-methyl AMPA** in treated leaves, new growth above the treatment, other new growth of the apple trees. No other metabolites were identified. No relevant food or feed items were investigated.

Grapes

Three different studies on metabolism in grape are available which investigated the different types of application to yield sufficient radioactive residues for further identification and characterisation:

In a first study ([redacted] **1990**) ^{14}C-glyphosate-trimesium labelled either in the *N*-phosphonomethylglycine (PMG) anion or the trimethylsulfonium (TMS) cation was applied as a soil drench to the bases of the trunks. The total radioactive residues after trunk application were low (<0.01 mg/kg). Therefore no characterisation of the residue was attempted.

In the second study ([redacted] **1991**) ^{14}C-glyphosate-trimesium was applied to the soil around mature vines 14 days or one year prior to sampling. In addition, bunches of grapes were deliberately oversprayed with glyphosate-trimesium. As seen in the first study the total radioactive residues after soil application were low (<0.01 mg/kg). Overspray application resulted in a TRR of 1.25 mg/kg (^{14}C-PMG-label) at 14 days after treatment. The radioactive residues in grapes (fruit) were identified as **glyphosate anion** accounting for 77.1 % of the TRR (0.964 mg/kg) and 2.5 % of the TRR (0.031 mg/kg) as **AMPA**.

In the third study ([redacted] **1974**) the uptake and metabolism *N*-(phosphono-^{14}C-methyl)glycine (glyphosate) in grapevines was investigated following soil, trunk, hydroponic or foliar application as well as uptake of ^{14}C-labelled aminomethylphosphonic acid after soil application. Different grape varieties were used as representative of juice, table, and wine grapes.

The uptake of ^{14}C-glyphosate or its metabolite AMPA after soil treatment was 0.12 % of the applied radioactivity, while after trunk treatment, uptake and translocation was also minimal with 1.57 % of the applied activity recovered in vines (leaves and stems), while up to 82.72 % of the applied radioactivity were found in treated trunk.

After hydroponical treatment significant ^{14}C-activity was observed in or on the roots of the grapevines; between 4.7 and 18.66 % of the applied ^{14}C-activity (0.83 – 4.10 mg/kg) was associated with the roots. Markedly less activity was observed in the aerial portions of the grapevines.

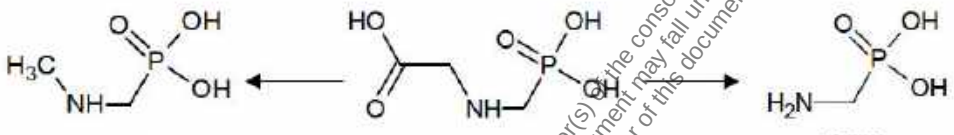
After foliar treatment the majority of the treatment remained on the treated leaves substantial uptake and translocation has occurred. The majority of the translocated ^{14}C-activity was associated with the stems and leaves (new growth) above the treated leaves and with the roots ^{14}C-activity translocation to the fruit was observed whenever fruit was present.

The major residue was **glyphosate** at different amounts of the TRR in treated leaves at 70.5 – 97.1 %, new growth above the treatment at 70.4 – 103.1 %, roots at 87.6 – 90.2 % and old stock and grapes (fruit) 64.6 – 79.5 %.
In root and old stock only **glyphosate** was present, while in treated leaf, new growth and grapes (fruit) the metabolite **AMPA** was identified as metabolite accounting for 1.5 – 9.2 % of the TRR, 1.0 – 2.0 %, <1.0 %, respectively.

Overall conclusion on metabolism in fruits

Within all studies investigating the metabolism of ^{14}C-glyphosate in fruits a similar picture on metabolism was found. In all studies low plant uptake was indicated after soil treatment.
Higher residue uptake was achieved using application scenarios such as hydroponic treatment or foliar treatment which allowed the investigation of the nature of residues.
Glyphosate parent compound accounted for the main part of radioactive residues. In some cases, **AMPA** was identified as minor metabolite.
***N*-methyl-AMPA** was indicated (not chromatographically separated from AMPA) in one apple study after foliar treatment and only in apple treated leaves, new growth and other new growth (leaves and stem).

Pathway for fruits – non-tolerant plants



N-methyl AMPA Glyphosate AMPA

AMPA: Minor metabolite in grapes (fruit) and other non-food and feed related commodities

AMPA/*N*-methyl AMPA: (not chromatographically separated) Minor in apple treated leaves, new growth above treatment, other new growth (no food or feed related items)

Root and tuber crops

Two metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **root and tuber crops**. An overview on the studies, the application scenarios and application rates used is given in the following table.
Within two confined rotational crops studies also primary crops beet and carrot were investigated (for details please refer to CA 6.6.1.).

Table 6.2.1-173: Overview on available plant metabolism studies for root and tuber crops

Plant	Application	Application rate	Reference
Potato	Soil application	*N*-(phosphono-^{14}C-methyl)glycine at 23.8 mg per pot or amino-^{14}C-methyl-phosphonic acid at 23.4 mg per pot *(application to bare soil)*	CA 6.2.1/008: [redacted] 1975, CP 67573 residue and metabolism Part 26: The metabolism of CP 67573 in potato plants, Report No. 376

Table 6.2.1-173: Overview on available plant metabolism studies for root and tuber crops

Plant	Application	Application rate	Reference
		N-(phosphono-^{14}C-methyl)glycine at 4.48 kg a.s./ha planting of pre-grown potatoes (BBCH 09) *(weeds treated with glyphosate and incorporated into soil to simulate ploughing)*	
	Foliar application	*N*-(phosphono-^{14}C-methyl)glycine 108 µg per plant at pre-bloom stage	
Sugar beets	Soil application	*N*-(phosphono-^{14}C-methyl)glycine or amino-^{14}C-methyl- phosphonic acid at 8.0 mg per pot corresponding to 4.48 kg glyphosate equiv./ha	CA 6.2.1/009: [redacted] 1976, CP 67573 residue and metabolism Part 29: The metabolism of CP 67573 in sugar beets, Report No. 394
	Foliar application	3.57 µg *N*-(phosphono-$^{13/14}$C-methyl)glycine (^{13}C/^{14}C ratio: 13:1) per plant and 0.89 µg *N*-(phosphono-^{14}C-methyl)glycine per leaf	
Beet (as primary crop in the rotational crop study)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application was performed 3 days before planting)	CA 6.6.1/005: [redacted], 1978, Uptake and metabolism of glyphosate in root, leaf and cereal type rotational crops, Report No. MSL-0882
Carrot (as primary crop in the rotational crop study)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application at the maximum plant growth of crops)	CA 6.6.1/006: [redacted] 1976, Metabolism of CP 67573 in representative vegetables and rotation crops, Report No.: 406

Potato

In the potato metabolism study ([redacted] **1974**) either *N*-(phosphono-^{14}C-methyl)glycine or amino-^{14}C-methyl-phosphonic acid was added to soil followed by mixing. In addition, glyphosate treated weeds were incorporated in two of the pots to simulate ploughing. This method of incorporating the radioactive herbicide into the soil produced results similar to results after soil treatment.

After soil application **AMPA** was the only metabolite in tubers, accounting for up to 35.3 % of the TRR. However, ***N*-methyl-AMPA**, which exist as trace impurity in certain samples of ^{14}C-glyphosate, was not separated from the ^{14}C-AMPA. Parent **glyphosate** was also found, but at lower levels (1.1-7.5 % of the TRR).

After foliar treatment the major part of residue in leaves was attributed to parent **glyphosate**. No identification of the radioactivity was conducted.

Sugarbeet

In a metabolism study on sugar beet ([redacted], **1976**) the uptake of radioactivity into sugar beets after soil application of *N*-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) or ^{14}C-aminomethylphosphonic acid (^{14}C-AMPA) or after foliar application of a mixture of ^{13}C- and ^{14}C-glyphosate was investigated.

After soil treatment for both analytes the observed uptake of radioactivity into roots and leaves of sugar beets was minimal. Less than 0.2 % of the applied activity following soil treatment were recovered in the plant samples.

After foliar treatment with the mixture of ^{13}C- and ^{14}C-glyphosate, the untreated leaves were found to contain 11.9 % of the applied radioactivity, while 31.2 % of the applied radioactivity had translocated to the roots and 30.2 % remained on the treated leaves.

The sugar beet root extract from the ^{14}C-glyphosate soil treatments indicated 30 % **glyphosate**, 10 % **AMPA**, and 60 % **neutral material**, while 70 % **glyphosate** and 30 % **neutral material** were found in the leaves. The aqueous extracts of the roots and the leaves from the ^{14}C-AMPA soil treatments contained 90 % **AMPA** and 10 % **neutral material**.

The neutral material observed was discussed as a result of photofixation of $^{14}CO_2$ from soil metabolism of the labelled compounds. The concentration of the neutral material in the roots of the plants grown in the ^{14}C-glyphosate treated soil indicates that this material is probably **sugars**.

The major labelled material detected after foliar treatment was **glyphosate** (85 - 90 % of extracted). The presence of AMPA was not detectable; similarly, no other labelled metabolites were observed.

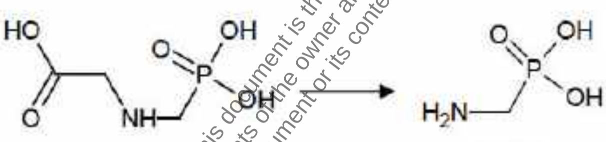
Overall conclusion on metabolism in root and tuber crops

Within both studies investigating the metabolism of ^{14}C-glyphosate in root and tuber crops a similar picture on metabolism was found. In both studies low plant uptake was indicated after soil treatment. After foliar treatment the main part of radioactive residues remained on the treated leave or was translocated to the root, while translocation to untreated leaves occurred to lower amount.

Glyphosate parent compound accounted for the main part of radioactive residues in sugar beet while in potato tuber after soil treatment the main part of identified radioactive residues was **AMPA**. **AMPA** was identified as major metabolite in potato tuber and a relevant metabolite in sugar beet root extracts. The presence of **natural products** such as sugars was indicated in sugar beets.

These findings were confirmed by results of the primary crops (beet and carrot) investigated as part of the confined rotational crop studies where only glyphosate and AMPA were determined in the extracts.

Pathway for root and tuber crops - non tolerant crops



Glyphosate AMPA

AMPA: Major metabolite in potato tuber (up to 35.3 % of the TRR) and identified metabolite in sugar beet roots (10 % in root extracts)

Leafy crops

Within two confined rotational crop studies also the fate and nature of glyphosate-derived residues in primary **leafy crops**. An overview on the studies, the application scenarios and application rates used is given in the following table (for further details please refer to CA 6.6.1.)

Table 6.2.1-174: Overview on available plant metabolism studies for leafy crops

Plant	Application	Application rate	Reference
Cabbage (as primary crop in the rotational crop study)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application was performed 3 days before planting)	CA 6.6.1/005: [redacted] 1978, Uptake and metabolism of glyphosate in root, leaf and cereal type rotational crops, Report No. MSL-0882
	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application at the maximum plant growth of crops)	CA 6.6.1/006: [redacted] 1976, Metabolism of CP 67573 in representative vegetables and rotation crops, Report No.: 406

Cereals and grass crops

Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **cereals and grass crops**. An overview on the studies is given in the following table.
Within a confined rotational crops study also primary cereals was investigated (for details please refer to CA 6.6.1.).

Table 6.2.1-175: Overview over available plant metabolism studies – cereals and grass crops

Plant	Application	Application rate	Reference
Wheat	Foliar application close to harvest	*N*-(phosphono-^{14}C-methyl)glycine trimesium salt at 5.64 kg/ha for the PMG-label (corresponding to 3.89 kg glyphosate equiv./ha)	CA 6.2.1/010: [redacted] 1989, ICIA0224: Metabolism on wheat following a pre-harvest foliar spray, Report No. RJ0778B
Barley Oats Rice Sorghum	Soil application	*N*-(phosphono-^{14}C-methyl)glycine at 4.5 kg a.s./ha	CA 6.2.1/011: [redacted] 1974, CP 67573 residue and metabolism Part 22: The metabolism of *N*-phosphonomethylglycine in barley, oats, rice and sorghum, Report No. 341
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine 0.183 mg/mL in hydroponic solution	
Wheat Maize/corn	Soil application	*N*-(phosphono-^{14}C-methyl)glycine 4.5 kg a.s./ha	CA 6.2.1/012: [redacted] 1973, CP 67573 residue and metabolism, part 10: The metabolism of CP 67573 in soybeans, cotton, wheat and corn, Report No. 304
		Amino-^{14}C-methylphosphonic acid 1.7 kg/ha (corresponding to 2.6 kg glyphosate equiv./ha)	
	Sand culture experiments	*N*-(phosphono-^{14}C-methyl)glycine 2.24 kg a.s./ha	

Table 6.2.1-175: Overview over available plant metabolism studies – cereals and grass crops

Plant	Application	Application rate	Reference
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine 3 mg/24 plants (maize) 3 mg/72 plants (wheat)	
Wheat (as primary crop in the rotational crop study)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application was performed 3 days before planting)	CA 6.6.1/005: [redacted] 1978, Uptake and metabolism of glyphosate in root, leaf and cereal type rotational crops, Report No. MSL-0882
Pasture (seed mixtures of fescue/alfalfa, bromegrass/red clover and timothy/white clover)	Soil application (pre-emergent)	*N*-(phosphono-^{14}C-methyl)glycine 4.48 kg a.s./ha	CA 6.2.1/013: [redacted] 1976, The metabolism of glyphosate in pasture crops, Report No. 404
Pasture (quackgrass, fescue/alfalfa mixture)	Foliar application to quackgrass followed by incorporation after 1 week and sowing of fescue/alfalfa mixture	*N*-(phosphono-^{14}C-methyl)glycine 1.68 kg a.s./ha	
Pasture (fescue and alfalfa)	Foliar application	*N*-(phosphono-^{13}C/^{14}C-methyl)glycine (90:10) 1.12 kg a.s./ha	
	Pre-harvest application	*N*-(phosphono-^{13}C/^{14}C-methyl)glycine (90:10) 1.12 kg a.s./ha	

In the first wheat study ([redacted] **1989**) *N*-(phosphono-^{14}C-methyl)glycine trimesium salt, the trimethylsulfonium salt of glyphosate was applied to wheat close to harvest (moisture content of grain <20 %). The main constituent of the TRR in grain, chaff and straw was glyphosate accounting for 90.8, 85.0 and 82.6 % of the TRR respectively (corresponding to 2.43, 278 and 103 mg/kg respectively). In addition to **glyphosate anion**, **AMPA** was identified as major metabolite in grain, chaff and straw which accounted for 2.8, 3.9 and 3.3 % of the TRR, respectively (corresponding to 0.08, 12.8 and 4.1 mg/kg respectively).

In cereal grains (barley, oats, rice and sorghum) of the second study ([redacted], **1974**) the uptake of radioactivity of *N*-(phosphono-^{14}C-methyl)glycine from treated soil was very limited. A range of 0.03 to 0.13 % of the applied radioactivity was found in the plants. Following application of ^{14}C-glyphosate via hydroponic solution, glyphosate gave a better uptake into the plants (2.70 to 4.68 % of the applied radioactivity into aerial parts and 6.53 to 23.05 % of the applied radioactivity into roots) and it was possible to investigate the nature of the residues in tops and roots of barley, oats, rice and sorghum.
In the aerial portion (tops) of all crops, **glyphosate** accounted for the main part of radioactivity (73.25 - 76.63 % of the TRR). 6.51 - 13.97 % of the TRR (0.027 to 0.243 mg/kg) was identified as **AMPA** and 1.41 to 5.43 % of the TRR (0.012 to 0.040 mg/kg) was ***N*-methyl-AMPA**. Barley and sorghum tops, contained the highest percentage of **AMPA** as well as the highest percentage of ***N*-methyl-AMPA**. Also, in the roots the main part of radioactivity was identified as **glyphosate** (19.10 - 52.60 % of the TRR). The most prominent metabolite was **AMPA** (2.18 to 7.42 % of the TRR; 0.037 to 0.273 mg/kg) and 0.43 - 1.41 % of the TRR (0.008 to 0.071 mg/kg) were identified as ***N*-methyl-AMPA**.

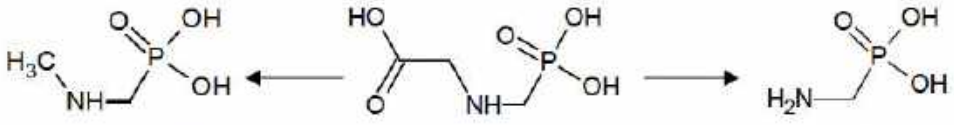
In the third cereal study [redacted], **1973**) the uptake and metabolism of *N*-(phosphono-^{14}C-methyl)glycine and amino-^{14}C-methylphosphonic acid in wheat and maize were investigated. Two soil uptake experiments per crop using *N*-(phosphono-^{14}C-methyl)glycine or Amino-^{14}C-methylphosphonic acid resulted in a very low uptake up to 0.12 % of the applied radioactivity for ^{14}C-glyphosate and up to 0.044 % of the applied radioactivity for ^{14}C-AMPA.
Uptake of *N*-(phosphono-^{14}C-methyl)glycine into plants growing in sand culture after application of an aqueous solution of *N*-(phosphono-^{14}C-methyl)glycine to the sand has also been examined. Only maize gave an uptake of 11.3 % of the applied dose into the aerial portion after 18 days. Wheat had an aerial uptake of only 0.03 % of the applied ^{14}C-activity after 18 days. The extraction data for the treated sand showed that *N*-(phosphono-^{14}C-methyl)glycine was not available for uptake into the plants.
Based on the higher plant uptake after hydroponic treatment (4.73 and 10.30 % of the applied radioactivity at day 28 in maize aerial part and roots, and 2.46 % and 2.52 % of the applied radioactivity at day 10 in wheat aerial parts and roots), plants were extracted, and the residues were analysed further. Parent **glyphosate** accounted for up to 81.1 % of the TRR in aerial parts of maize and wheat and up to 99.9 % of the TRR in roots. **AMPA** was found as major metabolite accounting for up to 27.0 % of the TRR in aerial parts and up to 10.1 % of the TRR in roots.
In addition, ***N*-methyl AMPA** accounted for up to 4.2 % of the TRR in aerial parts and up to 0.6 % of the TRR in roots.
Separate extractions to investigate the radioactivity in **natural products** indicated the incorporation of fragments or $^{14}CO_2$ into natural products (e.g. amino acids and peptides or citric acid cycle intermediates).

After pre-emergent application of *N*-(phosphono-^{14}C-methyl)glycine or planting pasture crops in soil containing incorporated perennials previously treated with *N*-(phosphono-^{14}C-methyl)glycine the uptake of residues by grass or legume pasture did not exceed 0.1 % of the applied radioactivity ([redacted] **1976**).
After foliar treatment of pasture crops with *N*-(phosphono-^{13}C/^{14}C-methyl)glycine (90:10) 41.8 - 69 % of the applied radioactivity were recovered in treated foliage one week after application. Regrowth after eradication of treated foliage showed residue levels at or below 0.2 % of the applied activity.
The majority of the radioactive residues extracted from treated fescue and alfalfa forage was shown to be **glyphosate**. Approximately 3 % of the radioactivity recovered in extracts of dried fescue forage showed a chromatographic behaviour corresponding to the metabolite **AMPA**.

Overall conclusion on metabolism in cereals – non-tolerant crops

In the first study wheat was treated close to harvest at moisture content <20 %. The foliar application yielded in a sufficient residue level to be investigated for nature of glyphosate residues. However, based on the late growth stage/low moisture content the metabolism was limited shown by high amounts of **glyphosate** present (> 82 % of the TRR). **AMPA** was identified accounting for ~3 % of the TRR (0.08 – 12.8 mg/kg in grain, chaff and straw) and was a major metabolite in grain.
In the other studies glyphosate was applied at earlier stage. In all experiments low plant uptake was indicated after soil treatment or sand culture treatment. Hydroponical treatment or foliar treatment allowed higher incorporation allowing further investigations on the nature of residues.
After growing in hydroponic solution **glyphosate** accounted for the main part of radioactive residues. **AMPA** was identified as major metabolite in wheat grain, maize roots and tops and in aerial part of barley. In addition, ***N*-methyl AMPA** was determined as minor metabolite in aerial and root parts of barley, oats, rice and sorghum as well as maize forage and root and wheat root after growing in hydroponic solution.
Also the radioactivity was also determined in **natural products** (e.g. amino acids and peptides or citric acid cycle intermediates.
The results are supported by results of the primary crop (wheat) also investigated in the confined rotational crop study; the only radioactive compounds found in the extracts were glyphosate and AMPA.
For further details please refer to the CA 6.6.1.

Pathway for cereals – non-tolerant crops



N-methyl AMPA Glyphosate AMPA

AMPA: Major metabolite in wheat grain (foliar application close to harvest), maize roots and tops (after hydroponic treatment); in aerial parts of barley (after hydroponic treatment).

***N*-methyl AMPA:** Minor metabolite in aerial and root parts of barley, oats, rice and sorghum (after hydroponic treatment); maize forage and root and wheat root (after hydroponic treatment) (no food or feed relevant items).

Pulses and oilseeds

Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **pulses and oilseeds.** In addition to primary crop studies, primary crops were also investigated in two confined rotational crop studies (for details please refer to CA 6.6.1.).
An overview on the studies, the application scenarios and application rates used is given in the following table:

Table 6.2.1-176: Overview on available plant metabolism studies for crop category pulses and oilseeds

Plant	Application	Application rate	Reference
Soybean	Soil application (drench)	*N*-(phosphono-^{14}C-methyl)glycine trimesium salt at 8.40 kg/ha (corresponding to 5.8 kg glyphosate equiv./ha) within two hours after planting the seeds	CA 6.2.1/014: [redacted] 1992, [^{14}C-Anion] ICIA0224: Nature of the residue: Soybeans, Report No. RR 91-092B
Soybean	Soil application	*N*-(phosphono-^{14}C-methyl)glycine at at 4.5 kg a.s./ha or amino-^{14}C-methyl- phosphonic acid at 1.7 kg/ha (corresponding to 2.6 kg glyphosate equiv./ha)	CA 6.2.1/015: [redacted] 1973, CP 67573 residue and metabolism, part 10: The metabolism of CP 67573 in soybeans, cotton, wheat and corn, Report No. 304
	Hydroponic sand culture	*N*-(phosphono-^{14}C-methyl)glycine at 2.24 kg a.s./ha	
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine at 12 mg/24 plants or 50 mg/99 plants or *N*-(phosphonomethyl)-^{14}C-carboxy-glycine at 12 mg/24 plants or *N*-(phosphonomethyl)-^{14}C-methyl-glycine 12 mg/24 plants or Mixture of *N*-(phosphono-^{13}/^{14}C-	

Table 6.2.1-176: Overview on available plant metabolism studies for crop category pulses and oilseeds

Plant	Application	Application rate	Reference
		methyl)glycine at 50 mg/198 plants or *N*-(phosphono-^{14}C-methyl)glycine at 12 mg/24 plants for 6 days	
Cotton	Soil application	*N*-(phosphono-^{14}C-methyl)glycine at 4.5 kg a.s./ha or amino-^{14}C-methyl- phosphonic acid at 1.7 kg/ha (corresponding to 2.6 kg glyphosate equiv./ha)	
	Sand culture	*N*-(phosphono-^{14}C-methyl)glycine at 2.24 kg a.s./ha	
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine at 12 mg/12 plants	
Pea (as primary crop in the rotational crop study)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application at the maximum plant growth of crops)	CA 6.6.1/006: [redacted] 1976, Metabolism of CP 67573 in representative vegetables and rotation crops, Report No.: 406
String bean (as primary crop in the rotational crop study)	Soil application	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application at the maximum plant growth of crops)	CA 6.6.1/006: [redacted] 1976, Metabolism of CP 67573 in representative vegetables and rotation crops, Report No.: 406

Soybean

In a metabolism study on soybean ([redacted] **1992**) the uptake of radioactivity into soybean grown in soil treated by drench with *N*-(phosphono-^{14}C-methyl)glycine trimesium salt (PMG) was investigated.

The TRR of forage samples accounted for 1.76 mg/kg, straw for 0.859 mg/kg, hulls for 0.487 mg/kg, green seeds for 0.772 mg/kg and yellow seeds for 1.31 mg/kg and allowed the investigation of the nature of glyphosate-derived residues.

Within extracts of forage, straw, hulls and yellow seeds **glyphosate anion** and its metabolite **AMPA** were identified accounting for 0.57 - 4.10 and 1.50 - 5.70 % of the TRR, respectively.

The remaining fractions of the extractable residue (34.13 - 48.9 % of the TRR), were shown to be radiolabelled **natural products** mainly consisting of **mono- and disaccharides** and **amino acids** and to a lower extent to **smaller proteins**.

The unextractable (bound) residues were characterised as natural products, 16.9 - 25.3 % of the TRR **carbohydrates**, 1.43 - 2.93 % of the TRR **lignin**, 16.0 - 24.0 % of the TRR **protein** and 7.6 - 21.8 % of the TRR **crude cellulose**.

A second study for pulses and oilseeds is available ([redacted] **, 1973**), investigating the uptake into soybean and cotton grown in soil, sand culture or hydroponic solution. Less than 0.3 % of the applied radioactivity was taken up by soybean and cotton plants at 4, 6 and 8 weeks after soil application. ^{14}C-AMPA, the major soil metabolite showed uptake considerably less than ^{14}C-glyphosate. The maximum uptake for any of soybean or cotton after eight weeks was 0.03 % of the applied dose on soybean.

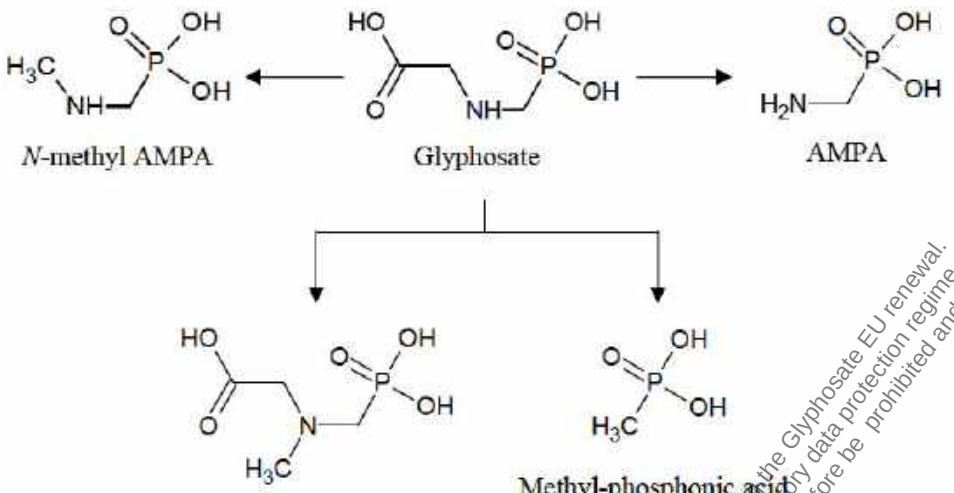
Uptake of ^{14}C-glyphosate into plants growing in sand culture after application of an aqueous solution to the sand has also been examined. Cotton and soybean had aerial uptakes of only 0.03 % and 0.07 % of the

applied ^{14}C-activity respectively, after 18 days. The extraction data (aqueous extraction followed by 1 N NH_4OH) for the treated sand show that glyphosate was not available for uptake into the plants.
In the hydroponic experiments the amount of ^{14}C-activity in cotton plants was found up to 2.98 % of the applied radioactivity in the aerial part and up to 19.34 % of the applied radioactivity in cotton roots, while they were found up to 4.19 % and up to 13.88 % of the applied radioactivity in soybean aerial parts and roots, respectively.
The major ^{14}C-containing component in the aqueous extracts after hydroponic treatment in all cases was **glyphosate** with up to 85.5 % of the TRR in soybean aerial parts and up to 97.3 % in roots and up to 80.7 % and 38.8 % of the TRR in cotton aerial parts and roots respectively. The major ^{14}C-containing degradate was **AMPA** with up to 8.0 - 27.0 % of the TRR in soybean and cotton aerial parts, and up to 8.9 - 10.1 % in roots of soybean and cotton.
Several minor metabolites, were also detected, and were indicated as ***N*-methyl AMPA** (up to 0.8 % of the TRR in cotton roots, up to 5.6 % of the TRR in soybean roots; up to 16.6 % of the TRR in soybean forage determined as AMPA/*N*-methyl-AMPA), **methyl phosphonic acid** (up to 0.3 % of the TRR in soybean roots and up to 2.0 % of the TRR in cotton roots), and ***N*-methyl glyphosate** (only up to 0.3 % of the TRR in cotton roots).
Within the study the occurrence of minor compounds was also discussed as artefacts from very small impurities in the starting ^{14}C-glyphosate or they may have been formed in the hydroponic solutions via microbial degradation of glyphosate.
Separate extractions to investigate the radioactivity in natural products indicated the incorporation of fragments or **$^{14}CO_2$** into **natural products (e.g. amino acids and peptides or citric acid cycle intermediates).**

Overall conclusion on metabolism in pulses and oilseeds

Within both studies investigating the metabolism of ^{14}C-glyphosate in soybean a similar picture on metabolism was found.
Glyphosate parent compound accounted for the main part of radioactive residues in most matrices. **AMPA** was found as minor metabolite in soybean straw, hull, hay, root and seeds as well as cotton forage and root. In soybean forage when **AMPA** was not chromatographically separated from ***N*-methyl AMPA**) major amounts were determined (after hydroponic treatment).
Metabolites such as**, methyl phosphonic acid,** and ***N*-methyl glyphosate** were indicated in soybean and cotton roots only after growing in hydroponical solution. ***N*-methyl AMPA** was also only indicated in soybean roots and forage after hydroponical treatment.
The incorporation of glyphosate into **natural products** (such as mono- and disaccharides, amino acids and to a lower extent to smaller proteins or citric acid cycle intermediates) was shown in both studies. The unextractable residues were characterised as natural products, such as carbohydrates, lignin, protein or crude cellulose.
The findings are confirmed by results of the primary crops (pea and string bean) which were part of the confined rotational crop studies; the only radioactive compounds found in the extracts were glyphosate and AMPA. For further details please refer to CA 6.6.1.

Pathway for pulses and oilseeds



AMPA: Minor metabolite in soybean forage, hull, and straw, hay, seed green, seed yellow, soybean and cotton aerial parts and roots (after hydroponic treatment).

AMPA/*N*-methyl-AMPA: (not chromatographically separated) Major in soybean forage after hydroponic treatment (no relevant food or feed item).

***N*-methyl-AMPA:** Minor metabolite in soybean root, cotton forage and root (no relevant food or feed items).

***N*-methyl glyphosate:** Minor metabolite, only found up to 0.3 % of the TRR in cotton root after hydroponic treatment (no relevant food or feed item).

Methyl-phosphonic acid: Minor metabolite, only found up to 0.3 % of the TRR in soybean root and up to 2.0 % of the TRR in cotton root after hydroponic treatment (no relevant food or feed items).

Miscellaneous crops

Two metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **miscellaneous crops (namely coffee and sugar cane)**. An overview on the studies is given in the following table:

Table 6.2.1-177: Overview over available plant metabolism studies - miscellaneous crops

Plant	Application	Application rate	Reference
Coffee	Foliar application	*N*-(phosphono-^{14}C-methyl)glycine at 0.32 mg/plant, only upper or only lower leaf surface 0.64 mg/plant, upper and lower surface treated 0.608 mg/plant, both surfaces treated, used for further extraction 1.9 mg/plant lower leaf surface on a	CA 6.2.1/016: [redacted], 1975, CP 67573 residue and metabolism part 24: The metabolism of CP 67573 in coffee plants, Report No. 344

Table 6.2.1-177: Overview over available plant metabolism studies - miscellaneous crops

Plant	Application	Application rate	Reference
		tree with beans	
	Stem application	*N*-(phosphono-^{14}C-methyl)glycine at 1.9 mg/plant (coating three of the lower segments of the stems application duration: 5 weeks)	
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine 1.1, 3.6 or 11.1 mg/L glyphosate. Treatment duration 3 weeks	
	Soil application	*N*-(phosphono-^{14}C-methyl)glycine at 4.5 kg a.s. or Aminomethyl- ^{14}C-phosphonic acid per ha at 4.5 kg/ha corresponding to 3.0 kg glyphosate/ha	
Sugarcane	Foliar application	*N*-(phosphono-^{14}C-methyl)glycine at 1.96 mg per plants	CA 6.2.1/017: Anonymous, 1976, Glyphosate residue and metabolism studies in sugarcane and soils, Report No. RD93
	Hydroponic treatment	*N*-(phosphono-^{14}C-methyl)glycine at 3 mg/plant	

A metabolism study ([redacted] **1975**) is available investigating the fate and nature of glyphosate-derived residues in **coffee**. In coffee plants treated via soil, stem, foliar or hydroponic application the uptake and translocation of radioactivity was minimal. After the soil treatment only 0.017 - 0.038 % of the applied radioactivity of *N*-(phosphono-^{14}C-methyl)glycine and aminomethyl-^{14}C-phosphonic acid was found in aerial part of the tree 8 weeks after the treatment. After the stem treatment the radioactivity in treated stem accounted for 87.2 % of applied radioactivity, whereas the TRR of leaves, untreated stems and roots accounted for 2.72 % of applied radioactivity.
For the foliar uptake of glyphosate several experiments with ^{13}C- and ^{14}C-glyphosate were used with different formulations and application techniques. In all samples, **glyphosate** was the major residue present (>71.7 - 95.0 % of the TRR). **AMPA/*N*-methyl AMPA** accounted for <0.7 to <1 % of the TRR.
Coffee trees carrying beans were also foliar treated with ^{14}C-glyphosate. In the immature beans 0.02 to 0.05 % of the applied radioactivity was found after 4 to 20 weeks after treatment, increasing to 0.94 % and to 0.68 % of applied radioactivity in green beans and pods as well as in ripe beans, respectively. **Glyphosate** was the major component of residue in all investigated bean matrices, comprising 91.2 to 98.0 % of the TRR. **AMPA/*N*-methyl AMPA** amounted to 0.98 to 5.0 % TRR.
After hydroponic treatment for three weeks most of the applied radioactivity was recovered in roots and in the remaining hydroponic solution. Only 0.1 to 0.2 and 4.3 to 11.7 % applied radioactivity were found in aerial parts and roots, respectively. The significant part of the residue in aerial parts and roots was characterised as the unchanged **glyphosate** (up to 74.0 and 81.9 % of the TRR). **AMPA** in aerial parts and roots accounted for up to 14.0 and 8.1 % of the TRR respectively.

The sugarcane study (**Anonymous, 1976**) consists of different experiments using either non-labelled glyphosate or ^{14}C-labelled glyphosate. Experiments using ^{14}C-labelled glyphosate were a root absorption experiment where sugarcane plants were grown in a hydroponic solution or foliar absorption of ^{14}C-glyphosate.

After 12 weeks sugarcane roots absorbed an amount of 13 %. 8 % of the applied radioactivity remained fixed in the roots; the stalk carried 1 % and the leaves 3 %. 81 % of the applied radioactivity had disappeared. Concentrations of 5 mg/kg (dry basis) occurred in roots, apical meristem, and the unexpanded leaf spindle, the regions of high metabolic growth activity but not of photosynthesis or sugar storage. Thin layer chromatography showed the residues to be in both the metabolite and parent forms with a possible predominance of metabolite **AMPA**.
In the experiment investigating the foliar absorption of ^{14}C-glyphosate, 18.6 % of residues applied were determined after 12 weeks. The treated leaves retained 4.6 %, roots accumulated 4.6 % and the primary stalk accumulated 4.3 %. Secondary suckers carried 3.2 % and the untreated leaves and spindle about 1.5 %. Accumulation took place in untreated younger leaves, spindle, primary apical meristem, stalk and roots. There was evidence of considerable translocation within the plant, probably in the phloem. The major translocated species was **glyphosate**, with only a minor contribution of the metabolite.
In addition, processing investigations were performed. An additional experiment for processing of mixed sugarcane juice performed with ^{14}C-labelled glyphosate showed that 36 % of the original glyphosate was removed in the liming solids. Most of the radioactive residues which were in the clarified juice found their way into the molasses fraction. The molasses film on raw sugar carried some portion of the radioactivity, and minor portions of molasses were occluded in the sugar crystal. Refining removed this minor residue by adsorption on the bone char resulting in no radioactivity detectable in refined sugar.

Overall conclusion on metabolism in miscellaneous crops

In sugarcane **glyphosate** and **AMPA** were the only identified compounds with a predominance of AMPA in the root absorption experiment.
Glyphosate was the major component of residue in all investigated matrices of coffee (treated and untreated leaves, aerial, stem, roots, beans, ripe pods and ripe beans). **AMPA** was not chromatographically separated from ***N*-methyl AMPA** and identified as minor in coffee treated and untreated leaves, aerial, stem, roots, beans, pods and ripe beans.

Pathway for miscellaneous crops

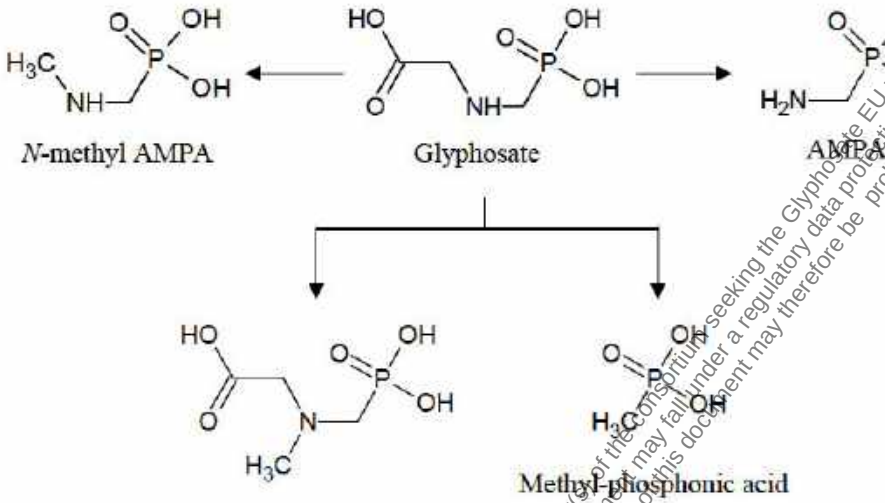
AMPA:	Predominant in sugar cane.
AMPA/*N*-methyl AMPA: (not separated in coffee study)	<u>Minor</u> in coffee treated and untreated leaves, aerial, stem, roots, beans, pods and ripe beans.

Overall conclusion on relevant metabolites for all crop groups for non-tolerant crops

The results of the different primary crop metabolism studies are very consistent. **Glyphosate** was the major ^{14}C-component in the different crops, and **AMPA** was the major or at least most prominent metabolite. The numerous plant uptake and metabolism studies demonstrate that glyphosate is slowly metabolised in plants to **AMPA**. ***N*-methyl AMPA** was identified in several commodities relevant to crop category pulses and oilseeds and cereals after foliar or hydroponic treatment. Nevertheless, in all cases ***N*-methyl AMPA** was only present in commodities not relevant as food or feed items (soybean forage, cereal aerial parts and roots). In apple leaves and new growth above the treatment, soybean forage as well as coffee commodities it was indicated as a mixture with AMPA.
***N*-methyl glyphosate** and **methyl-phosphonic acid** were determined in low amounts in one study only where soybean and cotton were grown in hydroponic solution. ***N*-methyl glyphosate** was only found up

to 0.3 % of the TRR in cotton roots and **methyl-phosphonic acid** was only found up to 0.3 % of the TRR in soybean roots and up to 2.0 % of the TRR in cotton roots. Both commodities are no relevant as food or feed items. These findings may also be discussed in context with impurities.
The incorporation of glyphosate into **natural products** (such as mono- and disaccharides, amino acids and to a lower extent to smaller proteins or citric acid cycle intermediates) was shown. The unextractable residues were characterised as natural products, such as carbohydrates, lignin, protein or crude cellulose.

Overall pathway considering all non-tolerant crop groups



AMPA:
Fruit crops: Minor metabolite in grapes (fruit) and other non-food and feed related commodities.
Root and tuber crops: Major metabolite in potato tuber (up to 35.3 % of the TRR) and identified metabolite in sugar beet roots (10 % in root extracts).
Cereals: Major metabolite in wheat grain (foliar application close to harvest), maize roots and tops (after hydroponic treatment); in aerial parts of barley (after hydroponic treatment).
Pulses and oilseeds: Minor metabolite in soybean forage, hull, and straw, hay, seed green, seed yellow; soybean and cotton aerial parts and roots (after hydroponic treatment).
Miscellaneous: Predominant in sugar cane.

***N*-methyl AMPA**:
Cereals: Minor metabolite in aerial and root parts of barley, oats, rice and sorghum (after hydroponic treatment); maize forage and root and wheat root (after hydroponic treatment) (no food or feed relevant items).
Pulses and oilseeds: Minor metabolite in soybean root, cotton forage and root (no relevant food or feed items).

AMPA/*N*-methyl-AMPA*:
Fruit crops: Minor metabolite in apple treated leaves, new growth above treatment, other new growth (no food or feed related items)
Pulses and oilseeds: Major in soybean forage after hydroponic treatment (no relevant food or feed item).
Miscellaneous: Minor in coffee treated and untreated leaves, aerial, stem, roots, beans, pods and ripe beans.
(*not chromatographically separated)

***N*-methyl glyphosate:** **Pulses and oilseeds**: Minor metabolite, only found up to 0.3 % of the TRR in cotton root after hydroponic treatment (no relevant food or feed item).

Methyl-phosphonic acid: **Pulses and oilseeds**: Minor metabolite, only found up to 0.3 % of the TRR in soybean root and up to 2.0 % of the TRR in cotton root after hydroponic treatment (no relevant food or feed items).

Genetically modified plants

Different metabolism studies are available including different genetical modifications such as CP4-EPSPS, CP4-EPSPS and GOX modification and GAT modification.
Based on the genetical modification different enzymes are involved and different metabolites are favoured.

- **CP4-EPSPS modified crops**

In CP4-EPSPS modified crops the glyphosate tolerance is based on a modified 5-enolpyruvylshikimate-3-phosphate synthase, which is much less susceptible to glyphosate than the enzyme natural occurring in plants.

- **CP4-EPSPS modified crops and GOX modified crops**

For the GOX modification, which is often used in combination with CP4-EPSPS, an alternative protein obtained from bacteria – the glyphosate oxidoreductase – is expressed in the plants, causing an accelerated degradation of glyphosate into AMPA.

- **GAT modified crops**

The GAT modification was implemented as an additional mode of glyphosate tolerance by expressing the enzyme "glyphosate *N*-acetyltransferase" (GAT). The new enzyme favours a metabolic pathway normally not observed in plants by acetylation of glyphosate and AMPA into *N*-acetyl glyphosate and *N*-acetyl-AMPA, both not showing herbicidal activity.

CP4 EPSPS modification and CP4 EPSPS modification/GOX modification

A metabolism study is available investigating the fate and nature of glyphosate-derived residues in **root and tuber vegetables of CP4 EPSPS modified sugar beet**. An overview on the application scenarios and application rates used is given in the following table:

Root and tuber vegetables

Table 6.2.1-178: Overview over available plant metabolism studies – root and tuber vegetables

Plant	Application	Application rate	Reference
CP4 EPSPS modification			
Sugar beet	Pre-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.9 kg a.s./ha	CA 6.2.1/018: [redacted] 2000a, Metabolism of Glyphosate in Roundup Ready Sugarbeet, Report No. MSL-16247
	Post-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 1.08 kg a.s./ha	

The nature of the residues in sugar beet plants (modified to express CP4 EPSPS: *N*-(phosphonomethyl)glycine) was investigated following the use of glyphosate applied either pre-emergent or twice post-emergent (**Mehrsheik, 2000a**).
The uptake of glyphosate from soil was very low in sugar beets. TRRs determined after pre-emergent treatment accounted for up to 0.006 mg/kg in tops and up to 0.009 mg/kg in roots.
After post-emergent treatment, TRRs were much higher (sugar beet tops up to 3.561 mg/kg and roots up to1.396 mg/kg). A translocation of radioactive residues into the roots was observed.
Glyphosate was the major component of the residue in both sugar beet tops and roots treated post-emergent, accounting for 79.65 % and 95.31 % of the TRR, respectively. The metabolite **AMPA** accounted for 1.84 % and 3.79 % of the TRR in tops and roots, respectively and was major in terms of concentration (>0.05 mg/kg in both tops and roots). **Glyphosate/AMPA acetylated conjugates** accounted for 0.80 % of the TRR in tops and 0.55 % of the TRR in roots. In addition, small amounts of ^{14}C-labelled **natural products** were indicated (1.38 % of the TRR in tops and 1.22 % of the TRR in roots) after post-emergent treatment.

Cereals

Two metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **cereals of CP4 EPSPS** as well as **CP4 EPSPS and GOX modified wheat and maize**, respectively.
An overview on the application scenarios and application rates used is given in the following table:

Table 6.2.1-179: Overview over available plant metabolism studies – cereals

Plant	Application	Application rate	Reference
CP4 EPSPS modification			
Wheat	Spray application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.84 kg a.s./ha at BBCH 15 and at BBCH 43 to the plant canopy	CA 6.2.1/019: Mehrsheik, A., 2000b, Metabolism of Glyphosate in Roundup Ready Wheat, Report No. MSL-16028, AA048760
CP4 EPSPS and GOX modification			
Maize/corn	Post-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.93 kg a.s./ha at BBCH 15-16 and 0.84 kg a.s./ha at BBCH 19 with and without soil protection	CA 6.2.1/20: [redacted] 1995, Nature of glyphosate residues in corn plants which are tolerant to Roundup® herbicide, Report No. MSL-14018

Within the wheat metabolism study (**Mehrsheik, 2000b**) the total radioactive residues in wheat forage, hay, straw and grain ranged from 12.12 to 34.81 mg/kg with straw containing the highest and grain the lowest level.
Glyphosate was the major component of the residue in all wheat matrices (forage, hay, straw and grain accounting for 69.19 % - 89.44 % of the TRR). **AMPA** was found to be the major metabolite in wheat grain (10.77 % of the TRR, 1.31 mg/kg).
In addition, ***N*-glyceryl AMPA** was identified as minor metabolite in wheat grain accounting for 0.34 % of the TRR (0.04 mg/kg). **Glyphosate/AMPA acetylated conjugates** and **other AMPA conjugates** were

characterised in wheat matrices, all accounting for less than 2.4 % of the TRR in any wheat commodity (0.08 – 0.84mg/kg).
The aqueous extracts of wheat matrices contained ^{14}C-labelled **natural products** (<2 % of the TRR). The radioactive natural products were considered to be derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from ^{14}C-glyphosate degradation into plant constituents.

Within the metabolism study on maize/corn modified to express CP4 EPSPS and GOX proteins ([redacted] **1995**) two foliar applications were done. Total radioactive residue in maize/corn forage, silage and fodder ranged from 9.11 mg/kg to 14.9 mg/kg for protected treatment, and 9.59 mg/kg to 19.1 mg/kg for non-protected treatment. Maize/corn grain contained much lower levels of radioactivity accounting for 0.685 mg/kg and 1.04 mg/kg for protected and non-protected treatments, respectively.
Glyphosate was observed to be the main radioactive residue in forage, silage and fodder accounting for 67.1 to 83.3 % of the TRR, whereas lower levels of glyphosate were present in grain (2.6 to 7.4 % of the TRR, 0.03 – 0.05 mg/kg). In contrast, **AMPA** was a major metabolite in all maize commodities found at approximately 11.2 % to 15.9 % of the TRR in forage, silage and fodder and 54.1 % to 60.3 % of the TRR in grain. Aqueous extracts also contained ***N*-glyceryl AMPA** accounting for 0.4 % to 1.6 % of the TRR in forage, silage and fodder and 6.9 % of the TRR in grain, where it was major in terms of concentration (0.05 – 0.07 mg/kg). In addition, low levels (< 2 % of TRR) of **glyphosate conjugates** and trace levels of other **AMPA conjugates** are mentioned.
In addition to this, aqueous extracts contained ^{14}C-labelled **natural products** (< 3.6 % of the TRR).
The radioactivity in oil extracted from grain was shown to be associated with **naturally occurring fatty acids**.
Unextractable residues were less than 5.4 % of the TRR in forage, silage and fodder, while they accounted for up to 25.27 % of the TRR (0.263 mg/kg) in grain. Acid hydrolysis of extracted grain released almost all of the bound radioactivity (90.24 % from grain). Majority of the acid-released radioactivity was shown to be **glucose**, derived from the incorporation $^{14}CO_2$ and other one carbon fragments of glyphosate into maize/corn **starch**.

Overall conclusion on cereals with CP4 EPSPS as well as CP4 EPSPS and GOX modification
The results of both studies are in good agreement. In both studies, glyphosate was the main component of the radioactive residue in wheat and maize matrices, except maize grain. **AMPA** was found as major metabolite in all maize as well as wheat grain. ***N*-glyceryl AMPA** was determined in lower amounts in wheat and maize grain, forage, silage and fodder. In wheat grain it was found to be minor metabolite but major metabolite in maize grain. In addition, **glyphosate/AMPA acetylated conjugates** and **other AMPA conjugates** were characterised.
Radioactive **natural products** were characterised in both studies which are considered to be derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from ^{14}C-glyphosate degradation into plant constituents.

Pulses and oilseeds

Three metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **oilseeds in CP4 EPSPS** as well as **CP4 EPSPS and GOX modified plants (rape, soybean and cotton)**. An overview on the studies is given in the following table:

Table 6.2.1-180: Overview over available plant metabolism studies – oilseeds

Plant	Application	Application rate	Reference
CP4 EPSPS and GOX modification			
Rape/canola	Post-emergence applications	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.455 kg a.s./ha at	CA 6.2.1/021: [redacted] 1994, Nature of Glyphosate residues in

Table 6.2.1-180: Overview over available plant metabolism studies – oilseeds

Plant	Application	Application rate	Reference
		BBCH 12-14 (14 days after planting or at 2x 0.90 kg a.s./ha at BBCH 12-14 and BBCH 16	Roundup herbicide tolerant canola, Report No. MSL-13318
CP4 EPSPS modification			
Soybean	Pre-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 5.38 kg a.s./ha	CA 6.2.1/022: [redacted] 1994, Nature of Glyphosate residues in soybeans tolerant to Roundup herbicide, Report No. MSL-13520
	Early post-emergence application	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.84 kg/ha (at BBCH 23, DALT 21)	
	Sequential post-emergence applications	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.84 kg a.s./ha (at BBCH 23, DALT 21) followed by 1.68 kg a.s./ha (BBCH 51, 43 days after planting)	
CP4 EPSPS modification			
Cotton	Post-emergence applications	Isotopic mixture of ^{12}C-, ^{13}C- and ^{14}C labelled *N*-(phosphono-methyl)glycine at 0.93 kg a.s./ha at BBCH 13-14 and 1.27 kg a.s./ha at BBCH 15-16 with and without soil protection	CA 6.2.1/023: [redacted] 1997, Nature of Glyphosate residues in cotton plants tolerant to Roundup herbicide, Report No. MSL-14113

Within the first study ([redacted] **1994**) tolerant rape (CP4 EPSPS and GOX modified) were treated once at early post-emergent application or twice as sequential post-emergence application. **AMPA**, ***N*-glyceryl AMPA**, ***N*-acetyl AMPA** and **sucrose** were identified in aqueous extracts of seeds. All of them were major in terms of concentration (0.06 – 0.58 mg/kg).

Saponifiable fatty acids were investigated after hexane extraction of the seeds and were identified as oleic and palmitic acids accounting for the major amount, as well as linoleic and linolenic acids. These four fatty acids are the major fatty acids in Westar variety canola with oleic acid accounting for >50 % of the total fatty acid content. Stearic acid was also found as a minor component.

In order to estimate the bioavailability of the residues after hexane and aqueous extraction, the extracted meal was treated with simulated gastric fluid (SGF) (containing pepsin) and simulated intestinal fluid (SIF) (containing pancreatin). Pepsin and pancreatin cause very little enzymatic release of bound

^{14}C-activity. Only small fraction of the ^{14}C-glyphosate-derived components in extracted meal would be biologically available if ingested by animals.
Extracted meal was enzymatically hydrolysed with protease, amylase, and cellulose, indicating the presence of radioactivity associated with **proteins**, **starch**, or **cellulose**. Acidic hydrolysis indicated the presence of radioactive **amino acids**, **organic acids** and **sugars**.
Treatment of extracted meal with 9:1 dioxane and water released radioactive residues associated with free or bound **lignin**.
Strong base hydrolysis of the extracted meal with 2.5 N NaOH at 85 °C for 65 hours. AMPA and formate were identified in the resulting extracts. Control experiments to determine the stability of AMPA showed that AMPA is gradually hydrolysed in base, and one of the hydrolysis products is formate.
The base hydrolysis results suggest that a significant amount of the unextracted residues in meal are due to bound AMPA. Upon hydrolysis, the AMPA is released and partially converted to formate.

Within one soybean study ([redacted] **1994**) the nature of residues resulting from soil uptake was investigated by a pre-emergence application to bare soil immediately before planting of soybeans containing the Roundup Ready gene (modified to express CP4 EPSPS protein). Also, the nature of residues resulting from foliar uptake was investigated by two different post-emergence treatment regimens.
The TRRs were the highest after sequential post-emergence treatment (10.416 - 23.651 mg/kg in forage, hay and seeds, while they accounted for only 0.205 - 0.748 mg/kg after pre-emerge treatment. Early post-emergence treatment resulted in the TRRs ranging between and 0.406 - 0.863 mg/kg in forage, hay and seeds respectively.
For plants after early post-emergence or two sequential post-emergence applications, **glyphosate** accounted for 88.5 - 89.1, 53.6 – 64.67 and 10.1 - 25.2 %. **AMPA** was a major metabolite in soybean seed (22.9 - 49.1 % of the TRR) and soybean hay (up to 12.8 % of the TRR), while it was minor in forage (2.3 - 6.8 % of the TRR). Additional metabolites were identified as ***N*-methyl-AMPA**, ***N*-glyceryl AMPA**, ***N*-acetyl-AMPA**, and ***N*-malonyl AMPA**, all less than 2 % of the TRR.
In soybean forage, hay and seed ***N*-methyl-AMPA** accounted for 0.13 – 0.14 mg/kg after sequential post-emergence application. ***N*-glyceryl AMPA** was major metabolite in seeds (0.278 mg/kg), ***N*-acetyl-AMPA** and ***N*-malonyl AMPA** in seeds (0.235 mg/kg and 0.309 mg/kg respectively). Moreover, 1.0 % (0.177 mg/kg) was attributed to other **AMPA conjugate** in seeds.
Additionally, up to 2.7 % was attributed to **natural products**. In seeds that received the two post-emergence applications 5.1 % of the TRR was shown to be associated with naturally occurring **organic and amino acids**. The radioactivity in hexane extracted oil from seeds was shown to be associated with **naturally occurring fatty acids** (0.8 % of the TRR).
The main part of the non-extracted remainder (after aqueous extraction) for selected samples of hay and seeds was released by sequential hydrolysis with protease, amylase and cellulose showing the presence of **natural plant constituents**.

The nature of the residues in glyphosate tolerant cotton (modified to express CP4 EPSPS protein) following the use *N*-(phosphono-^{14}C-methyl)glycine was studied ([redacted] **1997**). Two different experiments were performed, the one with soil protection, the other one without soil protection.
The total radioactive residue (TRR) in forage sample amounted to 15.2 mg/kg without soil protection and 30.4 mg/kg with soil protection. In contrast to the high residues in the forage, the residues in the final harvest stalk, seed and lint samples were all < 0.2 mg/kg in both experiments.
Investigation of the nature of residues in both experiments showed comparable results. In forage, 91.5 – 95.7 % of the TRR were present as **glyphosate**; the most abundant metabolite, **AMPA**, accounted for less than 2 % of the TRR. A **glyphosate-conjugate** accounted for up to 0.54 % of the TRR. **Natural products** accounted for up to 0.83 % of the TRR.
In seeds, there was very little **AMPA** (<1 – 1.38 % of the TRR) relative to **glyphosate** (12.0 – 23.7 % of the TRR). **Saponifiable fatty acids** accounted for up to 12.3 % of the TRR in seeds. **Natural products** accounted for up to 6.93 % of the TRR.

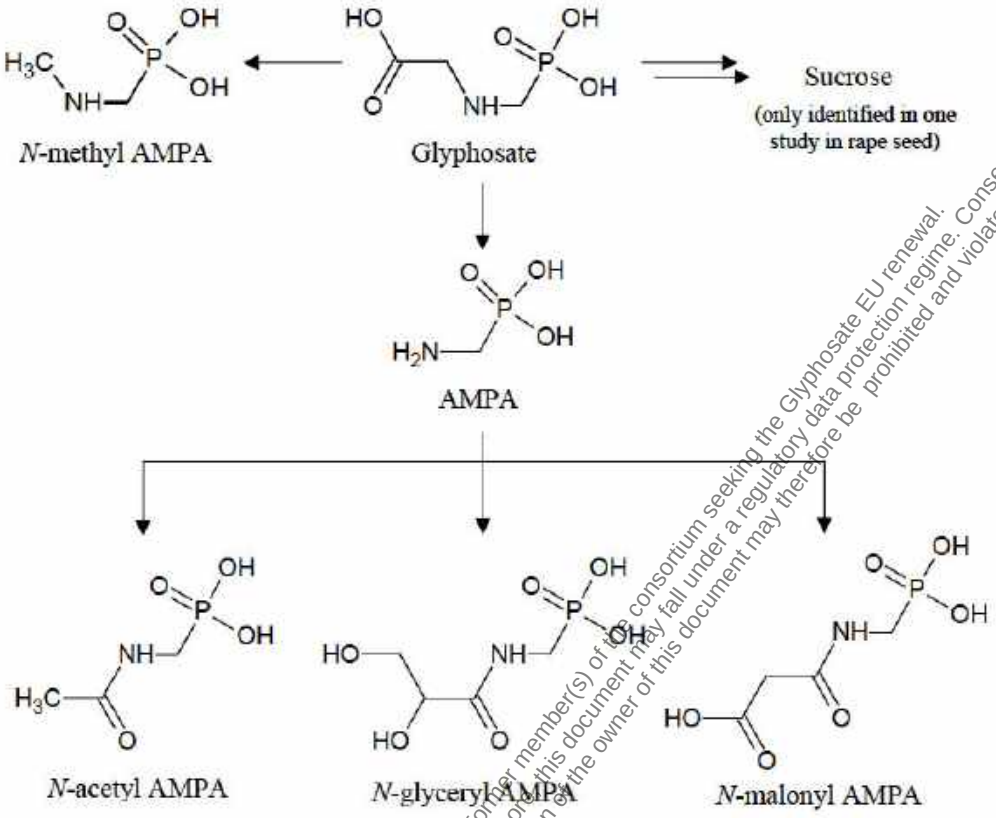
Overall conclusion on pulses and oilseeds with CP4 EPSPS as well as CP4 EPSPS and GOX modification

Within all three metabolism studies including rape, soybean and cotton, with CP4 EPSPS or CP4 EPSPS and GOX modification the metabolic pathway was found to be comparable.
Glyphosate accounted for the main part in soybean forage and hay, cotton seed and forage, while no glyphosate was identified in rape seeds.
In all crops the most abundant metabolite was **AMPA** which was major in soybean seed and hay and rape seed. Beneath **AMPA**, several metabolites were identified. ***N*-methyl-AMPA**, ***N*-glyceryl AMPA**, ***N*-acetyl AMPA** and ***N*-malonyl AMPA** were all identified as major metabolites in soybean seeds. ***N*-glyceryl AMPA** and ***N*-acetyl AMPA** were major also in rape seed.
Radioactive residues were incorporated into **natural products** (such as cellulose, lignin, starch, sugars, amino acids and fatty acids).

Overall conclusion on root and tuber crops, cereals/grass crops, pulses and oilseeds with CP4 EPSPS as well as CP4 EPSPS and GOX modification

Within all six metabolism studies including sugar beet, wheat, maize, rape, soybean and cotton, with CP4 EPSPS or CP4 EPSPS and GOX modification the metabolic pathway was found to be comparable.
Glyphosate accounted for the main part in sugar beet tops and roots, wheat forage, hay, straw and grain, maize forage, silage and fodder, soybean forage and hay, cotton seed and forage, while no glyphosate was identified in rape seeds.
In all crops the most abundant metabolite was **AMPA** which was major in wheat grain, maize forage, silage, fodder, grain, soybean seed and hay and rape seed. Four further metabolites were identified, i.e. ***N*-methyl-AMPA**, ***N*-glyceryl AMPA**, ***N*-acetyl AMPA** and ***N*-malonyl AMPA**, which were all major metabolites in soybean seeds. ***N*-glyceryl AMPA** was also major in maize grain and rape seed, while ***N*-acetyl AMPA** was major in rape seed.
Radioactive residues were incorporated into **natural products** (such as cellulose, lignin, starch, sugars, amino acids and fatty acids).

Pathway for root and tuber crops, cereals/grass crops, pulses and oilseeds with CP4 EPSPS as well as CP4 EPSPS and GOX modification



AMPA: Major metabolite in soybean (seed and hay), sugar beet (tops), wheat (grain), maize (fodder, grain, forage, silage), rape seed.
***N*-methyl AMPA:** Major metabolite in soybean seed.
***N*-acetyl AMPA:** Major in rape seed, soybean seed.
***N*-glyceryl AMPA:** Major in maize grain, rape seed, soybean seed.
***N*-malonyl AMPA:** Major in soybean seed.

GAT modification

Three metabolism studies are available investigating the fate and nature of glyphosate-derived residues in the crop categories **cereals and pulses and oilseeds with maize, rape and soybean including a GAT modification**. An overview on the application scenarios and application rates used is given in the following table.

Table 6.2.1.181: Overview over available plant metabolism studies

Plant	Application	Application rate	Reference
GAT modification			
Maize/corn	Pre-emergence application	*N*-(phosphono-^{14}C-methyl)glycine at 4.3 kg a.s/ha and three foliar applications each at 1.1 kg a.s/ha	CA 6.2.1/024: [redacted] 2007, The metabolism of [^{14}C]Glyphosate in Optimum GAT (Event DP- Ø9814Ø-6) field corn, DuPont-19529, ASB2008-2657

Table 6.2.1-181: Overview over available plant metabolism studies

Plant	Application	Application rate	Reference
Rape/canola	Soil pre-emergent application followed by 4 foliar applications	*N*-(phosphono-^{14}C-methyl)glycine At 4.50 kg a.s./ha (soil) followed by foliar three foliar applications at 0.94 to 1.03 kg a.s./ha	CA 6.2.1/025: [redacted] 2010, The metabolism of [14C]Glyphosate in 0827 canola, DuPont-26109, ASB2011-13744
Soybean	Soil pre-emergent application followed by 3 foliar applications	*N*-(phosphono-^{14}C-methyl)glycine at 3.290 kg a.s./ha (soil) (pre-emergent) followed by foliar three foliar applications at 1.410 kg a.s./ha (unifoliolate and seven trifoliolate leaves are fully developed), 2.284 kg a.s./ha (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf) and 0.880 to kg a.s./ha (one normal pod on the main stem that has reached its mature pod color))	CA 6.2.1/026: [redacted] A. M. G., 2007, The metabolism of [^{14}C]Glyphosate in GAT/GM-HRA (DP-356Ø43-5, PHP20163a) soybeans, DuPont-19530, ASB2008-2658

The magnitude and nature of the residues in maize plants modified to express the glyphosate *N*-acetyltransferase (gat) gene were examined following a single pre-emergence application and three foliar applications of *N*-(phosphono-^{14}C-methyl)glycine ([redacted] **2007**).
TRR in the immature foliage was low accounting for 0.022 mg/kg, in forage accounting for 3.476 mg/kg. At maturity, the majority of the TRR was present in stover (12.242 mg/kg) with 0.686 mg/kg in cobs and 0.275 mg/kg in grain.
The major component in forage was **glyphosate** (58.0 % of the TRR) with ***N*-acetyl glyphosate** present as major metabolite at 27.0 % of the TRR. **AMPA** and ***N*-acetyl AMPA** comprised 4.0 % and 1.7 % of the TRR respectively.
The major component of stover was **glyphosate** (74.9 % of the TRR) and ***N*-acetylglyphosate** was the most abundant metabolite (17.8 % of the TRR). The metabolites **AMPA** and ***N*-acetyl AMPA** were also detected but at much lower levels (3.4 % and 1.3 % of the TRR, respectively).
The major component of cobs and grain was ***N*-acetyl glyphosate** which comprised 63.8 % and 51.2 % of the TRR, respectively. ***N*-acetyl AMPA** was present as minor metabolite at 5.0 % and 9.4 % of the TRR respectively. **AMPA** and **glyphosate** were detected in grain at lower concentrations, 6.1 % and 0.1 % of the TRR, respectively.

A second metabolism of *N*-(phosphono-^{14}C-methyl)glycine was performed in rape modified to express the glyphosate *N*-acetyltransferase (gat) gene following a single pre-emergent soil application and three foliar applications ([redacted]**, 2010**).
The TRR measured in mature seed was 2.155 mg/kg. TRR levels in foliage and immature pods (with seed) were 1.550 to 5.979 mg/kg and 1.273 mg/kg, respectively. ***N*-Acetyl glyphosate** was the principal extractable component accounting for 51.1 % of the TRR (1.101 mg/kg) in seed and 79.6 to 93.0 % of the TRR (1.013 - 5.351 mg/kg) in the foliage and pod samples. At final harvest 7 days after the last foliar application increased levels of **glyphosate** were found at 20.8 % of the TRR (0.448 mg/kg) compared to levels seen in earlier harvests (3.0 % of the TRR, 0.179 mg/kg in immature foliage) taken after soil followed by two foliar applications while glyphosate was not detected in rape pod and foliage taken at the same sampling event. The other major metabolite was ***N*-acetyl AMPA**, accounting for 14.7 % of the TRR (0.316 mg/kg) in seed and 3.4 % of the TRR (0.203 mg/kg) in foliage sample. **AMPA** was detected as minor metabolite in the seed (1.9 % of the TRR, 0.041 mg/kg) and foliage (1.4 % of the TRR,

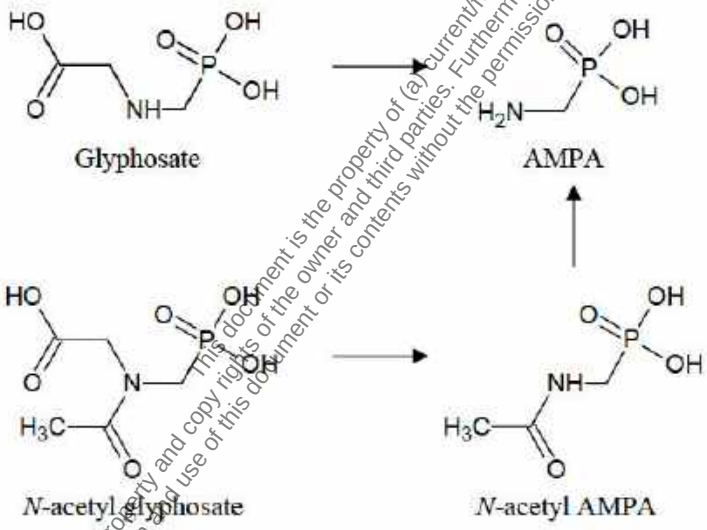
0.084 mg/kg), however [^{14}C]AMPA was also present in the treatment solutions at low concentrations (ca 3-5 %).

An additional metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues after treatments of soybean plants genetically modified to express the glyphosate *N*-acetyltransferase (gat) gene [redacted] 2007). The nature of residues resulting from soil uptake was investigated by a pre emergence application to soil followed by three foliar applications.
In forage (after soil treatment), the major metabolite was identified as **AMPA** (39.3 % of the TRR, 0.166 mg/kg) with high radioactive residues as **glyphosate** (9.1 % of the TRR, 0.039 mg/kg).
In hay (after soil treatment followed by one foliar application), glyphosate accounted for the main part of radioactive residues (72.5 % of the TRR, 9.740 mg/kg), while ***N*-acetyl glyphosate** was found as major metabolite (19.2 % of the TRR, 2.581 mg/kg). In addition, **AMPA** and ***N*-acetyl AMPA** were identified (0.7 to 5.3 % of the TRR, 0.096 to 0.704 mg/kg).
In grain (after soil treatment followed by two foliar applications) and grain, pod and foliage (after soil treatment followed by three foliar applications) ***N*-acetyl glyphosate** was the predominant metabolite (27.7 to 60.6 % of the TRR, 1.156 to 7.039 mg/kg) while **AMPA** accounted for 5.3 to 11.2 % of the TRR (0.103 to 2.25 mg/kg). ***N*-acetyl AMPA** accounted for 23.5 % of the TRR in grain at final harvest (0.738 mg/kg) and ranged between 1.4 and 3.3 % of the TRR (0.255 to 0.574 mg/kg) in foliage (after soil treatment followed by two foliar applications), pod and foliage (after soil treatment followed by three foliar applications).

Overall conclusion on cereals and oilseeds with GAT modification

In GAT modified maize, rape and soybean the metabolism of glyphosate was comparable. ***N*-acetyl glyphosate** was the predominant metabolite in almost all matrices (except soybean forage). In addition, ***N*-acetyl AMPA** and **AMPA** were relevant metabolites present in matrices of all three crops.

Pathway for cereals and oilseeds with GAT modification



AMPA: Major metabolite soybean forage, pods, foliage and grain.
***N*-acetyl AMPA:** Major metabolite in rape seed; soybean grain.
***N*-acetyl glyphosate:** Major metabolite in maize forage, stover, cob and grain; rape seed, foliage, and pod; soybean hay, grain, pod and foliage.

CA 6.2.2 Poultry

In total four studies on laying hens were conducted; one study was conducted using *N*-(phosphono-^{14}C-methyl)glycine, one study was conducted with a 9:1 mixture of *N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid, one study was conducted using *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt, and finally one study was conducted using *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine.

In the following the different metabolism studies on laying hen are summarised as full OECD summaries and are assessed again by the applicant.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.2/001
Report author	[redacted]
Report year	1994
Report title	(^{14}C)-Glyphosate: Distribution, metabolism and excretion following repeated oral administration to the laying hen
Report No	676/8-1011
Document No	276 GLY
Guidelines followed in study	EPA nature of the residues in livestock (171-4)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • Only five animals dosed per group • The radioactivity balance is 80.34 % for Group A and 65.23 % for Group B (sacrificed ca. 23.5 h and ca. 1 h after the final dose, respectively; GIT and its contents and carcasses were not measured) • Excreta were collected only once daily • The application period was seven days for Group A and five days for Group B; A plateau concentration was reached in egg white after ca. five days of dosing, but plateau levels were not achieved in egg yolk by day 7 (metabolites were investigated for Group B which was dosed for 5 days) • The radioactivity was not quantified separately in the different muscle types • Radioactive residues in muscle were below the limit of detection for Group A, but this limit accounted for 0.043 ppm equivalents; for Group B, radioactive residues of 0.041 ppm equivalents were determined and further analysed • Extractability of radioactive residues not reported in detail (multi-stage extraction procedure), recovery of radioactivity in the further investigated fractions after extraction was only moderate to low, and the organic phases (chloroform) and the residues after solvent extraction were not further measured [or not reported] and examined (it has been assumed in the report that the final extracts represented the residue in the original samples) • Evaluation of residues in "% Total", which means "percent of total area detected in analysed sample by chromatographic analysis"

	instead of "% TRR", is unusual (re-calculation was possible upon dossier compilation) • For egg white (plateau concentration of approximately 0.049 mg eq/kg in Group A, 0.056 mg eq/kg on the investigated Day 4 sample of Group B), total radioactive residues could be determined, but the levels of radioactivity recovered after the multi-stage extraction procedure (Group B, extraction efficiency only approximately 14 %) were too low to quantify by HPLC, while TLC indicated the presence of glyphosate • Duration of sample storage for excreta, liver and skin was 205 days until end of analytical phase; Note: Analysis of extracts showed that glyphosate was the major residue and thus degradation during storage was negligible
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The absorption, distribution, metabolism and excretion of radioactive residues have been studied following repeated oral administration of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) to laying hens (two groups of five animals each) once daily for seven (Group A) or five consecutive days (Group B), respectively. The nominal dose level was 200 mg ^{14}C-labelled glyphosate per kg feed consumed, and the actual daily dose levels were 29.57 mg/animal and 29.77 mg/animal (Group A and Group B, corresponding to 17.9 mg/kg bw and day and 17.2 mg/kg bw and day, respectively). One hen was kept as control without dosing the test substance (Group C). Animals of Group A were sacrificed at ca. 23.5 h after the final dose, and hens of Group B were sacrificed at plasma radioactivity c_{max} ca. 1 h after the last dosing.

Approximately 80 % of the administered dose was recovered in the case of Group A in total, and approximately 65 % was recovered in the case of Group B. The main part was rapidly excreted (63.65 – 76.45 % of the dose recovered in excreta, 0.81 – 2.98 % of the dose in cage washings and 0.75 – 0.88 % of the dose in cage debris). Radioactive residues associated with edible matrices (egg white, egg yolk and tissues) accounted in sum for less than 0.04 % of the administered dose for both groups.

Of the relevant edible matrices of the laying hens, highest total radioactive residues (TRR) were found in liver (1.242 mg eq/kg for Group A and 1.080 mg/kg for Group B). In skin (including subcutaneous fat), total radioactive residues of 0.212 mg/kg and 0.359 mg/kg were measured (Group A and Group B, respectively), while residue concentrations in peritoneal fat accounted for 0.153 mg/kg and 0.083 mg/kg (Group A and Group B, respectively). In skeletal muscle, residue levels of <0.043 mg/kg (below detection limit) and 0.041 mg/kg were found (Group A and Group B, respectively). The mean concentration of radioactive residues in egg white reached a plateau on day 5 of dosing (ca. 0.049 mg eq/kg), whilst levels in egg yolk increased up to day 7 (0.484 mg/kg, Group A). In the case of Group B, mean concentrations of radioactive residues in egg white (0.072 mg/kg) and egg yolk (0.228 mg/kg) were highest on day 5. The mean levels of radioactivity in plasma collected from hens of Group B peaked (0.475 mg eq/kg) at the first sampling interval (1 h post initial dose).

Excreta and edible matrices (tissues and eggs) of animals of Group B were extracted with 0.1 M HCl/chloroform followed by two ion exchange column chromatography steps for the aqueous phase

(multi-stage extraction procedure). Portions of ca. 44 – 100 % of the TRR were recovered in the final extracts of excreta, liver, skin, fat, muscle and egg yolk, and ca. 14 % TRR was recovered in the final extract of egg white. Quantification of the extraction efficiency was difficult due to the low levels of radioactivity present. It has been assumed in the report that losses incurred during the extraction procedure were not associated with one or more specific components and the final extracts represented the residues in the original samples. The remaining non-extractable residues were not further examined.
The major residue in the extracts of all matrices was unchanged glyphosate (approximately 61 - 99 % of the total area detected in the analysed samples ("% Total") according to HPLC data, absolute concentrations: 0.663 mg eq/kg in liver (HPLC; TLC: up to 0.966 mg eq/kg), up to 0.354 mg eq/kg in skin, up to 0.082 mg eq/kg in fat extracts, up to 0.040 mg eq/kg in muscle, up to 0.158 mg eq/kg in egg yolk). Indications for the occurrence of the metabolite AMPA from minor TLC regions (up to 14 % Total) in the extracts of excreta, liver and skin or of unknown components in the extracts of skin, fat and egg yolk were not substantiated by HPLC and therefore supposed to be chromatographic artefacts. The concentration of radioactive residues in the final extract of egg white was below the level of detection following HPLC analysis, and TLC analyses of this extract yielded only one radioactive region, which corresponded to the glyphosate standard. The results of the chromatographic analyses suggested that orally administered glyphosate was not substantially metabolised prior to elimination.

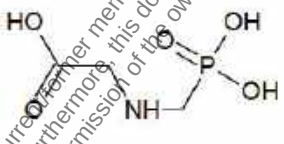
I. Materials and methods

A. Materials

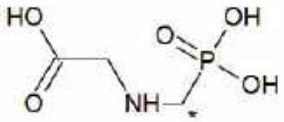
Test material

Chemical structure:

a) N-(phosphonomethyl)glycine (unlabelled)
Batch 206-JaK-25-1, chemical purity 97.5 %



b) N-(phosphono-^{14}C-methyl)glycine,
glyphosate (C-1, labelled), batch CFA 745 C6
and batch CFA 745 C8, solid



* Position of the radio label

Radiochemical purity: >97 % (confirmed by reanalysis);
purity in the aqueous formulation was also >97 %

Specific activity: Batch 1 12.3 MBq/mg (2.11 GBq/mmol)
Batch 2 12.3 MBq/mg (2.11 GBq/mmol)
Batch 3 12.1 MBq/mg (2.07 GBq/mmol),
all supplied as aqueous solutions

CAS-No.: 1071-83-6

Log $P_{o/w}$: -3.4 ± 0.1

Test animals:

Species:	Hen, *Gallus gallus*
Strain:	ISA strain
Breeding facility:	Not reported
Gender and numbers involved:	Female, 11 animals (5 treatment Group A, 5 treatment Group B, 1 control animal), identified by cage labels (coloured according to dose level) and uniquely numbered by means of colour coded leg markings
Body weight:	1.52 ± 0.21 kg on arrival, 1.65 ± 0.17 kg for Group A and 1.73 ± 0.12 kg for Group B (acclimatisation)
Age:	20 – 22 weeks
Location of the in-life phase:	Hazleton Europe, OtleyRoad, Harrogate, North Yorkshire, United Kingdom, HG3 1PY
Acclimatisation:	Approximately 7 days prior to first treatment in individual stainless steel metabolism cages suitable for the separate collection of excreta and eggs
Housing:	Individually housed in metabolism cages in an experimental room with fluorescent lighting at a 10.5/13.5 hours light/dark cycle Temperature: 12 – 24 °C, Humidity: 40 – 80 % (Group A: 90 % on one day), ≥10 air changes/h
Feed and water:	RS 11/18 % Layers Meal (Fridaythorpe Feeds Ltd, Fridaythorpe, Driffield, York, United Kingdom) containing grit (Fringhill Mill Farm Supplies, Darley, North Yorkshire, United Kingdom) (ca. 150 g/day) and mains water *ad libitum*

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	Nominal dose level of 200 mg/kg feed; The radiolabelled glyphosate was diluted with non-radiolabelled glyphosate; daily doses administered: Group A: mean of 0.738 MBq/animal or 29.57 mg/animal, Group B: mean of 3.978 MBq/animal or 29.77 mg/animal; Calculated using the body weights during acclimatisation: Group A: mean of 17.9 mg equiv./kg bw and day Group B: mean of 17.2 mg equiv./kg bw and day
Feed consumption:	Actual feed consumption was not reported
Vehicle:	Water
Timing:	Once daily (by gavage)

Five laying hens were dosed orally with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) once a day for seven consecutive days (Group A), and five further hens were treated once a day for five consecutive days (Group B, higher radioactivity) in the same manner. The target dose level was 200 mg/kg feed, based on a daily diet consumption of approximately 150 g/day. Actual daily dose levels of approximately 30 mg/animal were administered, corresponding to 17.9 mg equiv./kg bw and day and 17.2 mg equiv./kg bw and day for Group A and Group B, respectively. The test item was administered in the morning as a solution in water by oral gavage. The dosing apparatus was flushed with vehicle (water) to expel any residual dose into the animal. A control animal (Group C) was not dosed.
Animals were observed twice daily for mortality and morbidity. Body weights were recorded on arrival, during acclimatisation, on the first day of dosing (Group A only) and at necropsy.

2. Sampling and storage

Excreta were collected at time intervals of approximately 24 hours after initiation of dosing until termination (sampling prior to the next dosing). Eggs were collected twice daily, prior to dosing and approximately 3-6 h after each dose (or 1 h on the day of sacrifice). Daily egg samples were pooled for each animal (egg yolk and egg white separately). In the case of Group B, blood samples were collected from a wing vein at several intervals after the initial dose (1, 2, 3, 4, 6, 8 and 12 h post-dose). Blood was transferred into tubes containing lithium heparin and centrifuged to collect plasma. At the end of the collection period cages were rinsed thoroughly with water and then methanol.
Hens were sacrificed by cervical dislocation at ca. 23.5 h post-final dose for Group A or at plasma radioactivity c_{max} (ca. 1 h after the final dose) for Group B, respectively. At termination, the edible organs and tissues skeletal muscle (maximum amounts of breast and thigh, pooled by animal), fat (maximum amounts of peritoneal), liver and skin (including subcutaneous fat) were collected, macerated and sub-sampled at dissection prior to storage at ca. -20 °C. Eggs were kept refrigerated and subsequently homogenised (yolk) or macerated (white) prior to radioanalysis and storage at ca. -20 °C. Excreta were refrigerated prior to analysis and storage at approximately -20 °C.

3. Analytical procedures

The radioactive residues in samples of excreta, egg white, egg yolk, cage washings, macerated tissues and plasma were determined by combustion and/or liquid scintillation counting (LSC). Excreta and cage debris were homogenised in a minimum volume of water prior to combustion.
Excreta, egg white and egg yolk collected on day 4 and tissues collected/sampled at necropsy from animals of Group B were examined for ^{14}C-glyphosate and potential radiolabelled metabolites.
Radioactive residues were extracted from each matrix after addition of chloroform and 0.1 M HCl using a homogeniser. Samples were centrifuged and the aqueous phase of the supernatant retained and the radioactive residues in the aqueous phase were assessed. Each extract was adjusted to pH 2 (± 0.4) with 0.2 M HCl and transferred to a glass column for extraction using a chelating ion exchange resin (Fe(III)-Chelex 100). After washing with water, 0.2 M HCl and two small portions of 6 M HCl, the radioactive residues were eluted from the resin with 6 M HCl and the collected eluate adjusted to approximately 10 M HCl. The eluate was then transferred to a further glass column for extraction using a strong anion exchange resin (AG 1-X8, pre-rinsed with 6 M HCl). The sample was immediately eluted from the column using 6 M HCl. Extracts obtained after this multi-stage extraction procedure were evaporated to dryness (<40 °C), reconstituted in water, passed through a filter (0.45 µm) and submitted to chromatographic analysis.
Reversed-phase HPLC was performed on a Lichrosorb RP-18 column with on-line radiodetection and fluorescence detection. The samples were derivatised with 9-fluorenylmethyl chloroformate (FMOC) reagent prior to analysis. TLC was performed on cellulose plates using a developing solvent of methanol : water (solvent system 1) or ethanol : trichloroacetic acid : ammonium hydroxide : acetic acid (TLC system 2). After development, bands were visualised by autoradiography and ninhydrin spray reagent and quantified, where appropriate, using a radio-TLC linear analyser.
Glyphosate and aminomethylphosphonic acid (AMPA) were used as authentic reference items.
The radioactive residues in extracts of liver, skin and excreta (72 – 97 h) from Group B animals were isolated by semi-preparative TLC (system 1). The main radioactivity region was scraped from the plate

and extracted into methanol. The isolated residue was analysed by FT-IR using glyphosate and AMPA (solutions in methanol) as standards.

II. Results and discussion

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in the table below. In total, approximately 80 % of the administered dose was recovered in the case of Group A (study termination ca. 23.5 h after the final dose), and approximately 65 % was recovered in the case of Group B (study termination ca. 1 h after the final dose). The main part was excreted, accounting in sum for 80.32 % and 65.21 % of the dose (Group A and Group B, respectively; excreta plus cage washings and cage debris).
Radioactive residues associated with edible portions (egg white, egg yolk and tissues) accounted in sum for less than 0.04 % of the administered dose in both groups (including skin, fat, muscle, liver and eggs).

Table 6.2.2-1: Total recovered radioactivity following repeated oral administration of ^{14}C-glyphosate to laying hens at a nominal dose level of 200 mg/kg feed

Matrix / tissue	% dose (mean of treatment group)[1]	
	Group A (*17.9 mg/kg bw*, 7 days)[2]	**Group B (*17.2 mg/kg bw*, 5 days)[2]**
Excreta	76.45	63.65
Egg white	<0.01	<0.01
Egg yolk	<0.01	<0.01
Cage washings	2.98	0.81
Cage debris	0.88	0.75
Tissues	0.02	0.02
Total	80.34	65.23

Values in *italics* were calculated upon dossier compilation
[1] % dose = Percent of administered radioactivity (% AR)
(mean values Group A: 0.738 MBq/animal, Group B: 3.978 MBq ^{14}C-glyphosate/animal)
[2] Calculated using the mean weights of the test item administered (Group A: 29.57 mg/animal, Group B: 29.77 mg/animal) and the body weights recorded during acclimatisation (Group A: 1.65 kg, Group B: 1.73 kg)

In the table below the total radioactive residues (TRR) are summarised for samples of laying hens following administration of 200 mg ^{14}C-glyphosate (C-1 label) per kg feed once a day for seven consecutive days (Group A, corresponding to 17.9 mg equiv./kg bw and day) or for five days (Group B, corresponding to 17.2 mg eq/kg bw and day), respectively. TRRs are expressed as glyphosate equivalents. Highest TRR values were found in liver (1.242 mg eq/kg for Group A and 1.080 mg/kg for Group B). In skin (including subcutaneous fat), total radioactive residues of 0.212 mg/kg and 0.359 mg/kg were found (Group A and Group B, respectively), while residue concentrations in peritoneal fat accounted for 0.153 mg/kg and 0.083 mg/kg (Group A and Group B, respectively). In skeletal muscle, residue levels of <0.043 mg/kg (below detection limit) and 0.041 mg/kg were measured (Group A and Group B, respectively).
Eggs were separately analysed for radioactive residues in egg white and egg yolk. In the case of Group A, mean concentration of radioactive residues in egg white reached a plateau (ca. 0.049 mg eq/kg) on day 5 of dosing, whilst levels in egg yolk increased up to day 7 (0.484 mg/kg). In the case of Group B, mean concentrations of radioactive residues in egg white (0.072 mg/kg) and egg yolk (0.228 mg/kg) were highest on day 5.
Following the initial dose, mean levels of radioactivity in plasma collected from hens of Group B peaked (0.475 mg eq/kg) at the first sample interval (1 h post dose) and slowly declined thereafter (whereby one individual animal reached maximum plasma concentration after 2 h and one animal after 3 h).

Table 6.2.2-2: Total radioactive residue (TRR) levels in tissues following repeated oral administration of ^{14}C-glyphosate to laying hens at a nominal dose level of 200 mg/kg feed

Tissue	TRR in mg eq/kg (mean of treatment group)	
	Group A (*17.9 mg/kg bw*, 7 days)	**Group B (*17.2 mg/kg bw*, 5 days)**
Skin (including subcutaneous fat)	0.212	0.359
Fat (peritoneal fat)	0.153[1]	0.083
Muscle (skeletal muscle: breast and thigh muscle pooled by animal)	<0.043 (n.d.)	0.041
Liver	1.242	1.080

Values in *italics* were calculated upon dossier compilation
[1] Mean value of 4 animals in the case of fat Group A, since value of animal 003F was not used in determination of mean due to suspected contamination
n.d. = not detected (limit of detection given in addition)

Table 6.2.2-3: Total radioactive residue (TRR) levels in egg following repeated oral administration of ^{14}C-glyphosate to laying hens at a nominal dose level of 200 mg/kg feed

Days	TRR in mg eq/kg (mean of treatment group)			
	Group A (*17.9 mg/kg bw*, 7 days)		**Group B (*17.2 mg/kg bw*, 5 days)**	
	Egg white	**Egg yolk**	**Egg white**	**Egg yolk**
1	<0.024 (n.d.)	<0.062 (n.d.)	<0.010 (n.d.)	<0.011 (n.d.)
2	0.029	<0.062 (n.d.)	0.023[1]	0.006[1]
3	0.043	0.090	0.044	0.075
4	0.038[2]	0.198[2]	0.056	0.164
5	0.049	0.318	0.072	0.228
6	0.059	0.365	-	-
7	0.053	0.484	-	-

Values in *italics* were calculated upon dossier compilation
[1] The data for the egg collected from animal 010F in the morning of Day 2 were not included in the calculation of the mean value of egg white and egg yolk Group B due to a collection error
[2] Mean value of 4 animals in the case of egg white and egg yolk Group A Day 4, since values of animal 001F were not used in determination of mean due to suspected contamination
n.d. = not detected (limit of detection given in addition)

Table 6.2.2-4: Total radioactive residue (TRR) levels in blood plasma following the initial oral administration of ^{14}C-glyphosate to laying hens (Group B) at a nominal dose level of 200 mg/kg feed

Time (hours)	TRR in mg eq/kg (mean of treatment group)
	Group B (*17.2 mg/kg bw*, 5 days)
1	0.475
2	0.402
3	0.321
4	0.187
6	0.172
8	0.099
12	0.050

Values in *italics* were calculated upon dossier compilation

B. Extraction and characterisation of residues

Excreta and edible matrices (tissues and eggs) of animals of Group B were extracted with 0.1 M HCl/chloroform followed by two ion exchange column chromatography steps for the aqueous phase. Results of the acidified aqueous extraction and of the entire multi-stage extraction procedure are summarised in the table below. Portions of >63 %, >72 %, >100 %, >75 %, 43.7 %, >52 % and ca. 14 % of the TRR were extractable in excreta (72 – 97 h), liver, skin, fat, muscle, egg yolk (72 – 97 h) and egg white (72 – 97 h), respectively, by the multi-stage extraction procedure. Quantification of the extraction efficiency was difficult due to the low levels of radioactivity present. It has been assumed in the report that losses incurred during the extraction procedure were not associated with one or more specific components and the final extracts represented the residues in the original samples. The remaining non-extractable residues were not further examined.

Table 6.2.2-5: Extraction of radioactive residues from excreta, egg and tissues of laying hens (Group B) following repeated oral administration of ^{14}C-glyphosate at a nominal dose level of 200 mg/kg feed

Matrix / tissue	Extraction efficiency[1]	
	Acidified aqueous extraction	**Multi-stage extraction procedure**
Excreta (72 – 97 h)	not reported	>63 % (spiked control excreta: 57 %)
Liver	not reported	>72 % TRR (0.778 mg eq/kg)
Skin	not reported	>100 % TRR (0.359 mg eq/kg)
Fat	not reported	>75 % TRR (0.062 mg eq/kg)
Muscle	ca. 81 % (0.033 mg eq/kg)	43.7 % TRR (*0.018 mg eq/kg*)
Egg yolk (72 – 97 h)	not reported	>52 % TRR (0.085 mg eq/kg)
Egg white (72 – 97 h)	>69 % (0.039 mg eq/kg)	ca. 14 % TRR (ca. *0.008 mg eq/kg*)[2]

Values in *italics* were calculated upon dossier compilation

[1] Multi-stage complex extraction procedure starting with extraction with chloroform and 0.1 M HCl (further workup of the aqueous phase); Quantification of the extraction efficiency was difficult due to the low levels of radioactivity present; It has been assumed in the report that losses incurred during the extraction procedure were not associated with one or more specific components and the final extract represented the residue in the original sample

[2] Following HPLC analysis, the radioactive residue in egg white was below detection limits and could thus not be quantified; TLC analysis identified one region of radioactivity corresponding to the glyphosate standard

Aliquots of the concentrated extracts prepared by the multi-stage extraction procedure were derivatised with FMOC reagent and analysed by HPLC. Further aliquots of the extracts were analysed by TLC using two solvent systems. The results of the chromatographic analyses are summarised in the table below, and the concentrations of the components of the radioactive residues in mg eq/kg are calculated in a table below.

In the extract of excreta after the multi-stage extraction procedure, portions of more than 95 % of the total area detected in the analysed samples ("% Total", corresponding to "% of the TRR" under the assumption of the report that the final extract represented the residues in the original sample (no specific components lost); results of calculations of % TRR values for all matrices taking into consideration the extraction efficiencies are provided in the right column of the table below) were identified as glyphosate using three different analytical methods (HPLC and TLC) and comparison with the reference item. Evidence for the occurrence of the metabolite AMPA from minor TLC regions after developing with solvent system 1 and solvent system 2 (approximately 2 % or 1 % Total, respectively) was supposed to be a likely artefact of the chromatographic procedure as HPLC data supporting this assignment were lacking. In the case of liver, ca. 84 – 89 % Total were assigned to glyphosate by TLC (system 2 and system 1, respectively), and ca. 61 % Total were identified as glyphosate by HPLC (one further single unknown HPLC peak representing 37 % Total did not correspond to AMPA standard; since the existence of this unknown

region was not confirmed by TLC, it might have been a chromatography artefact). In the extracts of skin, fat, muscle and egg yolk after the multi-stage extraction procedure, portions of >96 % Total were identified as glyphosate by HPLC, and the presence of glyphosate was confirmed by both TLC systems (comparison with reference items). Portions of radioactive residues in the extracts of skin, fat and egg yolk designated as unknown components according to TLC analysis were located at the origin, most likely resulting from non-specific binding (e. g. due to disturbed cellulose sorbent layer; this effect was substantiated in the case of skin by over-laying sample extracts with cold glyphosate standard prior to developing in solvent system 2, which significantly reduced the binding). As for the excreta extract, the assignment of minor portions of radioactive residues in the extracts of liver (10 – 14 % Total) and skin (approximately 1 % Total) as AMPA according to TLC was not substantiated by HPLC. In the case of the egg white extract, the radioactive residue was below detection limits of HPLC analysis and thus could not be quantified. TLC analysis of the egg white extract identified one major region of radioactivity corresponding to the glyphosate standard. In the cases of the extracts of liver, skin and excreta, the identification of glyphosate was confirmed by the FT-IR spectra of isolated main regions from semi-preparative TLC (system 1) in comparison with those of the respective reference item.

The concentrations of glyphosate calculated using the "% Total" values accounted for 0.663 mg eq/kg in the extracts of liver (TLC: up to 0.966 mg eq/kg), 0.354 mg eq/kg in skin, 0.082 mg eq/kg in fat, 0.040 mg eq/kg in muscle and 0.158 mg eq/kg in egg yolk (worst-case calculations from the HPLC results without considering the extraction efficiency, reflecting the assumption of the report that losses during extraction were not specific to particular metabolites and the final extracts represented the residues in the initial samples).

Table 6.2.2-6: Identification of radioactive residues in excreta, liver, skin, fat, muscle and egg yolk following repeated oral administration of ^{14}C-glyphosate to laying hens (Group B) at a nominal dose level of 200 mg/kg feed

Sample / analysis	% Total[1]				% TRR[1]
	Glyphosate	AMPA	Unknown	Total (allocated)	Total (recovered and allocated)
Excreta (72 – 97 h), radio-HPLC	95.07	-	-	95.07	*59.89*
Excreta (72 – 97 h), TLC system 1	97.19	2.34	-	99.53	*62.70*
Excreta (72 – 97 h), TLC system 2	98.44	1.06	-	99.51	*62.69*
Liver, radio-HPLC	61.38	-	36.80[2]	98.18	*70.69*
Liver, TLC system 1	89.41	9.94	-	99.35	*71.53*
Liver, TLC system 2	83.72	14.26	-	97.98	*70.55*
Skin, radio-HPLC	98.65	-	-	98.65	*98.65*
Skin, TLC system 1	95.34	0.79	3.23[3]	99.36	*99.36*
Skin, TLC system 2, sample + reference item[4]	78.78	1.76	19.08[3]	99.62	*99.62*
Skin, TLC system 2, pure sample	56.84	1.48	41.35[3]	99.68	*99.68*
Fat, radio-HPLC	98.94	-	-	98.94	*74.21*
Fat, TLC system 1	62.21	-	34.86[3]	97.07	*72.80*
Fat, TLC system 2	66.55	-	31.69[3]	98.24	*73.68*
Muscle, radio-HPLC	97.81	-	-	97.81	*42.74*
Muscle, TLC system 1	97.91	-	-	97.91	*42.79*
Muscle, TLC system 2	45.15	-	-	45.15	*19.73*
Egg yolk (72 – 97 h), radio-HPLC	96.13	-	-	96.13	*49.99*
Egg yolk (72 – 97 h), TLC system 1	72.60	-	23.83[3]	96.43	*50.14*
Egg yolk (72 – 97 h), TLC system 2	24.23	-	72.93[3]	97.16	*50.52*

Egg white (72 – 97 h), radio-HPLC	Radioactive residue below detection limits				
Egg white (72 – 97 h), TLC system 1	100	-	-	100	*14*
Egg white (72 – 97 h), TLC system 2	100	-	-	100	*14*

Values in *italics* (% TRR) were calculated upon dossier compilation

[1] % Total = percent of total area detected in analysed sample by chromatographic analysis (radiodetection):
"% Total" = "% ROI" – "% Unallocated" (because Total Area = Region Of Interest + Unallocated);
The values in "% TRR" of the initial matrix sample could be calculated for the actually measured analytical sample (regarding the recovery after the multi- stage extraction procedure, given in Table 6.2.2-5) as
"% Total" x "% extraction efficiency" ÷ 100;
for instance, 97.81 % Total in muscle (radio-HPLC) x 43.7 % recovery after extraction ÷ 100 = 42.74 % TRR actually measured in extract sample after extraction and sample preparation for chromatographic analysis,
61.38 % Total for glyphosate and 36.80 % Total for unknown peak in liver (radio-HPLC) x 72 % recovery after extraction ÷ 100 = 44.19 % TRR for glyphosate and 26.50 % TRR for unknown peak,
89.41 % Total for glyphosate and 9.94 % Total for AMPA in liver (TLC system 1) x 72 % recovery after extraction ÷ 100 = 64.38 % TRR for glyphosate and 7.16 % TRR for AMPA,
83.72 % Total for glyphosate and 14.26 % Total for AMPA in liver (TLC system 2) x 72 % recovery after extraction ÷ 100 = 60.28 % TRR for glyphosate and 10.27 % TRR for AMPA actually measured in extract sample
skin: recovery after extraction >100 %, therefore calculated % TRR = % Total;
for the respective values calculated in mg eq/kg see subsequent Table 6.2.2-7

[2] One single unknown HPLC peak eluting after approximately 3.3 minutes, not corresponding to AMPA standard;
The existence of this unknown region was not confirmed by TLC, suggesting it may be a chromatography artefact

[3] One single unknown band located at the origin of the TLC plate (R_f near 0; most likely resulting from non-specific binding, therefore not calculated in % TRR individually)

[4] Sample extract and unlabeled reference items spotted on the same locations, which reduced non-specific binding

Table 6.2.2-7: Calculated concentrations of components of the radioactive residues in liver, skin, fat, muscle and egg yolk following repeated oral administration of ^{14}C-glyphosate to laying hens (Group B) at a nominal dose level of 200 mg/kg feed

Tissue / analysis	**Concentration in mg eq/kg[1]**	
	Glyphosate	**AMPA**
Liver, radio-HPLC	*0.663*	not detected[2]
Liver, TLC system 1	*0.966*	*0.107*
Liver, TLC system 2	*0.904*	*0.154*
Skin, radio-HPLC	*0.354*	not detected
Skin, TLC system 1[3]	*0.342*	*0.003*
Skin, TLC system 2, sample + reference item[4]	*0.283*	*0.006*
Skin, TLC system 2, pure sample[3]	*0.204*	*0.005*
Fat, radio-HPLC	*0.082*	not detected
Fat, TLC system 1[3]	*0.052*	not detected
Fat, TLC system 2[3]	*0.055*	not detected
Muscle, radio-HPLC	*0.040*	not detected
Muscle, TLC system 1	*0.040*	not detected
Muscle, TLC system 2	*0.019*	not detected

Table 6.2.2-7: Calculated concentrations of components of the radioactive residues in liver, skin, fat, muscle and egg yolk following repeated oral administration of ^{14}C-glyphosate to laying hens (Group B) at a nominal dose level of 200 mg/kg feed

Tissue / analysis	Concentration in mg eq/kg[1]	
	Glyphosate	AMPA
Egg yolk (72 – 97 h), radio-HPLC	*0.158*	not detected
Egg yolk (72 – 97 h), TLC system 1[3]	*0.119*	not detected
Egg yolk (72 – 97 h), TLC system 2[3]	*0.040*	not detected

All values (in *italics*) were calculated upon dossier compilation

[1] Calculated using the TRR in the respective tissue and egg samples of laying hens of Group B (given in Table 6.2.2-2 and Table 6.2.2-3, respectively) and the portion of the component in the analysed sample in % Total (given in Table 6.2.2-6);
Since calculation with the % TRR values (right column or footnote 1 in Table 6.2.2-6) would lead to lower mg/kg values
(e.g. 0.763 mg eq/kg total (recovered and allocated) or 0.477 mg eq/kg glyphosate in liver, 0.062 mg eq/kg glyphosate in fat, 0.018 mg eq/kg glyphosate in muscle and 0.082 mg eq/kg glyphosate in egg yolk (Day 4) according to radio-HPLC), the given values represent a worst-case calculation and reflect the assumption of the report that losses during extraction were not specific to particular metabolites and the final extracts represented the residues in the initial samples (compare footnote in Table 6.2.2-5)

[2] In the case of liver, 0.663 mg eq/kg glyphosate and 0.397 mg eq/kg unknown (calculation with 44.19 % TRR and 26.50 % TRR according to footnote 1 in Table 6.2.2-6 would yield values of 0.477 mg eq/kg glyphosate and 0.286 mg eq/kg unknown, respectively), were calculated according to radio-HPLC analysis; The single unknown HPLC peak eluting after approximately 3.3 minutes did not correspond to AMPA standard; The existence of this unknown region was not confirmed by TLC, suggesting it may be a chromatography artefact

[3] In the cases of skin, fat and egg yolk, TLC showed in addition one single unknown band located at the origin of the TLC plate (R_f near 0; most likely resulting from non-specific binding)

[4] Sample extract and unlabeled reference items spotted on the same locations, which reduced non-specific binding

C. Storage stability

Samples of macerated tissues were stored at ca. -20 °C following dissection and sub-sampling. Eggs (yolk and white) and excreta were stored at ca. 4 °C prior to analysis and subsequent storage at ca. -20 °C. The storage intervals between sample collection and start of extraction were 59 days for excreta and 65 days for all other samples, and the storage intervals between end of analytical phase and date of sample collection were 103 days to 109 days for fat, egg yolk, egg white and muscle, and 205 days in the cases of liver, skin and excreta. Analysis of extracts showed that the parent compound glyphosate was the major component of the residue and thus degradation during storage was negligible.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) was administrated to laying hens (two groups of five animals each) once daily for seven (Group A) or five consecutive days (Group B), respectively. The target dose level was 200 mg ^{14}C-labelled glyphosate per kg feed consumed, and the actual daily dose levels were 29.57 mg/animal and 29.77 mg/animal (Group A and Group B, corresponding to 17.9 mg/kg bw and day and 17.2 mg/kg bw and day, respectively. One control animal was not dosed. Animals of Group A were sacrificed at ca. 23.5 h after the final dose, and hens of Group B were sacrificed at plasma radioactivity c_{max} ca. 1 h after the last dosing.
Approximately 80 % of the administered dose was recovered in the case of Group A in total, and approximately 65 % was recovered in the case of Group B (study termination closer to the final dose). The major portions of radioactive residues were recovered in excreta (63.65 – 76.45 % of the dose), cage washings and cage debris, and less than 0.04 % of the administered dose was associated in both groups with edible matrices (egg white, egg yolk and tissues in sum). At study termination, the highest radioactive residues in the relevant edible matrices were detected in liver (1.080 – 1.242 mg eq/kg).

The major residue in the extracts of all matrices was unchanged glyphosate (approximately 61 – 99 % of the total area detected in the analysed samples ("% Total") according to HPLC data; absolute concentrations in liver: 0.663 mg eq/kg (HPLC; TLC: up to 0.966 mg eq/kg), in skin: up to 0.354 mg eq/kg, in fat extracts: up to 0.082 mg eq/kg, in muscle: up to 0.040 mg eq/kg, in egg yolk up to 0.158 mg eq/kg; egg white: glyphosate only radioactive region in TLC of final extracts, below detection limit of HPLC). Indications for the occurrence of the metabolite AMPA from minor TLC regions (up to 14 % Total) in the extracts of excreta, liver and skin or of unknown components in the extracts of skin, fat and egg yolk were not substantiated by HPLC and therefore supposed to be chromatographic artefacts.
In summary, glyphosate orally administered to laying hens was rapidly voided in excreta (primarily as unchanged test item) resulting in low residue levels in tissues and eggs. Chromatographic analyses revealed that the residues present in eggs and tissues primarily consisted of unchanged parent compound.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in laying hens has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals 503, with major deficits (only 5 animals were dosed per group; the radioactivity balance was 65 – 80 %; extractability was only moderate to low for excreta, liver, fat, muscle and eggs, and the non-extractable residues as well as the organic phases were nor measured neither characterised / investigated; For egg white, total radioactive residues could be determined, but the levels of radioactivity recovered after the multi-stage extraction procedure were too low to quantify). The residue identification and characterisation is poor with regard to the extractability, the recovery of residues from the ion exchange columns and the fractions not further examined. However, the study contributes data on the excretion and distribution of residues, the total radioactive residues in eggs and tissues and the time course of the residue concentration in plasma, and the identified residues do not contradict the results from other livestock metabolism studies.
The study is considered to be supportive for the assessment of the metabolic behaviour of glyphosate in laying hens.

Assessment and conclusion by RMS:

Study previously submitted to the EU

2. Information on the study

Data point:	CA 6.2.2/002
Report author	[redacted]
Report year	1988
Report title	Metabolism of $^{13}C/^{14}C$ -labeled Glyphosate and Aminomethylphosphonic acid in laying hens. Part I.
Report No	[redacted]6103-112
Document No	[redacted]-7591
Guidelines followed in study	Not specified
Data point:	CA 6.2.2/003
Report author	[redacted]
Report year	1988

Report title	Metabolism of $^{14}C/^{13}C$-labeled Glyphosate and Aminomethylphosphonic acid in laying hens. Part II.
Report No	[redacted]-7420
Document No	Not available
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • Only five animals dosed per treatment group • Excreta and eggs were collected only once daily • The radioactivity was not quantified separately in the different fat types • The radioactivity balance was 83.6 %, 85.9 % and 82.4 % for test 2, 3 and 4, respectively • The application period was 7 d, after which a plateau was not certainly reached in egg yolk • No flow chart depicting the overall extraction and fractionation strategies for each sample matrix was provided • For egg yolk, relevant amounts of non-extractable residues (test 2: 0.010 mg/kg or 9.7 % TRR, test 3: 0.014 mg/kg or 15 % TRR, test 4: 0.048 mg/kg or 14.0 % TRR, test 5: 0.021 mg/kg or 18.7 % TRR) were not characterised / not investigated • No quantification of the residues as concentration (mg/kg, as active ingredient equivalents) in the original sample matrix analysed (re-calculation possible)
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

Four treatment groups with laying hens were performed to investigate the behaviour of N-(phosphono-$^{13}C/^{14}C$-methyl)glycine ($^{13}C/^{14}C$-glyphosate) and amino-$^{13}C/^{14}C$-methylphosphonic acid ($^{13}C/^{14}C$-AMPA) in poultry. One additional group was performed as a control group without dosing with test substance.

In the low treatment groups (tests 2, 3 and 5), the hens each received a dose of 120 mg/kg feed = 14.2 – 15.6 mg test mixture/day ≙ 15.0 mg $^{13}C/^{14}C$-glyphosate disodium salt and 1.6 mg $^{13}C/^{14}C$-AMPA monosodium salt per day (8.62 – 9.84 mg/kg bw/day ≙ 7.76 – 8.86 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 0.86 – 0.98 mg $^{13}C/^{14}C$-AMPA/kg bw/day), while in the high treatment group (test 4) the hens each received 400 mg/kg feed dose level = 46.0 mg test mixture/day ≙ 49.9 mg $^{13}C/^{14}C$-glyphosate disodium salt and 5.3 mg $^{13}C/^{14}C$-AMPA monosodium salt per day (29.75 mg/kg bw/day ≙ 26.78 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 2.98 mg $^{13}C/^{14}C$-AMPA/kg bw/day). One capsule was administered each for 7 days. In tests 2, 3 (replicate of treatment group 2) and 4, the hens were sacrificed 22 to 24 hours after the last dose. In test 5, a 10-day depuration phase was added after the 7th dose after which the hens were sacrificed.

Only minor amounts of the administered radioactivity were found in egg yolk (0.01 – 0.02 % AR), egg white (<0.01 % AR) and tissues (up to 0.02 % AR). Elimination of radioactivity via excreta was the primary elimination route, ranging from 81.0 to 90.5 % of AR.

Of the relevant matrices of the hen, highest total radioactive residues were found in the kidney (0.069 – 7.004 mg eq/kg), followed by liver (0.079 – 1.914 mg eq/kg) and egg yolk (0.090 – 0.344 mg eq/kg). Residues in muscle, fat and egg white were much lower, generally not exceeding 0.1 mg eq/kg.

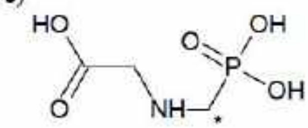
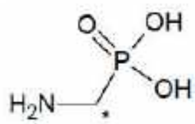
The radioactivity level in tissues of the depuration group were generally lower; the liver had the highest level (0.079 mg equiv./kg).

A plateau level in egg white was reached after approximately 6 days. A plateau level in egg yolk could not be observed. More than 81 % of TRR were extracted using chloroform and water and only low amounts of the residues remained unextractable. Generally, the majority of the residues in the tissues was extractable using water and only low amounts of radioactivity were found in the chloroform extracts, except for fat of hens of test 5.

Glyphosate and AMPA accounted for the majority of the radioactive residue in tissues. Some evidence for further metabolism of glyphosate and AMPA was observed in the muscles, where a minor unknown metabolite was detected (2.4 to 16.2 % of TRR or ≤0.005 mg equiv./kg).

I. Materials and methods

A. Materials

Test material	a) N-(phosphono-^{14}C-methyl)glycine b) N-(phosphono-^{13}C-methyl)glycine c) N-(phosphonomethyl)glycine d) Amino-^{14}C-methylphosphonic acid e) Amino-^{13}C-methylphosphonic acid
Chemical structure:	a, b, c)  d, e)  * Position of the radio label
Radiochemical purity:	≥98 %
Specific activity of the mixed test items:	a, b, c) 0.66 MBq/mg (17.7 µCi/mg = 3 mCi/mmol) and 2.19 MBq/mg (59.1 µCi/mg = 10 mCi/mmol) d, e) 1.00 MBq/mg (27.02 µCi/mg = 3 mCi/mmol) and 3.33 MBq/mg (90.1 µCi/mg = 10 mCi/mmol)
CAS No:	a, b, c) 1071-83-6 d, e) 1066-51-9
Log $P_{o/w}$:	a, b, c) -3.2 d, e) -2.47

Test animals:	
Species:	Hen, *Gallus gallus*
Strain:	White Leghorn
Breeding facility:	[redacted]
Gender and numbers involved:	Female, 25 animals, identified via numbered leg bands
Body weight:	1.546 – 1.607 kg (range of mean weight of hens per test at day 8 of acclimation)
Age:	Approx. 27 weeks
Location of the in-life phase:	[redacted]
Acclimatisation:	8 days before first treatment in their respective cages
Housing:	Individually housed in metabolic cages (28 cm x 43 cm x 38 cm) with artificial light at a 16/8 hours light/dark cycle Temperature: 19 – 24 °C, Humidity: 48 – 68 %
Feed and water:	Ralston Purina Layena, *ad libitum* and tap water, *ad libitum*

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	8.62 – 9.84 (test 2, 3 and 5) or 29.75 mg equiv./kg bw/day (test 4)
Feed consumption:	106 – 142 g/day
Vehicle:	Gelatine capsules
Timing:	Once daily
Duration:	7 days (+ 10 days depuration phase in test 5)
* Calculated based on average body weights of the hens per treatment group at day 8 of acclimation (1.585, 1.603, 1546 and 1.657 kg for test 2, test 3, test 3 and test 5, respectively), the actual dose level of 120 or 400 mg/kg feed consumed and the average feed consumption of the hens per treatment group during the testing period (130, 118, 115 and 119 g feed /day for test 2, test 3, test 3 and test 5, respectively).	

Four treatment groups, each with five laying hens were conducted with a 9:1 mixture of N-(phosphono-$^{13}C/^{14}C$-methyl)glycine ($^{13}C/^{14}C$-glyphosate) and amino-$^{13}C/^{14}C$-methylphosphonic acid ($^{13}C/^{14}C$-AMPA) to investigate their behaviour in poultry. For this, the ^{14}C-glyphosate and ^{14}C-AMPA were each diluted with the corresponding ^{13}C-enriched and unlabelled materials so as to produce a final ^{13}C enrichment of approximately 50 % with the above mentioned specific activities.
The test mixture was administered in a gelatine capsule that was deposited in the crop of each hen. One additional group was performed with five laying hens as a control group. Hens of the control group were given capsules containing dextrose powder.

Due to the low water solubility of glyphosate at neutral pH, $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA were converted to their respective sodium salt forms in order to ensure complete administration. The free acid forms of the test mixtures were neutralised to pH 7.0 with standard 5 N sodium hydroxide. At this pH, glyphosate was converted to its disodium salt and AMPA to its monosodium salt. The neutralised solutions of the test mixtures were adsorbed onto dextrose which was then filled in gelatine capsules.
The capsules used for treatment groups with a dose of 120 mg/kg feed were prepared using the 10 mCi/mmol 9:1 test mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA while the capsules used for the treatment group with a dose level of 400 mg/kg feed were prepared using the 3 mCi/mmol test mixture.
Following their preparation, the capsules were immediately frozen (-20 °C) and sent to Hazleton Laboratories (on dry ice) for the in-life part of the study where the doses in the capsules were verified. The dosing capsules were analysed to determine the actual total radioactivity in each of the dose capsules. Three capsules from each dose level were analysed by liquid scintillation counting (LSC).

In tests 1 (control group), 2, 3 (both tests are replicates with a dose level 120 mg/kg feed = 14.2 – 15.6 mg test mixture/day ≙ 15.0 mg $^{13}C/^{14}C$-glyphosate disodium salt and 1.6 mg $^{13}C/^{14}C$-AMPA monosodium salt per day or 8.83 – 9.84 mg/kg bw/day ≙ 7.95 – 8.86 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 0.88 – 0.98 mg $^{13}C/^{14}C$-AMPA/kg bw/day) and test 4 (400 mg/kg feed dose level = 46.0 mg test mixture/day ≙ 49.9 mg $^{13}C/^{14}C$-glyphosate disodium salt and 5.3 mg $^{13}C/^{14}C$-AMPA monosodium salt per day or 29.75 mg/kg bw/day ≙ 26.78 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 2.98 mg $^{13}C/^{14}C$-AMPA/kg bw/day), one capsule was administered each for 7 days. The hens were sacrificed 22 to 24 hours after the last dose using carbon dioxide. In test 5 (120 mg/kg feed or 8.62 mg/kg bw/day ≙ 7.76 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 0.86 mg $^{13}C/^{14}C$-AMPA/kg bw/day), one capsule was administered each day for 7 days. This was followed by a depuration period lasting 10 days after which the hens were sacrificed. A macroscopic examination of each hen at sacrifice was performed. Actual dose levels are summarised in the table below:

Table 6.2.2-8: Dose levels

	Test 2 (120 mg/kg feed)	Test 3 (120 mg/kg feed)	Test 4 (400 mg/kg feed)	Test 5 (120 mg/kg feed)
mg equiv./kg bw/day (glyphosate/AMPA)	9.84 (8.86/0.98)	8.83 (7.95/0.88)	29.75 (26.78/2.98)	8.62 (7.76/0.86)
Average body weight (kg)	1.585	1.603	1.546	1.657

Dose levels were calculated using average body weights of the hens per treatment group at day 8 of acclimation and an average feed consumption of the hens per treatment group during the testing period (130, 118, 115 and 119 g feed/day for test 2, test 3, test 3 and test 5, respectively).

Animals were observed twice daily for mortality and moribundity as well as once daily for general appearance and behaviour. Body weights were recorded on days 2, 6, 7 and 8 of acclimation and on days 8 and 17 of the test period. Feed consumption and egg production were recorded daily.
Excreta produced by each group were collected and pooled daily. At the end of the study, the surface was rinsed using 1 % trisodium phosphate. Egg yolk and egg white were collected daily, separated and pooled within each treatment group. Weights of cage rinse and eggs were recorded.
Hens of tests 1 to 4 were sacrificed 22 to 24 hours after last dosing and hens of test 5 were sacrificed 10 days after last dosing with the test material using carbon dioxide. A macroscopic examination of each hen was performed.

2. Sampling and storage

Blood and the following tissues and organs were collected from each animal at sacrifice: kidney (both), liver (without gallbladder), thigh and breast muscle, abdominal fat, ovaries, whole blood (heparinised), gizzard (lining removed) and the remaining gastrointestinal tract from glandular stomach to rectum (with contents). All samples were pooled separately by group.
Blood was stored refrigerated until after determination of radioactivity in the samples and was then stored below 0 °C. All other samples were stored below 0 °C at the site of the in-life part (Hazleton Laboratories). After determination of radioactivity in the samples, they were sent to Monsanto Co. (site of analysis) on dry ice via overnight freight. The samples were stored at -20 °C at Monsanto Co.

3. Analytical procedures

The total ^{14}C-activity present in samples was determined directly by combustion of homogenised samples (triplicates) of tissues, egg white and egg yolk, blood and excreta followed by LSC. Radioactivity in fat samples was determined by digestion of homogenised samples in radioactive dioxide absorber for 72 hours followed by LSC. The radioactivity in extracts of eggs, organ and tissue samples and excreta was determined by LSC.

Radioactive components from homogenised samples were extracted using chloroform and water. For excreta, a further extraction was performed using 1 N sodium hydroxide. From egg yolk, approx. 30 % of the daily sample within each group were pooled. For excreta, 1 % of each daily sample was pooled and diluted with water before extraction. For test 5, pooled samples were prepared from days 1 – 8 and from days 9 – 17.
The samples were extracted twice using a chloroform/water mixture (1:1; v:v) followed by a third extraction using only water. Water and chloroform phases and precipitate were separated by centrifugation. The combined chloroform extracts and the water extracts were analysed by LSC and the precipitate was combusted.
In general, protein was precipitated from the combined water extracts by treating the extract with methanol except for the extract of egg yolk where 2 N hydrochloric acid was used. After centrifugation to remove the proteins, the water extracts were concentrated to dryness. The resulting residues were solubilised in water and analysed by HPLC.

Chloroform extracts of egg yolk and fat (tests 2 and 5) were characterised by acid and base hydrolyses. Acid hydrolysis was conducted with 6 N hydrochloric acid at 100 °C for 2 hours. Afterwards, the hydrolysate was partitioned with chloroform/water and the radioactivity was determined by LSC.
Base hydrolysis was conducted with 7 N sodium hydroxide at 100 °C for 2 hours. Afterwards, the hydrolysate was partitioned with chloroform/water. The radioactivity in the chloroform phase was directly determined by LSC while the aqueous phase was neutralised with 6 N hydrochloric acid prior to LSC analysis.

Two HPLC methods were employed to characterise the residues in the extracts: an ion pair HPLC and a cation exchange HPLC.
For determination of the distribution of radioactive residues in the samples, the results of the analyses of the water extracts with the cation exchange HPLC were used.
For identification of residues in the extracts, HPLC fractions corresponding to glyphosate and AMPA were isolated from kidney extracts using ion pair HPLC. After removal of the ion pair reagent, the concentrated extracts were treated with trifluoroacetic anhydride and trifluoroethanol at 100 °C for 2.5 hours and the mixture were analysed by GC with radioactivity detection and GC/MS. Fractions containing glyphosate and AMPA were isolated from kidney extracts and were purified using a Fe(III)-Chelex column. After derivatisation as described above, they were analyses by GC with radioactivity detection and GC/MS. Peak assignment was based on retention time comparison with reference standards.

II. Results and discussion

Residues found in the control group (test 1) were below the detection limits of the analytical methods used except for a low level of contamination observed in excreta samples. The report states, that the contamination might have occurred during sample collection or preparation for analysis. Therefore only results of treated dose groups are presented.

A. Recovery of radioactivity and total radioactive residues (TRRS)

The total recovered radioactivity in eggs, tissues and excreta are summarised in relation to administered radioactivity (% AR). Between 82.4 and 90.5 % of the administered radioactivity was recovered. The major part of the administered radioactivity was detected in the excreta of all treatment groups. Between 81.1 and 90.5 % of the radioactivity was excreted (these values do not include residues found in the pan rinse fraction). In test 5 (with depuration for 10 days), in all matrices <0.01 % of AR was detected except for egg yolk where 0.02 % of AR was detected. In the other treatment groups, amounts in animal matrices ranged between <0.01 and 0.02 % AR except for the GI tracts with contents, where 1.30 to 2.11 % of AR could be detected.

The total radioactive residues are summarised for samples of laying hens, following administration of 120 or 400 mg/kg feed for 7 days. Highest TRR-values (except for GI tract with contents) were detected in kidneys for all treatment groups except test 5. Amounts ranged between 0.069 (test 5) to 7.004 mg equiv./kg (test 4) in kidneys.

Eggs were separately analysed for radioactive residues in egg white and egg yolk. A plateau level in egg white was reached after approximately 6 days. Residues in egg yolk increased until the end of dosing. Levels in egg yolk and egg white expressed as parent glyphosate equivalents are displayed below.
Residues in the high treatment group were higher than in the low treatment groups, approximately proportional to the dose rates. Residues declined during depuration, so that residues in the hens after depuration were significantly lower than in the treatment groups where the hens were sacrificed shortly after the last dose.

Table 6.2.2-9: Distribution of radioactive residues in tissues, excreta and eggs of laying hens after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels

	% AR[1]			
Matrix	**Test 2** (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)	**Test 3** (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)	**Test 4** (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)	**Test 5** (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)
Kidneys	0.02	0.02	0.02	<0.01
Liver	0.02	0.02	0.02	<0.01
Thigh muscle	<0.01	<0.01	<0.01	<0.01
Breast muscle	<0.01	<0.01	<0.01	<0.01
Fat	<0.01	<0.01	<0.01	<0.01
GI tract with contents	1.65	2.11	1.30	<0.01
Gizzard	<0.01	<0.01	<0.01	<0.01
Ovaries	0.02	0.02	0.01	<0.01
Egg white	<0.01	<0.01	<0.01	<0.01
Egg yolk	0.01	0.01	0.01	0.02
Excreta	81.8	83.6	81.0	90.5
Pan rinse	0.06	0.08	0.06	0.01
Total	83.6	85.9	82.4	90.4

[1] % AR = percent of administered radioactivity

Table 6.2.2-10: Total radioactive residue in samples of laying hens after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels

	TRR (mg equiv./kg)[1]			
Matrix	**Test 2** (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)	**Test 3** (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)	**Test 4** (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)	**Test 5** (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)
Kidneys	1.808	1.747	7.004	0.069
Liver	0.560	0.511	1.914	0.079
Thigh muscle	0.026	0.026	0.090	0.008
Breast muscle	0.018	0.019	0.055	0.006
Fat	0.020	0.015	0.063	0.005
GI tract with contents	18.8	23.9	52.7	0.038
Gizzard	0.352	0.361	1.134	0.032
Ovaries	0.264	0.271	0.939	0.016
Whole blood	0.135	0.146	0.524	0.049

[1] TRR = total radioactive residue

Table 6.2.2-11: Radioactive residues in egg yolk of laying hens after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels

	TRR (mg equiv./kg)			
Days	**Test 2** (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)	**Test 3** (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)	**Test 4** (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)	**Test 5** (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)
1	n.d.	n.d.	n.d.	n.d.
2	n.d.	n.d.	n.d.	n.d.
3	0.002	0.002	n.d.	0.002
4	0.033	0.020	0.096	0.028
5	0.077	0.050	0.252	0.071
6	0.118	0.096	0.444	0.121
7	0.170	0.133	0.625	0.172
8	0.229	0.191	0.753	0.196
9	---	---	---	0.224
10	---	---	---	0.236
11	---	---	---	0.206
12	---	---	---	0.173
13	---	---	---	0.128
14	---	---	---	0.089
15	---	---	---	0.060
16	---	---	---	0.038
17	---	---	---	0.019
At sacrifice	0.237	0.244	0.970	0.012

TRR = total radioactive residue
n.d. = not detectable
--- = not applicable

Table 6.2.2-12: Radioactive residues in egg white of laying hens after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels

	TRR (mg equiv./kg)			
Day	**Test 2** (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)	**Test 3** (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)	**Test 4** (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)	**Test 5** (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)
1	n.d.	n.d.	n.d.	n.d.
2	n.d.	n.d.	n.d.	n.d.
3	0.004	0.004	0.010	0.003
4	0.008	0.012	0.026	0.008
5	0.010	0.014	0.030	0.009
6	0.011	0.017	0.032	0.010
7	0.011	0.016	0.026	0.010
8	0.011	0.015	0.027	0.010
9	---	---	---	0.007
10	---	---	---	0.008
11	---	---	---	0.006
12	---	---	---	0.006
13	---	---	---	0.002
14	---	---	---	0.002
15	---	---	---	0.001
16	---	---	---	n.d.
17	---	---	---	n.d.
At sacrifice	0.007	0.013	0.024	0.001

TRR = total radioactive residue
n.d. = not detectable
--- = not applicable

B. Extraction and characterisation of residues

The analysis of the radioactive residues following extraction was reported in the second part of the study (part II). Kidney, liver, gizzard, fat, muscle and egg yolk samples were investigated for their composition of glyphosate and AMPA. Egg white samples were not further analysed due to low residues in the samples.

For easier comprehension, values from the report were re-calculated to give amounts relative to the TRR (% of TRR). Due to rounding, discrepancies may occur when re-calculating the values. In addition to % TRR values, TRR values were calculated in mg equiv./kg. The values for the respective total water extracts were considered for calculation. The report also contains values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for calculations. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

Total extraction rates ranged from 81.4 to 100 % of TRR. In all samples, the major part of the residue was extracted with water. Only low amounts were found in the chloroform extracts (0.0 to 6.0 % of TRR). Exceptions were chloroform extracts of fat and egg yolk. In fat of test 2 to test 4, 12.0 to 17.2 % of TRR were found in the chloroform extracts. From fat of hens of test 5, 57.2 % of TRR was extracted with chloroform. In egg yolk, 8.8 to 10.3 % of TRR were extracted using chloroform.

Glyphosate and AMPA were identified in an isolated and derivatised fraction of kidney using spectroscopic methods (GC with radioactivity detection and GC/MS). Identification rates ranged from 93.7 to 97.4 % of TRR in kidneys, from 94.0 to 95.7 % of TRR in liver, from 83.6 to 97.0 % of TRR in gizzard, from 82.1 to 86.2 % of TRR in fat (except test 5, 42.2 %), from 80.8 to 90.5 % of TRR in thigh muscle, from 73.7 to 88.3 % of TRR in breast muscle and from 71.8 to 80.2 % of TRR in egg yolks.

Amounts of glyphosate (28.1 – 93.2 % TRR) were generally higher than amounts of AMPA (4.2 – 53.1 % TRR) in all extracts. The ratios ranged from 9.6/0.4 (glyphosate/AMPA) to 5.8/4.2. Only in the depuration test (test 5), ratios changed further to 4.9/5.1 (breast muscle, without regard for unknowns) and 4.4/5.6 (liver).

In thigh and breast muscle, one unknown compound was detected. The amounts ranged from 2.4 to 16.2 % of TRR. Due to very low amounts (≤0.005 mg equiv./kg), it was not identified.
After acidic and base hydrolyses of chloroform extracts of fat (test 2 and 5) and egg yolk, most of the residues remained chloroform-soluble which suggests that the residues present in the chloroform extract of fat tissue are tightly bound to the natural constituents of the fat or the egg yolk, respectively.

From excreta, only very low amounts could be extracted using chloroform (0.02 %). The major part of radioactivity was extracted using water and 1 N sodium hydroxide. Between 96.3 and 105.6 % were accounted for through the extraction. Glyphosate and AMPA were identified by HPLC analysis. The ratio of the two amounted to approx. 9/1 in the extracts.

Table 6.2.2-13: Extraction of the radioactive residues in kidneys of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Kidney	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR	**1.808**	**100**	**1.747**	**100**	**7.004**	**100**	**0.069**	**100**
ERR	***1.781***	***98.5***	***1.702***	***97.4***	***6.864***	***98.0***	***0.066***	***95.7***
Chloroform extract	*0.002*	0.1	*0.002*	0.1	*0.007*	0.1	*<0.001*	0.3
Water extract	*1.779*	98.4	*1.700*	97.3	*6.857*	97.9	*0.066*	95.4
RRR	***0.025***	**1.4**	***0.045***	**2.6**	***0.147***	**2.1**	***0.003***	**4.4**
Accountability[1]	110.9 %		109.5 %		106.8 %		98.9 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-14: Extraction of the radioactive residues in liver of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Liver	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR (mg equiv./kg)	0.560	100	0.511	100	1.914	100	0.079	100
ERR	***0.551***	***98.4***	***0.498***	***97.4***	***1.864***	***97.4***	***0.077***	***97.4***
Chloroform extract	*0.003*	0.5	*0.003*	0.5	*0.011*	0.6	*0.000*	0.0
Water extract	*0.548*	97.9	*0.495*	96.9	*1.853*	96.8	*0.077*	97.4
RRR	***0.010***	**1.8**	***0.014***	**2.7**	***0.052***	**2.7**	***0.002***	**2.6**
Accountability[1]	107.6 %		108.5 %		109.4 %		101.6 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-15: Extraction of the radioactive residues in gizzard of laying hens following treatment with a 9:1-mixture $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Gizzard	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR (mg equiv./kg)	0.352	100	0.361	100	1.134	100	0.032	100
ERR	***0.347***	***98.5***	***0.351***	***97.3***	***1.107***	***97.6***	***0.028***	***86.7***
Chloroform extract	*<0.001*	0.1	*0.001*	0.2	*0.000*	0.0	*<0.001*	0.5
Water extract	*0.346*	98.4	*0.351*	97.1	*1.107*	97.6	*0.028*	86.2
RRR	*0.005*	1.5	*0.010*	2.7	*0.026*	2.3	*0.004*	13.4
Accountability[1]	100.3 %		108.5 %		105.4 %		104.7 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-16: Extraction of the radioactive residues in fat of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Fat	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR (mg equiv./kg)	0.020	100	0.015	100	0.063	100	0.005	100
ERR	***0.020***	***100.0***	***0.015***	***100.1***	***0.063***	***100.0***	***0.005***	***99.9***
Chloroform extract	*0.003*	17.2	*0.002*	16.2	*0.008*	12.0	*0.003*	57.2
Water extract	*0.017*	82.8	*0.013*	83.9	*0.055*	88.0	*0.002*	42.7
RRR	---	---	---	---	---	---	---	---
Accountability[1]	101.9 %		106.6 %		104.9 %		101.6 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-17: Extraction of the radioactive residues in thigh muscle of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Thigh muscle	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR (mg equiv./kg)	0.026	100	0.026	100	0.090	100	0.008	100
Total extracted	***0.026***	***100.0***	***0.026***	***100.0***	***0.090***	***100.0***	***0.008***	***100.0***
Chloroform extract	*0.001*	2.2	*<0.001*	1.8	*0.001*	1.3	*<0.001*	1.6
Water extract	*0.025*	97.8	*0.026*	98.2	*0.089*	98.7	*0.008*	98.4
RRR	---	---	---	---	---	---	---	---
Accountability[1]	109.1 %		111.2 %		95.0 %		93.6 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-18: Extraction of the radioactive residues in breast muscle of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Breast muscle	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR (mg equiv./kg)	0.018	100	0.019	100	0.055	100	0.006	100
ERR	***0.018***	***100.0***	***0.019***	***100.0***	***0.055***	***100.1***	***0.006***	***100.0***
Chloroform extract	*<0.001*	1.1	*<0.001*	0.8	*0.001*	1.4	*<0.001*	6.0
Water extract	*0.018*	98.9	*0.019*	99.2	*0.054*	98.7	*0.006*	94.0
RRR	---	---	---	---	---	---	---	---
Accountability[1]	104.5 %		99.7 %		96.6 %		95.2 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-19: Extraction of the radioactive residues in pooled egg yolk of laying hens following treatment with a 9:1-mixture $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Egg yolk pool (day1 – sacrifice)	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./ kg	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**	**mg equiv./ kg**	**% TRR**
TRR (mg equiv./kg) [a]	0.106	100	0.090	100	0.344	100	0.114	100
ERR	***0.096***	***90.2***	***0.077***	***85.1***	***0.296***	***86.0***	***0.093***	***81.4***
Chloroform extract	*0.011*	10.0	*0.009*	10.3	*0.030*	8.8	*0.011*	9.5
Water extract	*0.085*	80.2	*0.067*	74.8	*0.266*	77.2	*0.082*	71.9
RRR	*0.010*	9.7	*0.014*	15.0	*0.048*	14.0	*0.021*	18.7
Accountability[1]	94.9 %		94.3 %		99.1 %		98.1 %	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-20: Identification and characterisation of the radioactive residues in kidneys of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Kidney	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR	**1.808**	**100**	**1.747**	**100**	**7.004**	**100**	**0.069**	**100**
ERR	***1.781***	***98.5***	***1.702***	***97.4***	***6.864***	***98.0***	***0.066***	***95.7***
Chloroform extract	*0.002*	0.1	*0.002*	0.1	*0.007*	0.1	*<0.001*	0.3
Water extract	*1.779*	98.4	*1.700*	97.3	*6.857*	97.9	*0.066*	95.4
Water extract analysed by cation exchange HPLC:								
AMPA	*0.084*	4.6	*0.092*	5.3	*0.295*	4.2	*0.007*	9.8
Glyphosate	*1.663*	92.0	*1.596*	91.4	*6.528*	93.2	*0.058*	83.9
Water extract analysed by ion pair HPLC:								
AMPA[1]	*0.087*	*4.8*	---	---	*0.281*	*4.0*	*0.006*	*9.3*
Glyphosate[1]	*1.660*	*91.8*	---	---	*6.459*	*92.2*	*0.059*	*85.6*
Total identified	***1.747***	**96.6**	***1.688***	**96.7**	***6.823***	**97.4**	***0.065***	**93.7**
Total characterised[2]	***0.002***	***0.1***	***0.002***	***0.1***	***0.007***	***0.1***	*<0.001*	***0.3***
RRR	***0.025***	**1.4**	***0.045***	**2.6**	***0.147***	**2.1**	***0.003***	**4.4**
Recovery of the extracts (% of water extract)[3]								
Deproteination	---		---		---		95.7	
Concentration	---		---		---		89.1	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[2] Characterised by extraction and/or chromatographic behaviour.

[3] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-21: Identification and characterisation of the radioactive residues in liver of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Liver	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR (mg equiv./kg)	0.560	100	0.511	100	1.914	100	0.079	100
ERR	***0.551***	***98.4***	***0.498***	***97.4***	***1.864***	***97.4***	***0.077***	***97.4***
Chloroform extract	*0.003*	0.5	*0.003*	0.5	*0.011*	0.6	*0.000*	0.0
Water extract	*0.548*	97.9	*0.495*	96.9	*1.853*	96.8	*0.077*	97.4
Water extract analysed by cation exchange HPLC:								
AMPA	*0.150*	26.7	*0.144*	28.1	*0.608*	*31.8*[3]	*0.042*	53.1
Glyphosate	*0.384*	68.6	*0.340*	66.5	*1.225*	*64.0*[3]	*0.032*	40.9
Water extract analysed by ion pair HPLC:								
AMPA[1]	*0.162*	*29.0*	*0.145*	*28.4*	*0.634*	*33.1*	*0.043*	*53.9*
Glyphosate[1]	*0.380*	*67.9*	*0.346*	*67.6*	*1.204*	*62.9*	*0.032*	*41.0*
Total identified	***0.534***	**95.3**	***0.484***	**94.6**	***1.832***	**95.7**	***0.074***	**94.0**
Total characterised[2]	***0.003***	***0.5***	***0.003***	***0.5***	***0.011***	***0.6***	***0.000***	***0.0***
RRR	***0.010***	**1.8**	***0.014***	**2.7**	***0.052***	**2.7**	***0.002***	**2.6**
Recovery of the extracts (% of water extract)[4]								
Deproteination	95.3		101.3		99.9		98.7	
Concentration	88.7		81.6		96.2		78.4	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[2] Characterised by extraction and/or chromatographic behaviour.

[3] The values for amounts of AMPA and glyphosate (as well as the sum of identified residues) were re-calculated since it was not apparent which basis was used in the report for calculation of amounts. Report gives values for AMPA and glyphosate as 32.1 and 64.8 % of TRR.

[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-22: Identification and characterisation of the radioactive residues in gizzard of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Gizzard	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR (mg equiv./kg)	0.352	100	0.361	100	1.134	100	0.032	100
ERR	***0.347***	***98.5***	***0.351***	***97.3***	***1.107***	***97.6***	***0.028***	***86.7***
Chloroform extract	*<0.001*	0.1	*0.001*	0.2	*0.000*	0.0	*<0.001*	0.5
Water extract	*0.346*	98.4	*0.351*	97.1	*1.107*	97.6	*0.028*	86.2
Water extract analysed by cation exchange HPLC:								
AMPA	*0.132*	37.4	*0.145*	40.1	*0.449*	39.6	*0.005*	16.9
Glyphosate	*0.210*	59.6	*0.199*	55.2	*0.645*	56.9	*0.021*	66.7
Water extract analysed by ion pair HPLC:								
AMPA[1]	*0.140*	*39.7*	*0.139*	*38.5*	*0.437*	*38.6*	*0.004*	*13.4*
Glyphosate[1]	*0.207*	*58.7*	*0.212*	*58.6*	*0.670*	*59.0*	*0.023*	*72.8*
Total identified	***0.342***	**97.0**	***0.344***	**95.3**	***1.095***	**96.5**	***0.027***	**83.6**
Total characterised[2]	***<0.001***	***0.1***	***0.001***	***0.2***	***0.000***	***0.0***	***<0.001***	***0.5***
RRR	***0.005***	**1.5**	***0.010***	**2.7**	***0.026***	**2.3**	***0.004***	**13.4**
Recovery of the extracts (% of water extract)[3]								
Deproteination	---		---		---		---	
Concentration	62.7		64.4		69.2		72.4	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[2] Characterised by extraction and/or chromatographic behaviour.

[3] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-23: Identification and characterisation of the radioactive residues in fat of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Fat	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR (mg equiv./kg)	0.020	100	0.015	100	0.063	100	0.005	100
ERR	***0.020***	***100.0***	***0.015***	***100.1***	***0.063***	***100.0***	***0.005***	***99.9***
Chloroform extract	*0.003*	17.2	*0.002*	16.2	*0.008*	12.0	*0.003*	57.2
Water extract	*0.017*	82.8	*0.013*	83.9	*0.055*	88.0	*0.002*	42.7
Water extract analysed by cation exchange HPLC:								
AMPA	*0.002*	10.9	*0.002*	11.7	*0.006*	10.1	*0.001*	14.1
Glyphosate	*0.014*	71.6	*0.011*	70.3	*0.048*	76.1	*0.001*	28.1
Water extract analysed by ion pair HPLC:								
AMPA[1]	*0.003*	*12.7*	*0.002*	*12.7*	*0.008*	*12.4*	*0.001*	*15.0*
Glyphosate[1]	*0.014*	*68.5*	*0.011*	*70.4*	*0.047*	*75.0*	*0.001*	*27.7*
Total identified	***0.016***	**82.5**	***0.012***	**82.0**	***0.054***	**86.2**	***0.002***	**42.2**
Total characterised[2]	***0.003***	***17.2***	***0.002***	***16.2***	***0.008***	***12.0***	***0.003***	***57.2***
RRR	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**
Recovery of the extracts (% of water extract)[3]								
Deproteination	95.8		87.1		87.4		82.0	
Concentration	100.1		102.5		100.7		101.8	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[2] Characterised by extraction and/or chromatographic behaviour.

[3] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-24: Identification and characterisation of the radioactive residues in thigh muscle of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Thigh muscle	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR (mg equiv./kg)	0.026	100	0.026	100	0.090	100	0.008	100
Total extracted	***0.026***	***100.0***	***0.026***	***100.0***	***0.090***	***100.0***	***0.008***	***100.0***
Chloroform extract	*0.001*	2.2	*<0.001*	1.8	*0.001*	1.3	*<0.001*	1.6
Water extract	*0.025*	97.8	*0.026*	98.2	*0.089*	98.7	*0.008*	98.4
Water extract analysed by cation exchange HPLC:								
AMPA	*0.003*	12.7	*0.003*	13.2	*0.013*	14.8	*0.003*	32.6
Glyphosate	*0.018*	68.1	*0.018*	68.2	*0.067*	74.8	*0.005*	57.9
Unknown	*0.002*	9.0	*0.003*	9.9	*0.005*	5.2	*<0.001*	2.8
Total identified	***0.021***	**80.8**	***0.021***	**81.4**	***0.081***	**89.6**	***0.007***	**90.5**
Total characterised[1]	***0.003***	***11.2***	***0.003***	***11.7***	***0.006***	***6.5***	***<0.001***	***4.4***
RRR	---	---	---	---	---	---	---	---
Recovery of the extracts (% of water extract)[2]								
Deproteination	97.7		93.9		95.9		70.2	
Concentration	76.4		66.5		71.8		79.5	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] Characterised by extraction and/or chromatographic behaviour.
[2] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-25: Identification and characterisation of the radioactive residues in breast muscle of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Breast muscle	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR (mg equiv./kg)	0.018	100	0.019	100	0.055	100	0.006	100
ERR	***0.018***	***100.0***	***0.019***	***100.0***	***0.055***	***100.1***	***0.006***	***100.0***
Chloroform extract	*<0.001*	1.1	*<0.001*	0.8	*0.001*	1.4	*<0.001*	6.0
Water extract	*0.018*	98.9	*0.019*	99.2	*0.054*	98.7	*0.006*	94.0
Water extract analysed by cation exchange HPLC:								
AMPA	*0.002*	13.8	*0.002*	11.6	*0.009*	17.3	*0.003*	42.2
Glyphosate	*0.011*	63.1	*0.012*	62.1	*0.039*	71.0	*0.002*	41.1
Unknown	*0.002*	11.6	*0.003*	16.2	*0.002*	4.3	*<0.001*	2.4
Total identified	***0.014***	**76.9**	***0.014***	**73.7**	***0.049***	**88.3**	***0.005***	**83.3**
Total characterised[1]	***0.002***	***12.7***	***0.003***	***17.0***	***0.003***	***5.7***	***0.001***	***8.4***
RRR	**---**	**---**	**---**	**---**	**---**	**---**	**---**	**---**
Recovery of the extracts (% of water extract)[2]								
Deproteination	---		---		---		---	
Concentration	---		---		---		---	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] Characterised by extraction and/or chromatographic behaviour.
[2] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.2-26: Identification and characterisation of the radioactive residues in pooled egg yolk of laying hens following treatment with a 9:1-mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA at different dose levels for 7 days

Egg yolk pool (day1 – sacrifice)	Test 2 (8.86 mg glyphosate and 0.98 mg AMPA per kg bw and days, 7 days)		Test 3 (7.95 mg glyphosate and 0.88 mg AMPA per kg bw and days, 7 days)		Test 4 (26.78 mg glyphosate and 2.98 mg AMPA per kg bw and days, 7 days)		Test 5 (7.76 mg glyphosate and 0.86 mg AMPA per kg bw and days, 7 days + 10 days depuration)	
	mg equiv./kg	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**	**mg equiv./kg**	**% TRR**
TRR (mg equiv./kg)[1]	0.106	100	0.090	100	0.344	100	0.114	100
ERR	***0.096***	***90.2***	***0.077***	***85.1***	***0.296***	***86.0***	***0.093***	***81.4***
Chloroform extract	*0.011*	10.0	*0.009*	10.3	*0.030*	8.8	*0.011*	9.5
Water extract	*0.085*	80.2	*0.067*	74.8	*0.266*	77.2	*0.082*	71.9
Water extract analysed by cation exchange HPLC:								
AMPA	*0.014*	13.1	*0.013*	14.3	*0.047*	13.7	*0.013*	11.1
Glyphosate	*0.071*	67.1	*0.054*	60.3	*0.218*	63.3	*0.069*	60.6
Water extract analysed by ion pair HPLC:								
AMPA[2]	*0.017*	*16.4*	*0.016*	*17.5*	*0.051*	*14.7*	*0.015*	*13.4*
Glyphosate[2]	*0.067*	*63.6*	*0.052*	*57.3*	*0.214*	*62.3*	*0.067*	*58.5*
Total identified	***0.085***	**80.2**	***0.067***	**74.6**	***0.265***	**77.0**	***0.082***	**71.8**
Total characterised[3]	***0.011***	**10.0**	***0.009***	**10.3**	***0.030***	**8.8**	***0.011***	**9.5**
RRR	***0.010***	**9.7**	***0.014***	**15.0**	***0.048***	**14.0**	***0.021***	**18.7**
Recovery of the extracts (% of water extract)[4]								
Deproteination	108.9		101.3		97.3		97.4	
Concentration	99.6		98.7		101.3		107.2	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] The TRR values in pooled egg yolk samples were determined by combustion analyses. Approx. 30 % of the whole egg yolk samples were used for analysis, respectively.

[2] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[3] Characterised by extraction and/or chromatographic behaviour.

[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

C. Storage stability

The duration of sample storage was four months (maximum). Storage stability was investigated using a pooled excreta sample. The sample was analysed at the beginning of the study and was reanalysed at the end of the study to determine any possible changes in the radioactive residues (4 months storage at -20 °C). The sample was extracted with chloroform/water and 1 N sodium hydroxide. The water and the 1 N sodium hydroxide extracts were combined, neutralised and analysed by HPLC (cation exchange and ion pair). Only glyphosate and AMPA were present in the samples and the ratio remained unchanged throughout storage at about 9 to 1. These results demonstrated no change in the radioactive residues during storage.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

Four treatment groups with laying hens were performed to investigate the behaviour of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA in poultry. Two different dose levels were investigated; in the low treatment groups (tests 2, 3 and 5), the hens each received a dose of 120 mg/kg feed = 14.2 – 15.6 mg test mixture/day (8.62 – 9.84 mg/kg bw/day), while in the high treatment group (test 4) the hens each received 400 mg/kg feed dose level = 46.0 mg test mixture/day (29.75 mg/kg bw/day). One capsule was administered each for 7 days. In tests 2, 3 (replicate of treatment group 2) and 4, the hens were sacrificed 22 to 24 hours after the last dose. In test 5, a 10-day depuration phase was added after the 7th dose after which the hens were sacrificed.

Of the relevant matrices of the hen, highest total radioactive residues were found in the kidney (0.069 – 7.004 mg eq/kg), followed by liver (0.079 – 1.914 mg eq/kg) and egg yolk (0.090 – 0.344 mg eq/kg). Residues in muscle, fat and egg white were much lower, generally not exceeding 0.1 mg eq/kg.

The radioactivity level in tissues of the depuration group were generally low; the liver had the highest level (0.079 mg equiv./kg).

A plateau level in eggs white was reached after approximately 6 days. A plateau level in egg yolk could not be observed.

Elimination of radioactivity via excreta was the primary elimination route.

More than 81 % of TRR were extracted using chloroform and water and only low amounts of the residues remained unextractable. Generally, the majority of the residues in the tissues was extractable using water and only low amounts of radioactivity were found in the chloroform extracts, except for fat of hens of test 5.

Glyphosate and AMPA accounted for the majority of the radioactive residue in tissues. Some evidence for further metabolism of glyphosate and AMPA was observed in the muscles, where a minor unknown metabolite was detected.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behavior of glyphosate in laying hens has been previously evaluated at EU level. It was performed under GLP. The study does not entirely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 503 with deficits (the radioactivity balance was 82 – 90 %; for egg yolk, relevant amounts of non-extractable residues were not characterised / not investigated).

Nevertheless, the study is considered to be supportive for the metabolism in laying hens.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.2/004
Report author	[redacted]
Report year	1994
Report title	[^{14}C-PMG] Glyphosate-trimesium: Nature of the residue in tissues and eggs of laying hens
Report No	RR 93-064B
Document No	Not available
Guidelines followed in study	EPA nature of residues in livestock (171-4)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • Excreta was collected only once daily • The application period was 10 days, after which a plateau was not certainly reached in eggs • The radioactivity was not quantified separately in the different fat types • The storage duration of egg white was slightly exceeded (storage duration 6.5 months) • The recovery of radioactive residues after extraction of liver was only 78.1 %
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labeled glyphosate-trimesium) was administered orally for 10 days to 10 laying hens. The target dose level was 90 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg phosphonomethyl-glycine (PMG) per kg feed consumed). The actual dose level was 91.1 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62.4 mg PMG per kg feed consumed) or 5.9 mg ^{14}C-PMG-labeled glyphosate-trimesium/kg bw/day (4.1 mg PMG/kg bw/day), respectively.

One additional test was performed as a control group without dosing with test substance. The hens were sacrificed 12 to 15 hours after the last dosing.

104.0 % of the administered dose was recovered in total. The major portion of radioactive residue was recovered in excreta, cage rinse and GI tract with contents. Radioactive residue associated with edible portions (eggs and tissue) accounted in sum for 0.1286 %.

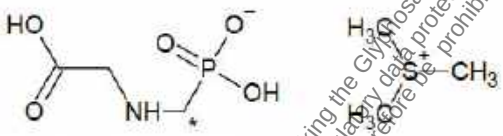
Of the relevant edible matrices of the hen, highest total radioactive residues were found in the kidney (2.17 mg/kg) and liver (0.440 mg/kg). Residues in thigh muscle, breast muscle, fat, egg yolk (day 10) and egg white (day 10) were between 0.0169 and 0.238 mg/kg.

Edible matrices were extracted with 0.1 M HCl/chloroform and 93.71, 70.29, 67.45, 91.6, 45.96 and 90.2 % of the TRR were extractable in liver, thigh muscle, breast muscle, fat, egg white and egg yolk,

respectively. The remaining non-extractable residue of liver and fat were 6.28 and 8.45 % TRR (or 0.0277 and 0.0025 mg/kg), respectively, and were not further examined. For thigh muscle, breast muscle, egg yolk and egg white, the residue after extraction was between 9.8 and 54.0 % TRR or 0.0095 – 0.02351 mg/kg) and was acid hydrolysed.
PMG (34.9 – 56.0 % TRR or 0.0101 – 0.2272 mg/kg) and AMPA (2.14 – 18.9 % TRR or 0.0008 – 0.0828 mg/kg) accounted for the majority of the radioactive residue in all matrices. Furthermore an unknown compound was assigned (1.1 – 4.3 % TRR or 0.0003 – 0.019 mg/kg).

I. Materials and methods

A. Materials

Test material	N-(phosphono-^{14}C-methyl)glycine trimesium salt
Chemical structure:	
	* Position of the radio label
Radiochemical purity:	95.3 %
Specific activity:	7.5 MBq/mg (0.204 mCi/mg = 49.9 mCi/mmol)
CAS No:	81591-81-3
Log $P_{o/w}$:	-2.9
Test animals:	
Species:	Hen, *Gallus gallus*
Strain:	White leghorn
Breeding facility:	[redacted]
Gender and numbers involved:	Female, 13 animals (3 control group and 10 treatment group), identified by cage card and leg band
Body weight:	1.51 – 1.91 kg (day 1 of dosing)
Age:	Approx. 9.5 months
Location of the in-life phase:	[redacted]
Acclimatisation:	7 days before first treatment
Housing:	Individually housed in metabolic cages (46 cm x 66 cm x 48 – 55 cm) with artificial light at a 16/8 hours light/dark cycle Temperature: 22 – 23 °C, Humidity: 48 – 56 %
Feed and water:	Purina Certified Layer Chow, *ad libitum* and water (Columbus Municipal Supply), *ad libitum*

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	5.9 mg N-(phosphono-^{14}C-methyl)glycine trimesium salt equiv./kg bw/day or 4.1 mg phosphonomethylglycine equiv./kg bw/day[1]
Feed consumption:	100 – 120 g/day
Vehicle:	Gelatine capsules
Timing:	Once daily

[1] Calculated based on body weight of 1.67 kg, the actual dose level of 91.1 mg ^{14}C-PMG-labeled glyphosate-trimesium or 62.4 mg PMG per kg feed consumed and the actual feed consumption of 109 g per day.

Ten laying hens were dosed with N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labeled glyphosate-trimesium) once a day for ten consecutive days, at a single dose level, by the oral route of administration. The target dose level was 90 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg phosphonomethylglycine (PMG) per kg feed consumed). The actual dose level was 91.1 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62.4 mg PMG per kg feed consumed), based on the actual dose and the actual feed consumption of 109 g per hen per day, or 5.9 mg ^{14}C-PMG-labeled glyphosate-trimesium/kg bw/day (4.1 mg PMG/kg bw/day).
The test item was administered in gelatine capsules containing ^{14}C-PMG-labeled glyphosate-trimesium and cellulose. Three additional laying hens were given capsules containing cellulose as the control group. The gelatine capsules were prepared at Western Research Center (Richmond, CA, USA) and shipped to Battelle Columbus Laboratories (Columbus, OH, USA) on dry ice overnight, where the capsules were stored frozen at approximately -20°C. Two capsules were extracted (predose capsules extracted with methanol, postdose capsules extracted with 1 M HCl) and three aliquots per capsule were radioassayed by LSC.
Animals were observed twice daily for mortality and moribundity. Body weights were recorded upon receipt, at randomisation, at day 1 and at termination. Feed consumption and clinical observations were recorded daily.

2. Sampling and storage

Excreta was collected prior to dosing and at 24 hours intervals after initiation of dosing until termination. Cage sides and floor as well as excreta collection pans were rinsed using deionised water. Cage rinse specimens were collected beginning on study day 8, every 24 hours until sacrifice. Eggs were collected twice daily. Eggs collected after dosing were stored at 4°C and pooled with eggs collected in the afternoon. The samples were separated into egg white and egg yolk.
The hens were sacrificed 12 to 15 hours after the last dosing. At termination, liver, kidney, breast muscle, thigh muscle, blood, GI tract and contents and fat (abdominal and perirenal) were collected and pooled by treatment group. All samples were stored frozen at approximately -20°C at the site of the in-life part (Battelle Columbus Laboratories). All tissue, eggs, excreta and cage rinse were sent frozen on dry ice to ZENECA, Inc. Western Research Center (Richmond, CA, USA). The samples were stored at -20°C at Western Research Center.

3. Analytical procedures

Specimens of excreta, tissues, eggs, and blood were homogenised and analysed for total radioactivity using tissue combustion and/or liquid scintillation counting (LSC). All samples were homogenised prior to combustion as follows. Excreta, kidney, breast muscle and thigh muscle were homogenised with water in a 1:1 ratio. Liver was homogenised with water in a 1:0.83 ratio. The GI tract and contents was homogenised with water in a 2.5:1 ratio. Samples of egg white, egg yolk, blood, fat and cage rinse were

homogenised without added water. The radioactivity in extracts of eggs and tissue samples was determined by LSC.

Liver, thigh muscle, breast muscle, fat, egg white (day 10) and egg yolk (day 10) were extracted two or three times with a 0.1 M HCl and chloroform mixture. The aqueous and chloroform phases were separated and the combined phases were analysed by LSC.
The aqueous phases were cleaned-up by Chelex® chromatography and 5 eluates were collected (fraction 5 – 9). For liver, thigh muscle, breast muscle, fat and egg yolk, one eluate (6 M HCl, fraction 8) was further purified on an anion-exchange column and 2 to 4 eluates were collected (fractions 10 – 12, 14). One concentrated eluate (liver, thigh muscle and breast muscle, fraction 10) or a combined and concentrated eluate (egg yolk, fractions 10 plus 11 = fraction 13) was analysed by HPLC and TLC. For thigh muscle, fat and egg yolk, these eluates were further analysed by GC-MS after derivatisation with heptafluorobutanol and trifluoroacetic anhydride. For liver, the first eluate (0.1 M HCl, fraction 5) after Chelex® chromatography was concentrated and hydrolysed with 6 M HCl for 3 hours. The hydrolysate was cleaned up by Chelex® chromatography and 4 eluates (fractions 26 – 29) were collected. Fraction 29 was further purified by anion-exchange chromatography before HPLC analysis.
The combined chloroform phases of fat and egg yolk were separated into non-polar and polar lipids according published methods. The non-polar and polar lipids were analysed by TLC.
The postextracted solid (fraction 4) was radioassayed. For thigh muscle, breast muscle, egg yolk and egg white, the residue after extraction was acid hydrolysed with 6 M HCl (at reflux for 7 – 10 h). The hydrolysate (fraction 16) of thigh muscle, egg yolk and egg white was adjusted to pH 1 before application on a Chelex® column. 4 to 52 eluates were collected (fractions 17 – 21) and for thigh muscle fraction 20 was further cleaned up by anion-exchange chromatography (fraction 22).

Peak assignment was based on retention time comparison with reference items.

II. Results and discussion

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in the table below. 104.0 % of the administered dose was recovered. The main part was excreted, accounting in sum for 99.9 % (excreta plus cage wash). Radioactivity recovered in GI tract with contents accounted for 3.90 %. Radioactivity associated with edible portions (eggs and tissue) accounted in sum for 0.1286 %.

The total radioactive residues (TRR) are summarised for samples of laying hens, following administration of 91.1 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed for 10 days. TRRs are expressed as PMG equivalents. Highest TRR values (except for GI tract with contents) were found in kidney (2.17 mg/kg) and liver (0.440 mg/kg). In thigh muscle, breast muscle and fat 0.0401, 0.0292 and 0.0220 mg/kg were found, respectively.
Eggs were separately analysed for radioactive residues in egg white and egg yolk. Egg yolk samples from days 5 to 10 contained residue levels greater than 0.1 mg/kg and the remaining samples contained less than 0.1 mg/kg. All egg white samples contained less than 0.02 mg/kg.

Table 6.2.2-27: Distribution of radioactive residues in tissues, excreta and eggs of laying hens after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

Matrix	% dose[1]
Kidney	0.039
Liver	0.0272
Thigh muscle	0.0128
Breast muscle	0.00935
Fat	0.00563
GI tract with contents	3.90
Blood	0.0249
Egg white (day 1 – 10)	0.0056
Egg yolk (day 1 – 10)	0.029
Excreta	99.3
Cage rinse	0.6
Total	104.0

[1] % dose = percent of administered dose

Table 6.2.2-28: Total radioactive residue in samples of laying hens after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

Matrix	TRR (mg/kg)[1]
Kidney	2.17
Liver	0.440
Thigh muscle	0.0401
Breast muscle	0.0292
Fat	0.0220
GI tract with contents	13.1
Blood	0.139
Egg white (day 10)	0.0169
Egg yolk (day 10)	0.238

[1] TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents; determined at Battelle Columbus Laboratories
For liver, thigh muscle, breast muscle, fat, egg white and egg yolk the TRR was further determined by combustion at Western Research Center. 0.4402, 0.0401, 0.0191, 0.0293, 0.0189 and 0.2400 mg/kg were found, respectively.

Table 6.2.2-29: Radioactive residues in egg white and egg yolk of laying hens after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

	TRR (mg/kg)	
Days	**Egg white**	**Egg yolk**
1	n.d.	n.d.
2	0.00374	0.00140
3	0.0106	0.0254
4	0.0105	0.0641
5	0.0133	0.110
6	0.0121	0.153
7	0.0144	0.194
8	0.0144	0.211
9	0.0171	0.229
10	0.0169	0.238

TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
n.d. = not detectable

B. Extraction and characterisation of residues

Edible matrices (tissue and eggs) were extracted with 0.1 M HCl/chloroform and the results are summarised in the tables below. Portions of 93.71, 70.29, 67.45, 91.6, 45.96 and 90.2 % of the TRR were extractable in liver, thigh muscle, breast muscle, fat, egg white (day 10) and egg yolk (day 10), respectively. The remaining non-extractable residues of liver and fat were 6.28 and 8.45 % TRR (or 0.0277 and 0.0025 mg/kg), respectively, and were not further examined. For thigh muscle, breast muscle, egg yolk and egg white, the residue after extraction was between 9.8 and 54.0 % TRR (or 0.0095 – 0.02351 mg/kg) and was acid hydrolysed.

The aqueous phases were purified by Chelex® chromatography. The first two eluates (0.1 M HCl eluate, fraction 5 and water eluate, fraction 6) were associated as polar conjugates. For liver, thigh muscle, breast muscle, fat and egg yolk, the eluate with the majority of the ^{14}C-residue (6 M HCl, fraction 8) was further purified on an anion-exchange column. A concentrated or a combined and concentrated extract was analysed by HPLC and TLC. For thigh muscle, fat and egg yolk, the eluates were further analysed by GC-MS after derivatisation. For liver, in addition to fraction 8, fraction 5 was cleaned-up by Chelex® chromatography and one concentrated eluate was analysed by HPLC. In TLC analyses, PMG and AMPA reference standards were used to confirm the identity of the ^{14}C-residue. In HPLC analyses, ^{14}C-PMG was used as standard. In the aqueous phase PMG (34.9 – 56.0 % TRR or 0.0101 – 0.2272 mg/kg) and AMPA (2.14 – 18.9 % TRR or 0.0008 – 0.0828 mg/kg) were identified in all matrices. Furthermore an unknown compound was assigned (1.1 – 4.3 % TRR or 0.0003 – 0.019 mg/kg). TLC and GC-MS analyses confirmed the peak identification.

The combined chloroform phases of fat and egg yolk were separated into non-polar and polar lipids and analysed by TLC (co-chromatography with cholesterol, sphingomyelin, phosphatidylcholine and phosphatidylethanolamine). For egg yolk, the majority of the ^{14}C-residue in non-polar lipids was identified as triglycerides and cholesterol while the majority of the ^{14}C-residue in polar lipids was identified as phosphatidylcholine. For fat, the non-polar lipids triglycerides, cholesterol and free fatty acids were identified.

Table 6.2.2-30: Extraction of the radioactive residues in tissue of laying hens following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 10 days

	Liver		Thigh muscle		Breast muscle		Fat	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	0.4402	100	0.04012	100	0.0292[1]	100	0.02934[2]	100
ERR	*0.4126*	*93.71*	*0.02820*	*70.29*	*0.0197*	*67.45*	*0.0268*	*91.6*
Aqueous phase	0.3993	90.7	0.02732	68.1	0.0195	66.8	0.0139	47.5
Chloroform phase	0.0133	3.01	0.00088	2.19	0.0002	0.65	0.0129	44.1
RRR	0.0277	6.28	0.01193	29.7	0.0095	32.5	0.0025	8.45
Accountability[3]	78.1 %		110.4 %		100.25 %		100 %	

Values in *italics* were calculated upon dossier compilation.
The % TRR values shown are the normalised values, obtained by dividing the "raw" % TRR by the recovery values at each step.
[1] Value determined by combustion at Battelle Columbus Laboratories was used.
[2] Value determined by extraction since direct combustion was not reproducible.
[3] Accountability = recovery after extraction with a 1 M HCl and chloroform mixture (not normalised values)
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents

Table 6.2.2-31: Extraction of the radioactive residues in eggs of laying hens following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 10 days

	Egg white (day 10)		Egg yolk (day 10)	
	mg/kg	% TRR	mg/kg	% TRR
TRR	0.0189	100	0.240	100
ERR	*0.00869*	*45.96*	*0.21648*	*90.2*
Aqueous phase	0.00849	44.9	0.15720	65.5
Chloroform phase	0.00020	1.06	0.05928	24.7
RRR	0.01021	54.0	0.02351	9.8
Accountability[1]	99.01 %		102.6 %	

Values in *italics* were calculated upon dossier compilation.
The % TRR values shown are the normalised values, obtained by dividing the "raw" % TRR by the recovery values at each step.
[1] Accountability = recovery after extraction with a 1 M HCl and chloroform mixture (not normalised values)
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents

Table 6.2.2-32: Identification and characterisation of the radioactive residues in liver and thigh muscle of laying hens following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 10 days

	Liver		Thigh muscle	
	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.4402**	**100**	**0.04012**	**100**
ERR	***0.4126***	***93.71***	***0.02820***	***70.3***
Aqueous phase (fraction 2)	0.3993	90.7	0.02732	68.1
Polar conjugates (= fraction 6 for liver; sum of fractions 5 and 6 for thigh muscle)[1]	0.0014[2]	0.31[2]	0.0067	16.59
Hydrol. Released fraction (= sum of fraction 26 and 27)[3]	0.0080	1.82	N/A	N/A
Fraction 28	0.0028	0.6	N/A	N/A
Fraction 29	0.0342	7.8	N/A	N/A
Fraction 29 analysed by HPLC				
PMG[4]	0.0238	5.4	N/A	N/A
AMPA[4]	0.005	1.2	N/A	N/A
Unknown 1	0.004	0.9	N/A	N/A

Table 6.2.2-32: Identification and characterisation of the radioactive residues in liver and thigh muscle of laying hens following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 10 days

	Liver		Thigh muscle	
	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.4402**	**100**	**0.04012**	**100**
Fraction 7	0.0101	2.3	0.00062	1.5
Fraction 8	0.3429	77.9	0.01999	49.8
Fraction 10	0.3430	77.9	0.01995	49.7
Fraction 13	0.2959	67.2	0.01697	42.3
Fraction 13 analysed by HPLC				
PMG[4]	0.2034	46.2	0.0150	37.5
AMPA[4]	0.0778	17.7	0.001	2.5
Unknown 1	0.015	3.4	0.001	2.3
Fraction 14	0.0470	10.7	0.00299	7.4
Fraction 11	N/A	N/A	0.00004	0.1
Fraction 9	N/A	N/A	0.00005	0.1
Chloroform phase (fraction 3)	0.0133	3.01	0.00088	2.19
RRR (fraction 4)	**0.0277**	**6.28**	**0.01193**	**29.7**
Hydrol. released fraction (= sum of fractions 17 and 18)[5]	N/A	N/A	0.0044	10.84
Fraction 19	N/A	N/A	0.00071	1.8
Fraction 21	N/A	N/A	0.00016	0.4
Fraction 22	N/A	N/A	0.00611	15.2
Total identified	***0.3100***	***70.5***	***0.0160***	***40.0***
Total characterised[6]	***0.1015***	***23.04***	***0.02366***	***58.42***
Final residue	**0.0277**	**6.28**	**0.0006**	**1.49**
Calculated theoretical values[7]				
PMG	0.2684	60.97	0.0245	61.00
AMPA	0.0992	22.53	0.0016	4.06
Unknown 1	0.0224	5.08	0.0015	3.79
Total identified	*0.3676*	*83.50*	*0.0261*	*65.06*
Total characterised[6]	*0.0436*	*9.91*	*0.01348*	*33.41*

[1] Sum of two eluates (fractions 5 and 6) after Chelex® chromatography of the aqueous phase.
[2] For liver, fraction 5 (0.0449 mg/kg or 10.2 % TRR) was further cleaned-up by Chelex® chromatography and 4 eluates (fractions 26 – 29) were collected.
[3] Sum of two eluates (fractions 26 and 27) after Chelex® chromatography of the hydrolysate.
[4] For liver, fraction 13 and 29 were analysed by HPLC. In sum 0.2272 mg/kg or 51.6 % TRR PMG and 0.0828 mg/kg or 18.9 % TRR AMPA, 0.019 mg/kg or 4.3 % TRR unknown 1 were found.
[5] Sum of two eluates (fractions 17 and 18) after Chelex® chromatography of the hydrolysate. Two further eluates (fractions 19 and 20) were used for the theoretical calculation of PMG, AMPA and unknown 1.
[6] Characterised by extraction and/or chromatographic behaviour.
[7] In the report, the measured ratio of PMG, AMPA and unknown 1 in fraction 13 of liver and thigh muscle was used to calculate the occurrence of PMG, AMPA and unknown 1 in other fractions (i.e. fractions 7, 13, 14, 28 and 29 for liver and fractions 7, 9, 11, 13, 14, 19, 21 and 22 for thigh muscle). In this part of the table the sum of each analyte in all fractions is listed, as shown in the report as well as total identified and total characterised based on the these values.

Values in *italics* were calculated upon dossier compilation. Minor deviations may occur due to rounding.
The % TRR values shown are the normalised values, obtained by dividing the "raw" % TRR by the recovery values at each step.
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents
N/A = not applicable

Table 6.2.2-33: Identification and characterisation of the radioactive residues in breast muscle and fat of laying hens following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 10 days

	Breast muscle		**Fat**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
TRR	**0.0292**	**100**	**0.02934**	**100**
ERR	***0.0197***	***67.45***	***0.0268***	***91.6***
Aqueous phase (fraction 2)	0.0195	66.8	0.0139	47.5
Polar conjugates (= sum of fractions 5 and 6)[1]	0.0063	21.54	0.0006	2.01
Fraction 7	0.0008	2.7	0.0004	1.3
Fraction 8	0.0123	42.1	0.0129	44.1
Fraction 10	0.0119	41.2	0.0129	44.0
Fraction 13	0.0117	40.5	0.0115	39.3
Fraction 13 analysed by HPLC				
PMG	0.0101	34.9	0.0103	35.1
AMPA	0.0013	4.5	0.0008	2.8
Unknown 1	0.0003	1.1	0.0004	1.3
Fraction 14	0.0002	0.7	0.0014	4.8
Fraction 11	0.0002	0.8	0.00001	0.03
Fraction 9	0.0002	0.5	0.00002	0.1
Chloroform phase (fraction 3)	0.0002	0.65	0.0129	44.1
Phospholipids	N/A	N/A	N/A	N/A
Non-polar lipids	N/A	N/A	0.0129	44.1
RRR (fraction 4)	**0.0095**	**32.5**	**0.0025**	**8.45**
HCl hydrolysate	0.0087	29.68	N/A	N/A
Total identified	***0.0114***	***39.4***	***0.0111***	***37.9***
Total characterised[2]	***0.0169***	***57.67***	***0.01573***	***53.64***
Final residue	**0.0008**	**2.86**	**0.0025**	**8.45**
Calculated theoretical values[3]				
PMG	0.0114	39.05	0.0119	40.66
AMPA	0.0015	5.00	0.0010	3.31
Unknown 1	0.0004	1.21	0.0004	1.46
Total identified	*0.0129*	*44.05*	*0.0129*	*43.97*
Total characterised[6]	*0.0156*	*53.08*	*0.0139*	*47.57*

[1] Sum of two eluates (fractions 5 and 6) after Chelex® chromatography of the aqueous phase.
[2] Characterised by extraction and/or chromatographic behaviour.
[3] In the report, the measured ratio of PMG, AMPA and unknown 1 in fraction 13 of breast muscle and fat was used to calculate the occurrence of PMG, AMPA and unknown 1 in other fractions (i.e. fractions 7, 9, 11, 13 and 14). In this part of the table the sum of each analyte in all fractions is listed, as shown in the report as well as total identified and total characterised based on the these values..

Values in *italics* were calculated upon dossier compilation. Minor deviations may occur due to rounding.
The % TRR values shown are the normalised values, obtained by dividing the "raw" % TRR by the recovery values at each step.
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents
N/A = not applicable

Table 6.2.2-34: Identification and characterisation of the radioactive residues in eggs of laying hens following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 10 days

	Egg white (day 10)		Egg yolk (day 10)	
	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.0189**	**100**	**0.240**	**100**
ERR	***0.00869***	***45.96***	***0.21648***	***90.2***
Aqueous phase (fraction 2)	0.00849	44.9	0.15720	65.5
Polar conjugates (= sum of fractions 5 and 6)[1]	0.0073	38.36	0.0076	3.22
Fraction 7	0.000066	0.4	0.00039	0.2
Fraction 8	0.00117	6.2	0.14873	61.9
Fraction 10	N/A	N/A	0.10360	43.2
Fraction 11	N/A	N/A	0.04474	18.6
Fraction 13	N/A	N/A	0.14282	59.5
Fraction 13 analysed by HPLC				
PMG	N/A	N/A	0.1345	56.0
AMPA	N/A	N/A	0.005	2.14
Unknown 1	N/A	N/A	0.003	1.28
Fraction 14	N/A	N/A	0.00552	2.3
Fraction 12	N/A	N/A	0.00039	0.2
Fraction 9	0.000	0.0	0.00053	0.2
Chloroform phase (fraction 3)	0.0002	1.06	0.05928	24.7
Phospholipids	N/A	N/A	0.01776	7.4
Non-polar lipids	N/A	N/A	0.04152	17.3
RRR (fraction 4)	**0.01021**	**54.0**	**0.02351**	**9.8**
Fraction 16	Not analysed by LSC		0.01825	7.6
Hydrol. released fraction (= sum of fractions 17 and 18)[2]	0.0071	37.83	0.0162	6.73
Fraction 19	0.00142	7.5	0.00115	0.5
Fraction 20	0.00165	8.7	0.00094	0.4
Total identified	***0.0000***	***0.00***	***0.1395***	***58.14***
Total characterised[3]	***0.018906***	***100.05***	***0.09500***	***39.73***
Final residue	**0.0000**	**0.00**	**0.0053**	**2.19**
Calculated theoretical values[4]				
PMG	0.0043	21.48	0.1429	59.54
AMPA	0.0002	0.82	0.0055	2.28
Unknown 1	0.0001	0.49	0.0033	1.37
Total identified	*0.0045*	*22.30*	*0.1484*	*61.82*
Total characterised[6]	*0.0147*	*77.74*	*0.08638*	*36.02*

[1] Sum of two eluates (fractions 5 and 6) after Chelex® chromatography of the aqueous phase.
[2] Sum of two eluates (fractions 17 and 18) after Chelex® chromatography of the hydrolysate. Two further eluates (fractions 19 and 20) were used for the theoretical calculation of PMG, AMPA and unknown 1.
[3] Characterised by extraction and/or chromatographic behaviour.
[4] In the report, the measured ratio of PMG, AMPA and unknown 1 in fraction 13 of egg yolk was used to calculate the occurrence of PMG, AMPA and unknown 1 in other fractions (i.e. fractions 7 – 9, 19 and 20 for egg white and fractions 7, 9, 12 – 14, 19, 20 for egg yolk). In this part of the table the sum of each analyte in all fractions is listed, as shown in the report as well as total identified and total characterised based on the these values.
Values in *italics* were calculated upon dossier compilation. Minor deviations may occur due to rounding.
The % TRR values shown are the normalised values, obtained by dividing the "raw" % TRR by the recovery values at each step.
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents
N/A = not applicable

C. Storage stability

All samples, stored frozen at approximately -20°C, were extracted and analysed within 4 to 6 months of sacrifice, except for egg white, the tissue with the lowest ^{14}C-residue, which was extracted at 6.5 months.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

N-(phosphono-^{14}C-methyl)glycine trimesium salt was administered orally for 10 days to 10 laying hens. The target dose level was 90 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg phosphonomethyl-glycine (PMG) per kg feed consumed). The actual dose level was 91.1 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62.4 mg PMG per kg feed consumed) or 5.9 mg ^{14}C-PMG-labeled glyphosate-trimesium/kg bw/day (4.1 mg PMG/kg bw/day), respectively.
104.0 % of the administered dose was recovered in total. Elimination of radioactivity via excreta was the primary elimination route. 0.1286 % of the administered dose was associated with edible portions (tissues and eggs).
PMG (34.9 – 56.0 % TRR or 0.0101 – 0.2272 mg/kg) and AMPA (2.14 – 18.9 % TRR or 0.0008 – 0.0828 mg/kg) accounted for the majority of the radioactive residue in all matrices. An unknown compound present at lower levels (1.1 – 4.3 % TRR or 0.0003 – 0.019 mg/kg) was characterised by its extraction behaviour and its retention time in two different chromatographic systems.
The other major fraction of the ^{14}C-residues in egg yolk and fat was due to natural incorporation into lipids. In egg yolk the ^{14}C incorporation was detected in the nonpolar lipid fraction (triglycerides and cholesterol), and in the phospholipid fraction (mainly in phosphatidylcholine). In fat, the ^{14}C-natural incorporation was shown in triglycerides, cholesterol and free fatty acids.

In conclusion, orally administered glyphosate-trimesium in hens is rapidly and essentially quantitatively excreted. The major residues consist of PMG and its primary metabolite AMPA. In addition, significant metabolic incorporation into natural products was observed as polar and non-polar lipids.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behavior of glyphosate in laying hens has been previously evaluated at EU level and was accepted. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 503 with minor deviations (the storage duration of egg white was slightly exceeded (storage duration 6.5 months), the recovery of radioactive residues after extraction of liver was only 78.1 %).
Egg white was extracted within 6.5 months and no HPLC analysis was performed as only 0.0189 mg eq/kg was found in egg white (0.00849 mg eq/kg in the aqueous phase, 0.00020 mg eq/kg in the chloroform phase and 0.01021 mg eq/kg in the RRR). Furthermore, this storage duration is well covered by available storage stability studies (see MCA 6.1).
The recovery of radioactive residues after extraction (accountability) of liver was only moderate (78.1 %). However, only small aliquots of homogenised liver were used for TRR measurement while 10 g homogenised liver was used for extraction. Based on the assumption that the calculated TRR (sum of fraction 2, 3 and 4) is the more reliable determination, the reported radioactive residues in mg/kg are probably overestimated and represent a worst case. Furthermore, supportive information is given in the summary of [redacted] 1988 (IIA 6.2.2/02) and [redacted] 1998 (IIA 6.2.2/03), where the recovery for liver was higher, and only glyphosate and AMPA were detected.
Therefore, the study is considered reliable and covers the guideline requirements for metabolism studies in laying hens.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.2/005
Report author	[redacted]
Report year	2007
Report title	The metabolism of [^{14}C]-*N*-acetylglyphosate (IN-MCX20) in laying hens
Report No	210573
Document No	[redacted]-19795
Guidelines followed in study	Residue Test Guideline, OPPTS 860.1300, Nature of the Residue – Livestock, U.S. Environmental Protection Agency, August 1996 and FAO Guidelines as Recommended by EU Commission Directive 96/68/EC Annex 1, Section 6.2 (21 October 1996)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • TG 503 recommends use of 10 birds, and 5 treated birds were used in this study. • Excreta was collected only once daily. • The application period was 7 days, after which a plateau was not certainly reached in eggs. • The radioactivity was not quantified separately in the different muscle and fat types. • Identification was done by HPLC retention comparison with authenticated standards in one system by HPLC. • Balance of components in matrices (egg white and muscle) with low absolute residue concentrations misses portions of up to 34.21 % TRR or 0.006 mg/kg (recovery or calculation issue).
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

N-acetylglyphosate [*N*-acetyl-*N*-(phosphomethyl)glycine)] was administered to five laying hens as an oral dose of [^{14}C]-*N*-acetylglyphosate twice daily for 7 consecutive days. The nominal dose level was 50 mg/kg of feed consumed per day. The actual dose level achieved based on feed consumption was 63.311 mg *N*-acetylglyphosate equivalent/kg feed (4.391 mg *N*-acetylglyphosate equivalent/kg bw/day). Excreta was collected once daily and eggs collected twice daily. The hens were sacrificed approximately 6 hours after the last dose. The total radioactive residues (TRR) in eggs (whites and yolks), liver, muscle (composite breast and thigh), and abdominal fat were determined.

Recovery of total administered dose in excreta, eggs, and tissues was 90.18 %. Excreta (including cage wash) contained 90.08 % of the total administered dose. Liver, muscle, fat, and eggs each contained ≤0.05 % of the administered dose. Higher residue concentrations were observed in the egg yolk than in egg white. The total radioactive residue concentration in the egg yolk increased steadily from 0.044 mg/kg after 48 hours to 0.342 mg/kg after 158 hours. Egg white total radioactive residue concentrations increased from 0.009 mg/kg after 48 hours to 0.019 mg/kg after 158 hours. The TRR in liver, muscle and fat were 0.511, 0.039 and 0.051 mg/kg, respectively.

Composite (Day 1-7) excreta was extracted with water. Tissues (liver, muscle, and fat) were extracted with 0.2 N hydrochloric acid. Composite (Day 1-7) egg whites were extracted with 0.2 N hydrochloric acid containing a mixture of dichloromethane and chloroform. Composite (Day 1-7) egg yolks were extracted with 0.2 N hydrochloric acid:methanol (1:1, v/v) containing dichloromethane. Approximately 81 – 96 % TRR was extracted from the eggs and tissues. The TRR remaining in the liver and egg yolk samples was subject to sequential treatment with pepsin and protease enzymes, which released additional radioactivity (0.27 – 11.61 % TRR). Metabolites were identified by HPLC co-chromatography with authentic radiolabelled and un-labelled reference standards then later confirmed in selected samples using mass spectrometry.

The HPLC profile of the excreta extract contained two radiolabelled components, the most abundant was *N*-acetylglyphosate and accounted for 82.38 % dose. Glyphosate was detected and accounted for 0.79 % dose.

Four radiolabelled components were detected in the HPLC-profile of the egg whites, the most abundant was *N*-acetylglyphosate and accounted for 41.48 % TRR (0.004 mg/kg). Glyphosate and *N*-acetyl AMPA were detected and accounted for 10.90 % TRR (0.001 mg/kg) and 4.34 % TRR (<0.001 mg/kg), respectively. A single, minor, unknown component, which was less polar than *N*-acetylglyphosate, accounted for 3.40 % TRR (<0.001 mg/kg).

Four radiolabelled components were detected in the HPLC-profile of the egg yolk extract, the most abundant was *N*-acetylglyphosate and accounted for 68.40 % TRR (0.157 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA were detected and accounted for 0.91 % TRR (0.002 mg/kg), 5.69 % TRR (0.013 mg/kg), and 1.10 % TRR (0.003 mg/kg), respectively.

The highest level of total radioactive residues in reconstructed whole eggs (sum of residues in egg whites and yolks) were observed after 7 days at 0.361 mg/kg. Concentrations of unchanged *N*-acetylglyphosate and the metabolites AMPA, glyphosate, and *N*-acetyl AMPA were calculated as 0.161, 0.002, 0.014 and 0.003 mg/kg, respectively in whole eggs.

Four radiolabelled components were detected in the HPLC-profile of the liver extract, the most abundant was *N*-acetylglyphosate and accounted for 63.82 % TRR (0.323 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA, were detected and accounted for 6.74 % TRR (0.034 mg/kg), 16.34 % TRR (0.084 mg/kg), and 4.04 % TRR (0.020 mg/kg), respectively.

The HPLC-profile of the muscle extract contained eight radiolabelled components, the most abundant was *N*-acetylglyphosate, and accounted for 25.22 % TRR (0.009 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA, were detected and accounted for 16.69 % TRR (0.005 mg/kg), 7.19 % TRR (0.002 mg/kg), and 1.89 % TRR (0.001 mg/kg), respectively. The remaining four components were minor in nature with none accounting for more than 8.95 % TRR (0.003 mg/kg).

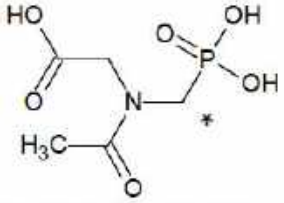
Six radiolabelled components were detected in the HPLC profile of the abdominal fat extract, the most abundant was glyphosate and accounted for 39.43 % TRR (0.023 mg/kg). AMPA, *N*-acetyl AMPA, and *N*-acetylglyphosate, were detected and accounted for 11.29 % TRR (0.007 mg/kg), 10.18 % TRR (0.006 mg/kg), and 23.45 % TRR (0.014 mg/kg), respectively. The remaining two components were

minor in nature with none accounting for more than 0.71 % TRR (<0.001 mg/kg).

Based on results of this study, it is concluded that there was not a significant transfer of *N*-acetylglyphosate and its metabolites to eggs or edible tissues (liver, muscle, and fat). Eggs and edible tissues contained <0.1 % of the total administered dose.

I. Materials and methods

A. Materials

Test material	[^{14}C]-*N*-Acetylglyphosate
Chemical structure:	
	* position of the radio label
Radiochemical purity:	>99 % (HPLC assay conducted by Charles River Laboratories)
Specific activity:	0.51 MBq/mg (13.83 µCi/mg)
Lot number	3562-059
CAS No:	129660-96-4 (non-radiolabeled)
Log $P_{o/w}$:	-6.26, pH 7 at 25 °C (*N*-acetylglyphosate)
Test animals:	
Species:	Laying hen, *Gallus gallus*
Strain:	Not reported
Breeding facility:	[redacted]
Gender and numbers involved:	Female, 7 animals (2 control group and 5 treatment group), identified by leg ring
Body weight:	1.71 – 2.42 kg at Study Day 1, 1.82 – 2.59 kg (Study Day 7)
Age:	Not reported
Location of the in-life phase:	[redacted]
Acclimatisation:	14 days prior to the start of dosing
Housing:	Individually housed in stainless steel cages during the pre-trial and on study periods. Throughout the acclimation and dosing periods, the hens were kept on a 16 hours light/8 hours dark cycle. Temperature and humidity were recorded daily, with ranges of 16 – 23 °C and 24 – 68 %, respectively.
Feed and water:	Commercially available hen diet (Layers Pellets; Batch No. 1756D 260753) offered at *ca* 100 g twice daily, i.e. a total of *ca* 200 g/day. Water (Mains tap water), *ad libitum*.

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	63.311 mg *N*-acetylglyphosate/kg feed or 4.391 mg *N*-acetylglyphosate/kg bw/day[1] If expressed as glyphosate equivalent, 50.649 mg glyphosate equivalent/kg feed or 3.513 mg glyphosate equivalent/kg bw/day[2]
Feed consumption:	134 g/day (average for the 7-day dosing period)
Vehicle:	Gelatine capsules
Timing:	Twice per day by balling gun

[1] Dose level in diet/feed calculated as an average over the 7-day dosing period from daily feed consumption and daily dose administered by individual animal. Dose level expressed on basis of animal bodyweight calculated as an average based on individual animal daily dose over the 7-day dosing period and corresponding individual animal average body weight during the dosing period (average of body weight on Study Days 1 and 7).

[2] Dose level was also expressed as glyphosate equivalents, derived from calculation using a conversion factor of 0.8 based on *N*-acetylglyphosate and glyphosate molecular weight of 211.11 and 169.07, respectively.

[^{14}C]-*N*-acetylglyphosate (sodium salt) was used to dose each of five laying hens twice per day during the 7-day dosing interval. The target dose level based on feed consumption was 50 mg *N*-acetylglyphosate/kg in the diet (or 40 mg/kg in the diet if expressed as glyphosate equivalents). The actual dose level based on feed consumption was 63.311 mg *N*-acetylglyphosate/kg feed based on average daily feed consumption during the 7-day dosing interval. If expressed as glyphosate equivalent in the diet, the actual dose was 50.649 mg glyphosate equivalent/kg feed. Based on average bodyweight by individual animal on Study Days 1 and 7 (during the dosing period) and corresponding daily dosage by individual animal, the actual dose administered was 4.391 mg *N*-acetylglyphosate/kg bw/day (or if expressed as glyphosate equivalent was 3.513 mg glyphosate equivalent/kg bw/day).

The dose solution contained [^{14}C]-*N*-acetylglyphosate in aqueous solution. The radioactive content and homogeneity of the dosing solution was confirmed by HPLC following storage and during the dosing period. The dose solution was stored at *ca* 4 °C during the dosing period. The radiochemical purity after 3 and 7 days was 99.4 % and 99.1 %, respectively, indicating stability of the dose formulation during the dosing period.

Dosing solution was dispensed into a gelatine capsule containing hen feed as soon as possible prior to dose administration. Dose solution (51-75 µL) was dispensed into small (Size 0) gelatine capsules, which were subsequently placed inside larger (Size 00) capsules to ensure no loss of dose prior to administration.

The test hens received a single oral dose of [^{14}C]-*N*-acetylglyphosate via a gelatine capsule twice daily (at *ca* 9 a.m. and 4 p.m.) for 7 consecutive days using a balling gun. Two control animals were dosed separately with gelatine capsules only.

The hens were subjected to a veterinarian inspection on arrival and deemed healthy and to have good egg producing capacity. The hens were acclimated to the study accommodation at Charles River Laboratories for 14 days prior to the start of dosing.

The hens were observed at least twice daily for general health and appearance during the pre-trial and on-study periods and were in good general health throughout the acclimation and dosing periods of the study.

Feed consumption, egg yield, and bodyweight was monitored for 14 days of acclimation and throughout the duration of the dosing period. Feed consumption, egg yield, and bodyweight remained relatively constant throughout the acclimation and dosing periods. Based on these observations and findings, it can be inferred that treatment with [^{14}C]-*N*-acetylglyphosate did not result in adverse effects on the hens.

2. Sampling and storage

Excreta samples were collected once daily from pre-dose until Study Day 7 (the day of sacrifice). After collection of excreta, each cage was rinsed with water and the cage wash pooled per treatment group and retained for total radioactivity analysis. Each composite cage wash sample was left for *ca* 24 hours prior to total radioactivity analysis. At the time of egg collection, eggs were wiped and the tissue added to the appropriate cage wash. At each timepoint, the excreta samples from the treated hens and the control hens were pooled (separately) and each composite sample weighed. Water was added to each composite sample at a ratio of *ca* 1:1 (solvent:excreta) and the sample was homogenised. At each timepoint, a sub-sample (10 %) was removed from the treated hen composite excreta sample and the weight recorded. The sub-sample was added to a total composite excreta sample container and was stored at *ca* -20 °C between subsequent sub-sample additions.

Eggs were collected twice daily from Study Day 14 (pre-trial) to Study Day 7 (the day of sacrifice). The eggs collected in the afternoon were pooled with eggs collected the next morning. Each egg collected from Day 1 until Day 7 was separated into egg yolk, egg white, and shell with care taken not to contaminate any egg yolk or egg white samples with shell particles. The egg yolk and egg white for the treated hens and the egg yolk and egg white for the control hens were pooled (10 % by weight of each hen's production) at each time point per group, and the composite weight of each sample was recorded. Composite samples of egg whites and egg yolks were homogenised (separately). The eggshell samples were not analysed for radioactive content.

The five treated hens and one control hen were sacrificed on Study Day 7, *ca* 6 hours after administration of the final capsule (*ca* 158 hours post first dose). Each hen was sacrificed by dislocation of the neck. Following sacrifice, each carcass was plunged into hot water (*ca* 80 °C) and the feathers plucked. Tissues and organs collected included the liver (whole), muscle, fat, and eggs. The muscle sample was a composite of approximately equal portions of thigh and breast muscle (all available thigh muscle was collected along with an approximately equal quantity of breast muscle). The skin with the subcutaneous fat was removed from each muscle sample. The fat sample consisted of the complete abdominal fat pad from each hen. Any whole eggs from the oviduct were collected and processed with the eggs collected from the last 24-hour period. Any partially formed eggs were processed separately. Each liver, muscle, and abdominal fat sample was weighed and samples from the treated hens were pooled (by tissue type).

Excreta and tissue samples not analysed immediately were stored at *ca* -20 °C following collection. Egg samples were stored at *ca* 4 °C prior to analysis. After analysis, samples were stored at *ca* -20 °C. Cage wash samples were stored at ambient temperature.

3. Analytical procedures

Specimen of excreta, cage wash, egg whites, egg yolk, and tissues were analysed in triplicates to quantify total radioactivity. Aliquots of pooled cage wash, egg whites, and egg yolk (each mixed with water) from each timepoint were taken and radioactivity was quantified using liquid scintillation counting (LSC). Following homogenisation, pooled excreta aliquots from each timepoint were taken for combustion analysis and LSC. Pooled tissue samples from treated hens were prepared for analysis (grated/chopped/homogenised to produce a fine powder). Aliquots were collected form composite liver and muscle samples for combustion analysis and quantification of total radioactivity by LSC. Aliquots of composite abdominal fat samples were analysed using LSC.

Following quantification of total radioactivity, further analysis was conducted on excreta, egg white, egg yolk, and tissue samples to determine extraction, characterisation, and identification of residues. The composite excreta, egg white, and egg yolk samples produced from pooled samples collected throughout

the dosing period (Study Days 1-7) were allowed to thaw and homogenised to ensure homogeneity. Prior to chromatographic analysis, the composite samples were analysed to confirm concentration and homogeneity of each. Triplicate aliquots of composite samples of excreta, egg white, and egg yolk were taken. Excreta and egg yolk were analysed using combustion and LSC, and egg white samples were analysed using LSC.

The composite excreta sample was extracted three times with water. On each occasion, samples were homogenised, centrifuged, and the supernatant decanted. The extracts were combined, the total volume measured, and triplicate aliquots removed for LSC. The extract was concentrated to dryness by rotary evaporation then reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v). The extracted radioactive residues were determined by assaying triplicate aliquots of each extract by LSC. Triplicate aliquots (*ca* 0.3 g) of the post-extracted solid (PES) were assayed by combustion followed by LSC analysis.

An aliquot of the composite egg white sample was extracted three times with 0.2 N hydrochloric acid using a homogeniser. Approximately 20 mL dichloromethane and 90 mL chloroform were added to the extract and the sample shaken. The sample was centrifuged and the aqueous layer removed. The extraction process with 0.2 N hydrochloric acid was repeated two additional times. The aqueous extracts were combined, and the radioactive content determined by LSC. The dichloromethane/chloroform layer was added to the extract and the mixture partitioned two times against an equal volume of hexane to remove fatty material. The radioactive content of the hexane and dichloromethane/chloroform fractions were determined by LSC analysis. The aqueous extract was then concentrated to dryness by rotary evaporation, reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v), and analysed by LSC. Prior to HPLC analysis, an aliquot of the concentrated extract was reduced to dryness under a stream of nitrogen and reconstituted in HPLC mobile phase A (0.025 M potassium phosphate (pH 2.3): methanol (96:4, v/v)). Triplicate sub-samples of the PES were removed and submitted for combustion and LSC analysis.

An aliquot of the composite egg yolk sample was extracted three times with 0.2 N hydrochloric acid:methanol (1:1, v/v) on ice using a homogenizer. Dichloromethane was added to the extract and the sample shaken. The sample was centrifuged and the aqueous layer removed. The extraction process with 0.2 N hydrochloric acid:methanol (1:1, v/v) was repeated two additional times. The aqueous extracts were combined, and the radioactive content determined by LSC. The aqueous extract remaining was then concentrated to dryness by rotary evaporation, reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v), and analysed by LSC. Prior to HPLC analysis, an aliquot of the concentrated extract was reduced to dryness under a stream of nitrogen then reconstituted in HPLC mobile phase. The radioactive content of the residue recovered from the dichloromethane layer (PES) was determined by combustion prior to LSC analysis.

Liver, muscle, and fat samples were extracted three times with 0.2 N hydrochloric acid. On each occasion, the sample was macerated followed by centrifugation and decanting of the extract. The abdominal fat was heated to *ca* 37 °C in a water bath prior to extraction. The extracts were combined and radioactivity was determined using LSC. The extract (for muscle and fat) was partitioned three times against an equal volume of hexane to remove fatty material. The radioactive content of the hexane fraction was determined by LSC analysis. The aqueous extract remaining was then concentrated to dryness by rotary evaporation, reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v), and analysed by LSC. Prior to HPLC analysis, an aliquot of the concentrated extract was reduced to dryness under a stream of nitrogen then reconstituted in HPLC mobile phase A. Triplicate sub-samples of the PES were removed and submitted for combustion and LSC analysis.

Egg yolk and liver non-solvent extractable radioactive residues were in excess of 0.01 mg/kg and as such, these residues were further characterised by enzyme hydrolysis (pepsin and protease).
The PES from egg yolk and liver were mixed with pepsin and 0.1 N hydrochloric acid. Samples were incubated (37 °C) in a shaking water bath for approximately 30 hours. Following incubation, the

radioactive content of the samples was determined by LSC before and after filtration. The post-extracted solids (including previously used filter paper) and protease enzyme were added to phosphate buffer (pH 7.5). Samples were incubated (37 °C) in a shaking water bath for approximately 24 hours and the radioactive content of both samples was measured before and after filtration.

Attempts were made to clean up the enzyme digests using iron loaded Chelex 100 ligand exchange resin followed by AG1X8 resin columns. Procedural recoveries following column clean up were low (*ca* 17 – 37 %) resulting in low levels of radioactivity present. Additionally, an aliquot of the liver pepsin digest was mixed with a dehydration agent in an attempt to reduce the volume and retain suitable samples of the water-soluble components for chromatographic analysis. This procedure failed to provide sufficiently clean samples to allow HPLC analysis. As a consequence of the low levels of radioactivity, it was not possible to profile these samples.

Extracts from excreta, egg whites, egg yolk, and tissue were analysed by HPLC. Peak assignment was done by HPLC retention comparison with authentic radiolabelled and un-labelled reference standards in one system by HPLC. Furthermore, aliquots of excreta extract (containing glyphosate and mainly *N*-Acetylglyphosate) and reference standards were analysed by LC-MS/MS.

II. Results and discussion

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in the table below. A total of 90.18 % of the administered dose was recovered. The majority of the administered dose was collected in excreta (84.14 %) and cage wash (5.94 %). Egg samples only accounted for a total of 0.05 % of the administered dose (0.01 % in egg white and 0.04 % in egg yolk). Distribution of radioactive residues in tissues (liver, muscle, and fat) accounting for 0.05 % of the applied dose in total.

Additionally, listed in the table below are concentrations of total radioactive residues (TRR) in egg white, egg yolk, and tissues (liver, muscle, and fat), expressed as *N*-acetylglyphosate equivalents. Higher concentrations were observed in the egg yolks than in whites. By the end of the dosing period (Study Day 7), TRR, expressed as *N*-acetylglyphosate equivalents, in egg whites was 0.019 mg/kg and in egg yolk was 0.342 mg/kg. Among tissues, the highest concentration of TRR was observed in liver (0.511 mg/kg). Muscle samples were determined to have the lowest concentration of TRR (0.039 mg/kg), while fat contained TRR at 0.051 mg/kg.

In addition to the concentration of TRR expressed as *N*-acetylglyphosate equivalents, TRR concentration is also displayed in the table below (and in other tables that follow) in glyphosate equivalents. The glyphosate equivalent values were not included in the study report, but were calculated from TRR expressed as *N*-acetylglyphosate equivalents and a conversion factor of 0.8, based on the molecular weights of glyphosate and *N*-acetylglyphosate.

Table 6.2.2-35: Distribution and concentration of radioactive residues in excreta, cage wash, eggs, and tissues of laying hens after oral administration of [^{14}C]-*N*-acetylglyphosate for 7 consecutive days

Matrix	% Administered dose	TRR (mg *N*-acetylglyphosate equivalents/kg)[1]	*TRR (mg glyphosate equivalents/kg)*[2]
Excreta	84.14	NA[3]	*NA*
Cage wash	5.94	NA	*NA*
Egg white[4]	0.01	0.019	*0.015*
Egg yolk[4]	0.04	0.342	*0.274*
Liver	0.05	0.511	*0.409*
Muscle	NA	0.039	*0.031*
Abdominal fat	NA	0.051	*0.041*
Total recovery	90.18	NA	*NA*

[1] TRR = total radioactive residue, expressed as *N*-acetylglyphosate equivalents.
[2] Total radioactive residues, expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.
[3] NA = not applicable
[4] Egg white and egg yolk TRR concentrations are the results for the sample collected at the end of the dosing period (Study Day 7). The % administered dose value is the cumulative dose collected across all 7 dosing days.

In the table below, the concentration of total radioactive residues (TRR), expressed as *N*-acetylglyphosate equivalents, are summarised for daily egg samples (egg whites and egg yolk) collected over the dosing period (Study Days 1-7). Residue concentration in both egg whites and egg yolk continued to increase during the 7-day dosing period and reached the highest observed concentration on the last day of dosing, Study Day 7. Therefore, it is unclear if residues in egg matrices reached a plateau level by the end of the dosing period. In addition to the concentration of TRR expressed as *N*-acetylglyphosate equivalents, TRR concentration is also displayed in the table below in glyphosate equivalents (calculated value added during dossier compilation).

Table 6.2.2-36: Radioactive residues in egg white and yolk of laying hens during oral administration [^{14}C]-*N*-acetylglyphosate over a period of 7 consecutive days

Study Day	TRR (mg/kg)			
	Egg white		Egg yolk	
	N-acetylglyphosate equivalents[1]	*Glyphosate equivalents*[2]	*N*-acetylglyphosate equivalents[1]	*Glyphosate equivalents*[2]
1	0.001	*0.001*	0.000	*0.000*
2	0.009	*0.007*	0.044	*0.035*
3	0.013	*0.010*	0.093	*0.074*
4	0.015	*0.012*	0.197	*0.158*
5	0.015	*0.012*	0.294	*0.235*
6	0.015	*0.012*	0.295	*0.236*
7	0.019	*0.015*	0.342	*0.274*

[1] TRR = total radioactive residue, expressed as *N*-acetylglyphosate equivalents
[2] TRR = total radioactive residues, expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.

B. Extraction and characterisation of residues

Results of extraction and characterisation/identification of residues in excreta, egg whites, egg yolk, and edible tissues (liver, muscle, and fat) are described and summarised below.

A summary of the results of extraction and identification of residues in excreta is shown the table below. Extraction of the composite excreta sample from Study Days 1-7 recovered 83.17 % of the administered dose (a total of 84.14 % of the administered dose was found in faeces). Processing (concentration) of the extract resulted in loss of 5.58 % of the administered dose (6.6 % TRR in excreta), which was considered minor. The concentrated extract was assumed to be representative of the initial extract and study results were presented as such. Two radiolabelled components were detected in the excreta radiochromatogram, the most abundant was *N*-acetylglyphosate and accounted for 82.38 % dose. Glyphosate was detected and accounted for 0.79 % dose. Unextracted residues accounted for 0.97 % of the administered dose.

Table 6.2.2-37: Extraction and identification of the radioactive residues in composite excreta from laying hens dosed with [^{14}C]-*N*-acetylglyphosate for 7 consecutive days

Fraction / component	% Administered dose
	Excreta
TRR	**84.14**
ERR	**83.17**
Concentrated aqueous extract	77.59
Glyphosate	0.79
N-acetylglyphosate	82.38
Total identified	***83.17***
Total characterised	**-**
RRR	**0.97**
Differences during processing[1]	5.58

[1] Differences during processing reflect a loss (6.6 % TRR) incurred during concentration and/or sample clean up for HPLC analysis. The concentrated extract was assumed to be representative of the initial extract and metabolite concentrations were calculated as such.
Values in italics were calculated upon dossier compilation.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue

A summary of the results of extraction and identification of residues in egg white and egg yolk is shown in the table below.

Extraction of the composite egg white sample (Study Days 1-7) recovered 94.33 % TRR (0.009 mg/kg *N*-acetylglyphosate equivalents). Subsequent processing of the extract resulted in no loss of radioactivity. Four radiolabelled components were detected in the radiochromatogram for egg white, the most abundant was *N*-acetylglyphosate and accounted for 41.48 % TRR (0.004 mg/kg). Glyphosate and *N*-acetyl AMPA were detected and accounted for 10.90 % TRR (0.001 mg/kg) and 4.34 % TRR (<0.001 mg/kg), respectively. A single minor unknown component, which was less polar than *N*-acetylglyphosate, accounted for 3.40 % TRR (<0.001 mg/kg). The remaining non-extractable residue was determined as 5.67 % TRR (0.001 mg/kg), which was not investigated further.

Extraction of the composite egg yolk sample (Study Days 1-7) recovered 81.47 % TRR (0.187 mg/kg *N*-acetylglyphosate equivalents). Subsequent processing of the extract resulted in no significant loss of radioactivity. Four radiolabelled components were detected in the radiochromatogram for egg yolk, the most abundant was *N*-acetylglyphosate and accounted for 68.40 % TRR (0.157 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA were detected and accounted for 0.91 % TRR (0.002 mg/kg), 5.69 % TRR (0.013 mg/kg), and 1.10 % TRR (0.003 mg/kg), respectively. The remaining non-extractable residue was determined as 18.53 % TRR (0.042 mg/kg), which was further investigated using pepsin and protease enzyme hydrolysis. Pepsin digest of egg yolks released an additional 11.61 % TRR (0.027 mg/kg). Attempts were made to suitably concentrate and clean the sample to allow HPLC analysis; however, losses were significant such that the cleaned sample contained 4.33 % TRR (0.010 mg/kg). The low levels in the cleaned sample precluded further characterisation of the yolk residues released by pepsin

digestion. Protease digestion of the egg yolk residues (recovered from pepsin digestion) yielded 3.10 % TRR (0.007 mg/kg). In light of the results of the pepsin clean up, no further processing of this fraction was undertaken. Unextracted residues accounted for 3.82 % TRR (0.008 mg/kg).

Table 6.2.2-38: Extraction and identification of the radioactive residues in composite egg white and yolk from laying hens dosed with [^{14}C]-*N*-acetylglyphosate for 7 consecutive days

Fraction / component	Egg white			Egg yolk		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
TRR	**100**	**0.010**	***0.008***	**100**	**0.229**	***0.183***
ERR	**94.33**	**0.009**	***0.007***	**81.47**	**0.187**	***0.150***
Concentrated aqueous extract	94.33	0.009	*0.007*	80.01	0.183	*0.146*
AMPA	-	-	-	0.91	0.002	*0.002*
Glyphosate	10.90	0.001	*0.001*	5.69	0.013	*0.010*
N-acetyl AMPA	4.34	<0.001	*<0.001*	1.10	0.003	*0.002*
N-acetylglyphosate	41.48	0.004	*0.003*	68.40	0.157	*0.126*
Minor unknown(s)	3.40[3]	<0.001[3]	*<0.001*[3]	-	-	-
Hexane fraction	<0.01	<0.001	*<0.001*	-	-	-
RRR	**5.67**	**0.001**	***0.001***	**18.53**	**0.042**	***0.034***
Pepsin digest	-	-	-	11.61	0.027	*0.022*
Processed pepsin digest	-	-	-	4.33	0.010	*0.008*
Protease digest	-	-	-	3.10	0.007	*0.006*
Total identified	***56.72***	***0.006***	***0.005***	***76.10***	***0.175***	***0.140***
Total characterised	***3.40***	***<0.001***	***<0.001***	***14.71***	***0.034***	***0.028***
Final residue	**5.67**	**0.001**	***0.001***	**3.82**	**0.008**	***0.006***
Differences during processing[4]	<0.01	<0.001	*<0.001*	1.47	0.004	*0.003*

[1] Values expressed as *N*-acetylglyphosate equivalents.
[2] Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.
[3] Comprised of a single component.
[4] Differences during processing reflect losses incurred during concentration and/or sample clean up for HPLC analysis. The concentrated extract was assumed to be representative of the initial extract and metabolite concentrations were calculated as such.
Values in italics were calculated upon dossier compilation. Values <0.001 mg/kg were set as 0.001 mg/kg.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue

A summary of the results of extraction and identification of residues in liver and muscle is shown in the table below.

Initial extraction of liver recovered 95.56 % TRR (0.483 mg/kg *N*-acetylglyphosate equivalents). Subsequent concentration of the liver extract resulted in losses (31.7 % TRR) postulated as resulting from non-selective adsorption to particulate matter in the concentrate. Attempts were made to recover the losses on concentration by rinsing the particulates and the apparatus with 0.1 % trifluoroacetic acid in water:methanol (96:4, v/v), however the adsorption to particulates was not reversible. Concentrated extracts were assumed to be representative of the initial extract and were calculated as such. Four radiolabelled components were detected in the radiochromatogram for liver, the most abundant was *N*-acetylglyphosate and accounted for 63.82 % TRR (0.323 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA were detected and accounted for 6.74 % TRR (0.034 mg/kg), 16.34 % TRR (0.084 mg/kg)

and 4.04 % TRR (0.020 mg/kg), respectively. The remaining non-extractable residue was determined as 4.44 % TRR (0.022 mg/kg), which was further investigated using pepsin and protease enzyme hydrolysis. Following pepsin digest of liver, a further 3.81 % TRR (0.019 mg/kg) was released. Attempts were made to concentrate and clean the sample to allow HPLC analysis; however, losses were significant such that the cleaned sample contained 0.63 % TRR (0.003 mg/kg). The low levels in the cleaned sample precluded further characterisation of the liver residues released by pepsin digestion. Protease digestion of the liver residues (recovered from pepsin digestion) yielded a further 0.27 % TRR (0.001 mg/kg). In light of the results of the pepsin clean up, no further processing of this fraction was undertaken. Unextracted residues accounted for 0.36 % TRR (0.002 mg/kg).

Extraction of muscle recovered 87.47 % TRR (0.029 mg/kg *N*-acetylglyphosate equivalents). Subsequent concentration of the muscle extract resulted in minor losses of radioactivity, however, the levels of radioactivity in the initial extracts were too low to accurately determine the losses and as such, the procedural recovery was regarded as quantitative. Eight radiolabelled components were detected in the radiochromatogram for muscle extract, the most abundant was *N*-acetylglyphosate and accounted for 25.22 % TRR (0.009 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA were detected and accounted for 16.69 % TRR (0.005 mg/kg), 7.19 % TRR (0.002 mg/kg), and 1.89 % TRR (0.001 mg/kg), respectively. The remaining four unknown components were minor in nature with none accounting for more than 8.95 % TRR (0.003 mg/kg). The remaining non-extractable residue was determined as 12.53 % TRR (0.004 mg/kg), which was not investigated further.

Table 6.2.2-39: Extraction and identification of the radioactive residues in liver and muscle from laying hens dosed with [^{14}C]-*N*-acetylglyphosate for 7 consecutive days

Fraction / component	Liver			Muscle		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
TRR	**100**	**0.505**	***0.404***	**100**	**0.033**	***0.026***
ERR	**95.56**	**0.483**	***0.386***	**87.47**	**0.029**	***0.023***
Concentrated aqueous extract	63.86[3]	0.322	*0.258*	87.47	0.029	*0.023*
AMPA	6.74	0.034	*0.027*	16.69	0.005	*0.004*
Glyphosate	16.34	0.084	*0.067*	7.19	0.002	*0.002*
N-acetyl AMPA	4.04	0.020	*0.016*	1.89	0.001	*0.001*
N-acetylglyphosate	63.82	0.323	*0.258*	25.22	0.009	*0.007*
Minor unknown(s)		-	-	14.86[3]	0.006[3]	*0.005[3]*
Hexane fraction	-	-	-	<0.01	<0.001	*<0.001*
RRR	**4.44**	**0.022**	***0.018***	**12.53**	**0.004**	***0.003***
Pepsin digest	3.81	0.019	*0.015*	-	-	-
Processed pepsin digest	0.63	0.003	*0.002*	-	-	-
Protease digest	0.27	0.001	*0.001*	-	-	-
Total identified	***90.94***	***0.461***	***0.369***	***50.99***	***0.017***	***0.014***
Total characterised	***4.08***	***0.020***	***0.016***	***14.86***	***0.006***	***0.005***
Final residue	**0.36**	**0.002**	***0.002***	**12.53**	**0.004**	***0.003***

Table 6.2.2-39: Extraction and identification of the radioactive residues in liver and muscle from laying hens dosed with [^{14}C]-*N*-acetylglyphosate for 7 consecutive days

Fraction / component	Liver			Muscle		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
Differences during processing[4]	31.70[5]	0.161[5]	*0.129*	<0.01	<0.001	*<0.001*

[1] Values expressed as *N*-acetylglyphosate equivalents.
[2] Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.
[3] Comprising up to 4 components, with no component accounting for greater than 8.95 % TRR (0.003 mg/kg).
[4] Differences during processing reflect losses incurred during concentration and/or sample clean up for HPLC analysis. The concentrated extract was assumed to be representative of the initial extract and metabolite concentrations were calculated as such.
[5] Losses (31.7 % TRR) during processing were postulated as resulting from non-selective adsorption to particulate matter in the concentrated extract.
Values in italics were calculated upon dossier compilation.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue

A summary of the results of extraction and identification of residues in abdominal fat is shown the table below.

Extraction of abdominal fat recovered 92.42 % TRR (0.053 mg/kg *N*-acetylglyphosate equivalents). Subsequent processing of the extract resulted in no significant loss of radioactivity. Six radiolabelled components were detected in the radiochromatogram for fat, the most abundant was glyphosate and accounted for 39.43 % TRR (0.023 mg/kg). AMPA, *N*-acetyl AMPA, and *N*-acetylglyphosate were detected and accounted for 11.29 % TRR (0.007 mg/kg), 10.18 % TRR (0.006 mg/kg), and 23.45 % TRR (0.014 mg/kg), respectively. The remaining two components were minor in nature with none accounting for more than 0.71 % TRR (<0.001 mg/kg). The remaining non-extractable residue was determined as 7.58 % TRR (0.004 mg/kg), which was not investigated further.

Table 6.2.2-40: Extraction and identification of the radioactive residues in abdominal fat from laying hens dosed with [^{14}C]-*N*-acetylglyphosate for 7 consecutive days

Fraction / component	Abdominal fat		
	% TRR	**mg/kg (*N*-acetyl-glyphosate equivalents[1])**	***mg/kg (glyphosate equivalents[2])***
TRR	**100**	**0.057**	***0.046***
ERR	**92.42**	**0.053**	***0.042***
Concentrated aqueous extract	92.42	0.053	*0.042*
AMPA	11.29	0.007	*0.006*
Glyphosate	39.43	0.023	*0.018*
N-acetyl AMPA	10.18	0.006	*0.005*
N-acetylglyphosate	23.45	0.014	*0.011*
Minor unknown(s)[3]	1.37	0.001	*0.001*
Hexane fraction	<0.00	<0.000	*<0.000*
Total identified	***84.35***	***0.050***	***0.040***
Total characterised	***1.37***	***0.001***	***0.001***
RRR	**7.58**	**0.004**	***0.003***
Differences during processing[4]	<0.01	<0.001	*<0.001*

[1] Values expressed as *N*-acetylglyphosate equivalents.
[2] Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.
[3] Comprised of up to 2 components, with no component accounting for greater than 0.71 % TRR (<0.001 mg/kg).
[4] Differences during processing reflect losses incurred during concentration and/or sample clean up for HPLC analysis. The concentrated extract was assumed to be representative of the initial extract and metabolite concentrations were calculated as such.
Values in italics were calculated upon dossier compilation.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue

C. Storage stability

The samples remained frozen prior to analysis. An analysis of storage stability was not conducted as part of this study since samples were analysed within 4 months of collection.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

N-acetylglyphosate was administered twice daily to laying hens as an oral dose of [^{14}C]-*N*-acetylglyphosate via gelatine capsule for 7 consecutive days. The actual dose level achieved was 63.311 mg *N*-acetylglyphosate/kg feed based on average daily feed consumption during the 7-day dosing interval. Based on average bodyweight by individual animal on Study Days 1 and 7 (during the dosing period) and corresponding daily dosage by individual animal, the actual dose administered was 4.391 mg *N*-acetylglyphosate/kg bw/day.

N-acetylglyphosate and its metabolites were eliminated rapidly by the hens, primarily in the excreta, accounting for 90.08 % of the administered dose (including cage wash). Total radioactive recovery was 90.18 % of the dose, not including the radioactivity in the gastrointestinal contents (which were not analysed).

Higher concentrations of total radioactive residues were observed in the egg yolks (0.044 – 0.342 mg/kg) than in whites (0.001 – 0.019 mg/kg). In both egg white and egg yolk, the most abundant residue identified was *N*-acetylglyphosate that was found at 41.48 % TRR (0.004 mg/kg) in egg white and at 68.40 % TRR (0.157 mg/kg) in egg yolk. Additionally, the metabolites identified in egg white were glyphosate (0.001 mg/kg) and *N*-acetyl AMPA (<0.001 mg/kg). In egg yolk, the metabolites identified were glyphosate (0.013 mg/kg), *N*-acetyl AMPA (0.003 mg/kg), and AMPA (0.002 mg/kg).

The total radioactive residues in the edible tissues ranged from 0.039 mg/kg (muscle) to 0.511 mg/kg (liver). The predominant residue found in liver and muscle was *N*-acetylglyphosate (0.323 mg/k and 0.009 mg/kg, respectively), and glyphosate (0.023 mg/kg) in fat. *N*-acetyl AMAP, and AMPA, as well as *N*-acetylglyphosate, and glyphosate were observed in all tissues evaluated (muscle, liver, and abdominal fat).

Based on results of this study, it is concluded that there was not a significant transfer of *N*-acetylglyphosate and its metabolites to eggs or edible tissues (liver, muscle, and fat). Eggs and edible tissues contained <0.1 % of the total administered dose.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behavior of glyphosate in laying hens has been previously evaluated at EU level. It was performed under GLP. The study does not entirely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 503 with deficits (TG 503 recommends use of 10 birds, and 5 treated birds were used in this study; identification was done by HPLC retention comparison with authenticated standards in one system by HPLC; balance of components in matrices (egg white and muscle) with low absolute residue concentrations misses portions of up to 34.21 % TRR or 0.006 mg/kg (recovery or calculation issue)).
Nevertheless, the study is considered to be supportive for the metabolism in laying hens.

Assessment and conclusion by RMS:

Overall assessment and conclusion on metabolism studies

Overall summaries are given in the following. Data are summarised also in Appendix G, and those studies where the nature of residues was investigated are considered for the definition of residues (see CA 6.7.1).

Five metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **poultry**. An overview on the studies is given in the following table:

Table 6.2.2-41: Overview over available poultry metabolism studies

Poultry	Application	Application dose	Reference
Laying hen	7 daily applications (once daily) via gavage	*N*-(phosphono-^{14}C-methyl)glycine 17.9 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.2/001: [redacted] 1994, (^{14}C)-Glyphosate: Distribution, metabolism and excretion following repeated oral administration to the laying hen, Report No. 676/8-1011
	5 daily applications (once daily) via gavage	*N*-(phosphono-^{14}C-methyl)glycine 17.2 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	
Laying hen	7 daily application (once daily) via gelatine capsules	*N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (9:1 mixture) 9.84 mg/kg bw/day (8.86 mg glyphosate/kg bw/day and 0.98 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.2/002: [redacted] 1988, Metabolism of ^{13}C/^{14}C -labeled glyphosate and aminomethylphosphonic acid in laying hens. Part I., Report No. [redacted]-7591 and CA 6.2.2/003: [redacted], 1988, Metabolism of ^{14}C/^{13}C-labeled glyphosate and aminomethylphosphonic acid in laying hens. Part II., Report No. [redacted]-7420
		N-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (9:1 mixture) 8.83 mg /kg bw/day (7.95 mg glyphosate/kg bw/day and 0.88 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	
		N-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (9:1 mixture) 29.75 mg/kg bw/day (26.78 mg glyphosate/kg bw/day and 2.98 mg AMPA/kg bw/day) or 400 mg/kg feed (expressed in glyphosate equiv.)	
	7 daily application (once daily) via gelatine capsules + 10 days depuration	*N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (9:1 mixture) 8.62 mg/kg bw/day (7.76 mg glyphosate/kg bw/day and	

Table 6.2.2-41: Overview over available poultry metabolism studies

Poultry	Application	Application dose	Reference
		0.86 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	
Laying hen	10 daily applications (once daily) via gelatine capsules	*N*-(phosphono-^{14}C-methyl)glycine (as trimesium salt) 4.1 mg/kg bw/day or 62.4 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.2/004: [redacted] 1994, [^{14}C-PMG] Glyphosate-trimesium: Nature of the residue in tissues and eggs of laying hens, Report No. RR-93-064B
Laying hen	7 daily applications (twice daily) via gelatine capsules	*N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine 3.513/kg bw/day or 50.649 mg/kg feed (expressed in glyphosate equiv.) 4.391 bw/day or 63.311 mg/kg feed (expressed in *N*-acetyl-glyphosate equiv.)	CA 6.2.2/005: [redacted] 2007, The metabolism of [^{14}C]-*N*-acetylglyphosate (IN-MCX20) in laying hens, Report No. [redacted] 19795

In [redacted] **1994,** *N*-(phosphono-^{14}C-methyl)glycine was administrated to laying hens (two groups of five animals each) once daily for seven (Group A) or five consecutive days (Group B), respectively. Animals of Group A were sacrificed at ca. 23.5 h after the final dose, and hens of Group B were sacrificed at plasma radioactivity c_{max} ca. 1 h after the last dosing.
Approximately 80 % of the administered dose was recovered in the case of Group A in total, and approximately 65 % was recovered in the case of Group B (study termination closer to the final dose). The major portions of radioactive residues were recovered in excreta (63.65 – 76.45 % of the administrated dose), cage washings (0.81 – 2.98 % of the administrated dose) and cage debris (0.75 – 0.88 % of the administrated dose). Less than 0.04 % of the administered dose was associated in both groups with edible matrices (egg white, egg yolk and tissues in sum). At study termination, the highest radioactive residues in the relevant edible matrices were detected in liver (1.080 – 1.242 mg eq/kg).
Excreta and edible matrices (tissues and eggs) of animals of Group B were extracted and the extracts were further analysed for metabolite identification. The major residue in the extracts of all matrices of this treatment group was unchanged **glyphosate** (approximately 61 – 99 % of the total area detected in the analysed samples ("% Total"); % Total are presented and not % TRR as these values represent a worst case) according to HPLC data; in egg white: glyphosate only radioactive region in TLC of final extracts, below detection limit of HPLC). Indications for the occurrence of the metabolite **AMPA** from minor TLC regions (up to 14 % Total) in the extracts of liver and skin or of unknown components in the extracts of skin, fat and egg yolk were not substantiated by HPLC and therefore supposed to be chromatographic artefacts.

Four treatment groups with laying hens were performed to investigate the behaviour of *N*-(phosphono-^{13}C/^{14}C-methyl)glycine (^{13}C/^{14}C-glyphosate) and amino-^{13}C/^{14}C-methylphosphonic acid (^{13}C/^{14}C-AMPA) in poultry ([redacted] **1988** and [redacted] **1988**).
In the three low treatment groups and in the high treatment group the hens each received one capsule each for 7 days. In two low treatment groups (replicates) and the high treatment group, the hens were sacrificed 22 to 24 hours after the last dose. In the third low treatment group, a 10-day depuration phase was added after the 7th dose after which the hens were sacrificed.
Only minor amounts of the administered radioactivity (AR) were found in egg yolk (0.01 – 0.02 % AR), egg white (<0.01 % AR) and tissues (up to 0.02 % AR). Elimination of radioactivity via excreta was the primary elimination route, ranging from 81.0 to 90.5 % of AR.

Of the relevant matrices of the hen, highest total radioactive residues were found in the kidney (0.069 – 7.004 mg/kg), followed by liver (0.079 – 1.914 mg/kg) and egg yolk (0.090 – 0.344 mg/kg). Residues in muscle, fat and egg white were much lower, generally not exceeding 0.1 mg/kg. The radioactivity level in tissues of the depuration group were generally lower; the liver had the highest level (0.079 mg/kg).
Glyphosate (28.1 – 93.2 % TRR) and **AMPA** (4.2 – 53.1 % TRR) were identified in edible matrices. Some evidence for further metabolism of glyphosate and AMPA was observed in the muscles, where a minor unknown metabolite was detected (≤0.005 mg/kg).

In [redacted] **1994**, *N*-(phosphono-^{14}C-methyl)glycine (^{14}C-PMG-labeled glyphosate) was administered orally for 10 days to 10 laying hens as its trimesium salt. The hens were sacrificed 12 to 15 hours after the last dosing.
104.0 % of the administered dose was recovered in total. The major portion of radioactive residue was recovered in excreta, cage rinse and GI tract with contents. Radioactive residue associated with edible portions (eggs and tissue) accounted in sum for 0.1286 %.
Of the relevant edible matrices of the hen, highest total radioactive residues were found in the kidney (2.17 mg/kg) and liver (0.440 mg/kg). Residues in thigh muscle, breast muscle, fat, egg yolk (day 10) and egg white (day 10) were between 0.0169 and 0.238 mg/kg.
PMG (glyphosate-anion) (34.9 – 56.0 % TRR or 0.0101 – 0.2272 mg/kg) and **AMPA** (2.14 – 18.9 % TRR or 0.0008 – 0.0828 mg/kg) accounted for the majority of the radioactive residue in all matrices. An unknown compound present at lower levels (1.1 – 4.3 % TRR) was characterised by its extraction behaviour and its retention time in two different chromatographic systems.
In egg yolk and fat a major fraction of the ^{14}C-residues was due to natural incorporation into lipids. In egg yolk the ^{14}C incorporation was detected in the nonpolar lipid fraction (**triglycerides and cholesterol**), and in the phospholipid fraction (mainly in **phosphatidylcholine**). In fat, the ^{14}C-natural incorporation was shown in **triglycerides**, **cholesterol** and **free fatty acids**.

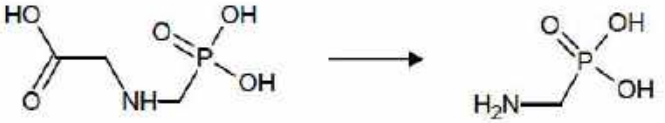
In [redacted] **2007** [^{14}C]-*N*-acetyl-*N*-(phosphomethyl)glycine ([^{14}C]-*N*-acetylglyphosate) was administered to five laying hens (twice daily) for 7 consecutive days. The hens were sacrificed approximately 6 hours after the last dose.
N-acetylglyphosate and its metabolites were eliminated rapidly by the hens, primarily in the excreta, accounting for 90.08 % of the administered dose (including cage wash). Total radioactive recovery was 90.18 % of the dose, not including the radioactivity in the gastrointestinal contents (which were not analysed).
Higher concentrations of total radioactive residues were observed in the egg yolks (0.035 – 0.274 mg/kg) than in egg white (0.001 – 0.015 mg/kg). These values, as well as the other values are expressed as glyphosate equivalent (calculated based on a conversion factor of 0.8). In both egg white and egg yolk, the most abundant residue identified was ***N*-acetylglyphosate** that was found at 41.48 % TRR (0.003 mg/kg) in egg white and at 68.40 % TRR (0.126 mg/kg) in egg yolk. Additionally, the metabolites identified in egg white were **glyphosate** (10.90 % TRR or 0.001 mg/kg) and ***N*-acetyl AMPA** (4.34 % TRR or <0.001 mg/kg). In egg yolk, the metabolites identified were **glyphosate** (5.69 % TRR or 0.010 mg/kg), ***N*-acetyl AMPA** (1.10 % TRR or 0.002 mg/kg), and **AMPA** (0.91 % TRR or 0.002 mg/kg).
The total radioactive residues in the edible tissues were 0.031 mg/kg, 0.041 mg/kg and 0.408 mg/kg in muscle, fat and liver, respectively. The predominant residue found in liver and muscle was ***N*-acetylglyphosate** (63.82 and 25.22 % TRR, respectively, or 0.258 mg/kg and 0.007 mg/kg, respectively), and **glyphosate** (39.43 % TRR or 0.018 mg/kg) in fat. ***N*-acetyl AMPA** (1.10 – 10.18 % TRR or <0.001 – 0.016 mg/kg), and **AMPA** (0.091 – 16.69 % TRR or 0.002 – 0.027 mg/kg), as well as ***N*-acetylglyphosate** (23.45 – 68.40 % TRR or 0.003 – 0.258 mg/kg), and **glyphosate** (5.69 – 39.43 % TRR or 0.001 – 0.067 mg/kg) were observed in all tissues evaluated (muscle, liver, and abdominal fat) as radioactive residues.

Overall conclusion on metabolism in poultry

Within all studies investigating the uptake and metabolism of ^{14}C-glyphosate in poultry it was shown that elimination of radioactivity via excreta was the primary elimination route.

After administration of glyphosate, a mixture of glyphosate and AMPA or PMG-labelled glyphosate (as trimesium salt) to laying hens a similar picture on metabolism was found. **Glyphosate** accounted for the main part of radioactive residues in all studies. Furthermore, **AMPA** was identified as a major metabolite in egg yolk, kidney, liver and muscle. In one study, the radioactivity was also detected in **natural products** (e.g. triglycerides, cholesterol phosphatidylcholine and free fatty acids).

Pathway for livestock (feeding with glyphosate) - poultry

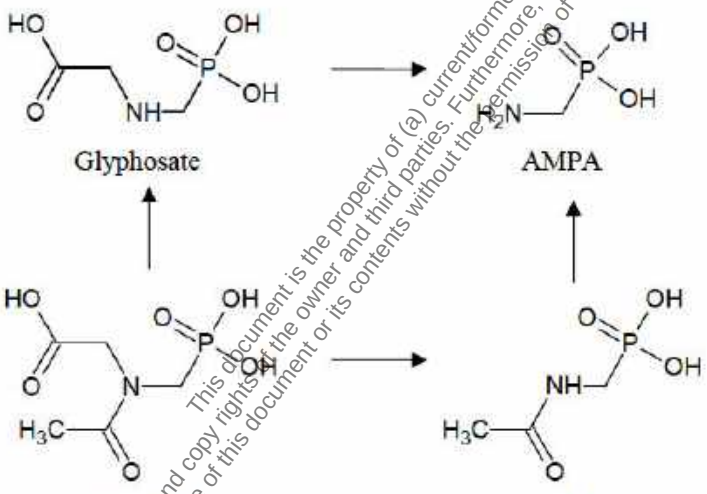


Glyphosate AMPA

AMPA: Major metabolite in egg yolk, kidney, liver and muscle. Minor metabolite in egg white, fat and skin (up to 0.009 mg/kg).

After administration of *N*-acetyl-glyphosate to laying hens ***N*-acetyl glyphosate** accounted for the main part of radioactive residues. **Glyphosate** was identified as major metabolite in liver and fat. In addition **AMPA** and ***N*-acetyl AMPA** were determined in egg white (only *N*-acetyl AMPA), egg yolk, liver, muscle and fat.

Pathway for livestock (feeding with *N*-acetyl glyphosate) - poultry



Glyphosate AMPA

N-acetyl glyphosate *N*-acetyl AMPA

Glyphosate: Major metabolite in liver and abdominal fat. Minor metabolite in egg white, egg yolk and muscle (up to 0.010 mg/kg)

AMPA: Minor metabolite in egg yolk, liver, muscle and fat (up to 0.006 mg/kg).

***N*-acetyl AMPA**: Minor metabolite in egg white, egg yolk, liver, muscle and fat (up to 0.016 mg/kg).

CA 6.2.3 Lactating ruminants

In total four studies on lactation ruminants were conducted; one study was conducted using *N*-(phosphono-^{14}C-methyl)glycine, one study was conducted using *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt, one study was conducted with a 9:1 mixture of *N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid and finally one study was conducted using *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine.

In the following the different metabolism studies on lactating ruminants are summarised as full OECD summaries and are assessed again by the applicant.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.3/001
Report author	[redacted]
Report year	1994
Report title	(^{14}C)-Glyphosate: Absorption, distribution, metabolism and excretion following repeated oral administration to the dairy goat
Report No	676/9-1011
Document No	279 GLY
Guidelines followed in study	EPA nature of the residues in livestock (171-4)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • The radioactivity balance is 89.9 % for Goat 1 and 57.60 % for Goat 2 (sacrificed ca. 23.5 h and ca. 8 h after the final dose, respectively; GIT and its contents and carcasses were not measured) • Urine and faeces were collected only once daily • The radioactivity was not quantified separately in the different muscle types (hind and fore quarter) and fat types • Radioactive residues in fat were below the limits of detection for both animals, but this limits accounted for 0.028 ppm equivalents for Goat 1 and 0.036 ppm equivalents for Goat 2, respectively, which is today above the trigger value • Extractability of radioactive residues not reported in detail (multi-stage extraction procedure), recovery of radioactivity in the further investigated fractions after extraction was only moderate, the organic phases (chloroform) were not further examined, and the residues after solvent extraction were not further measured or examined (it has been assumed in the report that the final extracts represented the residue in the original samples) • Evaluation of residues in "% Total", which means "percent of total area detected in analysed sample by chromatographic analysis" instead of "% TRR", is unusual (re-calculation was possible upon dossier compilation) • For milk (0.036 – 0.086 mg eq/kg) and muscle (0.035 or 0.061 mg eq/kg), total radioactive residues could be determined, but the levels of radioactivity recovered after the multi-stage extraction procedure (Goat 2) were too low to quantify (glyphosate visualised

	on TLC) • Duration of sample storage for faeces, urine and kidney was 207 – 208 days until end of analytical phase; Note: Analysis of extracts showed that glyphosate was the major residue and thus degradation during storage was negligible
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The absorption, distribution, metabolism and excretion of radioactive residues have been studied following repeated oral administration of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) to lactating goats twice daily for five (Goat 1) or three consecutive days (Goat 2), respectively. The nominal dose level was 200 mg ^{14}C-labelled glyphosate per kg feed consumed, and the actual daily dose levels were 355.4 mg/animal and 399.6 mg/animal (Goat 1 and Goat 2, corresponding to 7.6 mg/kg bw and day and 6.4 mg/kg bw and day, respectively). Goat 1 was sacrificed at ca. 23.5 h after the final dose, and Goat 2 was sacrificed at plasma radioactivity c_{max} ca. 8 h after the last dosing.

Approximately 90 % of the administered dose was recovered in the case of Goat 1 in total, and approximately 58 % was recovered in the case of Goat 2 (study termination closer to the final dose). The main part was rapidly excreted (52.58 – 78.16 % of the dose recovered in faeces, 4.74 – 9.44 % of the dose in urine, 0 – 1.74 % of the dose in cage debris and 0.29 – 0.48 % of the dose in cage washings). Radioactive residues associated with edible matrices (kidney, liver and milk) accounted in sum for less than 0.1 % of the administered dose for both animals.

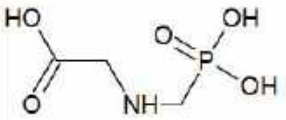
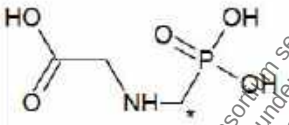
Of the relevant edible matrices of the dairy goat, highest total radioactive residues (TRR) were found in kidney (3.852 mg eq/kg for Goat 1 and 12.15 mg/kg for Goat 2) and liver (0.404 mg eq/kg for Goat 1 and 0.225 mg/kg for Goat 2). Lower residue concentrations were measured in skeletal muscle (0.035 mg eq/kg for Goat 1 and 0.061 mg/kg for Goat 2), and in fat the residue levels were below the detection limit (<0.028 mg eq/kg for Goat 1 and <0.036 mg/kg for Goat 2). Transfer of radioactive residues into milk was very low. The concentration of radioactive residues in whole milk reached a plateau concentration on day 2 of dosing (ca. 0.065 mg eq/kg, mean of day 2-4, Goat 1). In the case of Goat 2, the concentration of radioactive residues in whole milk was highest on day 3 (0.086 mg/kg). The level of radioactivity in plasma collected from Goat 2 peaked approximately 6 h post dose (0.102 mg eq/kg).

Urine, faeces and edible matrices (tissues and milk) of Goat 2 were extracted with 0.1 M HCl/chloroform followed by two ion exchange column chromatography steps for the aqueous phase (multi-stage extraction procedure). Portions of ca. 39 – 63 % of the TRR were recovered in the final extracts of liver, kidney, urine (24 – 48 h) and faeces (24 – 48 h) (radioactive residues retained in the chloroform phase were <24 % TRR), while the levels of radioactivity in the extracts of milk, fat and muscle were too low to be quantified. It has been assumed in the report that losses incurred during the extraction procedure were not associated with one or more specific components and the final extracts represented the residues in the original samples. The remaining non-extractable residues were not further examined.

The major residue in all samples was unchanged glyphosate (approximately 72 – 97 % of the total area detected in the analysed samples ("% Total"); absolute concentrations in liver: 0.215 – 0.217 mg eq/kg and in kidney: 11.128 – 11.777 mg eq/kg). Low levels of the metabolite AMPA were tentatively assigned in the extracts of urine, faeces and kidney by TLC, but not confirmed by HPLC. The results of the chromatographic analyses suggested that orally administered glyphosate was not metabolised prior to elimination.

I. Materials and methods

A. Materials

Test material:	
Chemical structure:	a) N-(phosphonomethyl)glycine (unlabelled) Batch 206-JaK-25-1, chemical purity 97.5 %  b) N-(phosphono-^{14}C-methyl)glycine; glyphosate (C-1, labelled), batch CFA 745 C6  * Position of the radiolabel
Radiochemical purity:	>97 % (confirmed by reanalysis); purity in the aqueous formulation was also >97 %
Specific activity:	Batch 1 12.3 MBq/mg (2.11 GBq/mmol) Batch 2 12.3 MBq/mg (2.11 GBq/mmol), both supplied as aqueous solutions
CAS No:	1071-83-6
Log $P_{o/w}$:	-3.4 ± 0.1

Test animals:	
Species:	Goat, *Capra aegagrus hircus*
Strain:	British Saanen strain
Breeding facility:	Not reported (recognised supplier)
Gender and numbers involved:	Two female lactating goats, cages identified by a coloured label showing information including project number and animal number
Body weight:	46.5 kg and 62 kg on day 1 of dosing
Age:	ca. 3 years
Location of the in-life phase:	Hazleton Europe, OtleyRoad, Harrogate, North Yorkshire, United Kingdom, HG3 1PY
Acclimatisation:	3 days in stainless steel metabolism cages immediately prior to dosing

Housing:	Animals were placed in stainless steel metabolism cages housed in an experimental pen with fluorescent lighting at a 10/14 hours (goat 1) or a 16/8 hours day/night cycle (goat 2), respectively Temperature: 10 – 24 °C, relative humidity: 40 – 80 %, ≥10 air changes/h
Feed and water:	Goats were fed a measured quantity (ca. 0.75 kg) of a commercially available diet, Supergoat 20 % (B Dugdale and Son, Bellman Mill, Clitheroe, Lancs) or Horse and Pony Nuts (I Anson Bros Ltd., The Mill, Thorpe Road, Masham) mixed with sugar beet pulp (ca. 0.75 kg) and an adequate quantity (ca. 0.5 kg) of hay every day. Goat 1 diet was supplemented with mixed flakes (Fringill Farm Supplies Ltd, Fringill Mill, Darley, Harrogate, United Kingdom). Diet was fed in two portions (morning and afternoon). Mains water was available *ad libitum*.

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	Nominal dose level of 200 mg/kg feed (based on a daily food consumption of 2 kg dry matter); The radiolabelled glyphosate was diluted with non-radiolabelled glyphosate; daily doses administered: Goat 1: mean of 16.66 MBq / 355.4 mg, Goat 2: mean of 36.19 MBq / 399.6 mg; Calculated using the body weights on the first day of dosing: Goat 1: mean of 7.6 mg equiv./kg bw and day Goat 2: mean of 6.4 mg equiv./kg bw and day
Feed consumption:	Actual feed consumption was not reported
Vehicle:	Water
Timing:	Twice daily (by gavage)

One lactating goat was dosed orally with N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) twice a day for five consecutive days (Goat 1, animal 001F or 27F), and one further goat was treated twice a day for three consecutive days (Goat 2, animal 002F or 95F; higher radioactivity) in the same manner. The target dose level was 200 mg/kg feed, based on a daily diet consumption of 2 kg/day. Actual daily dose levels of 355.4 mg/animal and 399.6 mg/animal were administered, corresponding to 7.6 mg equiv. /kg bw and day and 6.4 mg equiv. /kg bw and day for Goat 1 and Goat 2, respectively. The test item was administered as a solution in water by oral gavage after milk and excreta collections, where appropriate, but prior to feeding (morning and afternoon). The dosing apparatus was flushed with vehicle (water) to expel any residual dose into the animal.
Animals were observed twice daily for mortality and morbidity. Body weights were recorded on arrival, at the start of acclimatisation, on the first day of dosing and at necropsy.

2. Sampling and storage

Control samples of urine, faeces, cage wash, cage debris and milk were collected from Goat 1 prior to the first dosing occasion. Following the initial dose, urine and faeces were collected at 24 h intervals up to 96 h post dose for Goat 1 and up to 48 h post dose for Goat 2, respectively. A final collection was performed following necropsy (138 h and 61 h post dose for Goat 1 and Goat 2, respectively). At each excreta collection, cage debris was removed and following the collection the cage was rinsed with water. Animals were milked twice daily, in the morning and afternoon, and milk samples were pooled to provide 24 h collections. In the case of Goat 2, blood samples were collected by puncture of the jugular vein at several intervals (1, 2, 3, 4, 6, 8, 12 and 24 h after the initial dose). Blood was transferred into tubes containing lithium heparin anticoagulant, and centrifuged to collect plasma.

Goats were sacrificed by stunning with a captive bolt followed by immediate exsanguination via severance of the major neck blood vessels at ca. 23.5 h after the final dose for Goat 1 or at plasma radioactivity c_{max} (ca. 8 h after the final dose) for Goat 2, respectively. At termination, the edible organs and tissues skeletal muscle (maximum amounts of hind and fore quarter, pooled by animal), fat (maximum amounts of omental and kidney, pooled by animal), liver and kidney were collected, macerated and sub-sampled at dissection prior to storage at ca. -20 °C. Tissue homogenates, urine, faeces and milk were stored at -20 °C following processing and subsampling.

3. Analytical procedures

The radioactive residues in urine, faeces, whole milk, cage washings, cage debris, macerated tissues and plasma, where appropriate, were determined by combustion and/or liquid scintillation counting (LSC). Faeces and cage debris were homogenised in a minimum volume of deionised water prior to combustion.

Urine, faeces and milk collected on day 2 and tissues collected/sampled at necropsy from Goat 2 were quantitatively examined for ^{14}C-glyphosate and potential radiolabelled metabolites.

Radioactive residues were extracted from each matrix after addition of chloroform and 0.1 M HCl using a PTFE homogeniser. Samples were centrifuged and the aqueous phase of the supernatant retained and the radioactive residues in the aqueous phase and the organic phase were assessed. Approximately 80 % by weight of each extract was adjusted to pH 2 (± 0.4) with 0.2 M HCl and transferred to a glass column for extraction using a chelating ion exchange resin (Fe(III)-Chelex 100). After washing with water, 0.2 M HCl and two defined portions of 6 M HCl, the radioactive residues were eluted from the resin with 6 M HCl and the collected eluate adjusted to approximately 10 M HCl. The eluate was then transferred to a further glass column for extraction using a strong anion exchange resin (AG 1-X8, pre-rinsed with 6 M HCl). The sample was immediately eluted from the column using 6 M HCl. Extracts obtained after this multi-stage extraction procedure were evaporated to dryness (<40 °C), reconstituted in water, passed through a filter (0.45 µm) and submitted to chromatographic analysis.

Reversed-phase HPLC was performed on a Lichrosorb RP-18 column with on-line radiodetection and fluorescence detection. The samples were derivatised with 9-fluorenylmethyl chloroformate (FMOC) reagent prior to analysis. TLC was performed on cellulose plates using a developing solvent of methanol : water (TLC system 1) or ethanol : trichloroacetic acid : ammonium hydroxide : acetic acid (TLC system 2). After development, bands were visualised by autoradiography and ninhydrin spray and quantified by radio-TLC linear analyser.

Glyphosate and aminomethylphosphonic acid (AMPA) were used as authentic reference items.

The radioactive residues in extracts of kidney, urine (24 – 48 h) and faeces (24 – 48 h) from Goat 2 were isolated by semi-preparative TLC (system 1). The main radioactivity region was scraped from the plate and extracted into methanol. The isolated residue was analysed by FT-IR using glyphosate and AMPA (solutions in methanol) as standards.

II. Results and discussion

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in the table below. In total, approximately 90 % of the administered dose was recovered in the case of Goat 1 (study termination ca. 23.5 h after the final dose), and approximately 58 % was recovered in the case of Goat 2 (study termination ca. 8 h after the final dose). The main part was excreted (Goat 1: 78.16 % of the dose in faeces, 9.44 % in urine and 2.22 %

in cage debris and cage wash; Goat 2: 52.58 % of the dose in faeces, 4.74 % in urine and 0.29 % in cage wash), accounting in sum for 89.82 % and 57.61 % of the dose (Goat 1 and Goat 2, respectively).
Radioactive residues associated with edible portions (milk and tissues) accounted in sum for less than 0.1 % of the administered dose in both animals (including kidney, liver and whole milk).

Table 6.2.3-1: Total recovered radioactivity following repeated oral administration of ^{14}C-glyphosate to lactating goats at a nominal dose level of 200 mg/kg feed

Matrix / tissue	% dose[1]	
	Goat 1 (*7.6 mg/kg bw*, 5 days)[2]	**Goat 2 (*6.4 mg/kg bw*, 3 days)[2]**
Urine	9.44	4.74
Faeces	78.16	52.58
Cage wash	0.48	0.29
Cage debris	1.74	-
Whole milk	0.03	0.03
Tissues (kidney and liver)	0.05	-
Total	89.90	57.64

Values in *italics* were calculated upon dossier compilation
[1] % dose = Percent of administered radioactivity (% AR)
(mean values Goat 1: 16.66 MBq/animal, Goat 2: 36.19 MBq ^{14}C-glyphosate)
[2] Calculated using the mean weights of the test item administered (Goat 1: 355.4 mg/animal, Goat 2: 399.6 mg) and the body weights recorded on the first day of dosing (Goat 1: 46.5 kg, Goat 2: 62.0 kg)

In the table below the total radioactive residues (TRR) are summarised for samples of lactating goats following administration of 200 mg ^{14}C-glyphosate (C-1 label) per kg feed twice a day for five consecutive days (Goat 1, corresponding to 7.6 mg equiv./kg bw and day) or for three days (Goat 2, corresponding to 6.4 mg eq/kg bw and day), respectively. TRRs are expressed as glyphosate equivalents. Highest TRR values were found in kidney (3.852 mg eq/kg for Goat 1 and 12.15 mg/kg for Goat 2). In liver, total radioactive residues of 0.404 mg/kg and 0.225 mg/kg were measured (Goat 1 and Goat 2, respectively), while residue concentrations in skeletal muscle accounted for 0.035 mg/kg and 0.061 mg/kg (Goat 1 and Goat 2, respectively). In omental and kidney fat, the residue levels were below the detection limit (<0.028 mg/kg and <0.036 mg/kg for Goat 1 and Goat 2, respectively).
The transfer coefficient for milk was low (ca. 0.07 %, mean total mg eq/kg in milk in relation to mean dose), which is consistent with the lipophobic nature of glyphosate. The concentration of radioactive residues in whole milk reached a plateau concentration on day 2 of dosing (ca. 0.065 mg eq/kg, mean of day 2-4, Goat 1). In the case of Goat 2, the concentration of radioactive residues in whole milk was highest on day 3 (0.086 mg/kg).
Following the initial dose, levels of radioactivity in plasma collected from Goat 2 peaked approximately 6 h post dose (0.102 mg eq/kg, plateau level at 8 h post dose).

Table 6.2.3-2: Total radioactive residue (TRR) levels in tissues following repeated oral administration of ^{14}C-glyphosate to lactating goats at a nominal dose level of 200 mg/kg feed

Tissue	TRR in mg eq/kg	
	Goat 1 (*7.6 mg/kg bw*, 5 days)	**Goat 2 (*6.4 mg/kg bw*, 3 days)**
Fat (omental and kidney fat)	<0.028 (not detected)	<0.036 (not detected)
Kidney	3.852	12.15
Liver	0.404	0.225
Skeletal muscle (hind and fore quarter)	0.035	0.061

Values in *italics* were calculated upon dossier compilation

Table 6.2.3-3: Total radioactive residue (TRR) levels in whole milk following repeated oral administration of ^{14}C-glyphosate to lactating goats at a nominal dose level of 200 mg/kg feed

Days	TRR in mg eq/kg	
	Goat 1 (*7.6 mg/kg bw*, 5 days)	Goat 2 (6*.4 mg/kg bw*, 3 days)
1	0.036	0.040
2	0.060	0.066
3	0.064	0.086
4	0.072	not applicable
5	0.041	not applicable

Values in *italics* were calculated upon dossier compilation

Table 6.2.3-4: Total radioactive residue (TRR) levels in blood plasma following the initial oral administration of ^{14}C-glyphosate to the lactating Goat 2 at a nominal dose level of 200 mg/kg feed

Time (hours)	TRR in mg eq/kg
	Goat 2 (6*.4 mg/kg bw*, 3 days)
1	0.051
2	0.059
3	0.069
4	0.080
6	0.102
8	0.101
12	0.094

Values in *italics* were calculated upon dossier compilation

B. Extraction and characterisation of residues

Urine, faeces and edible matrices (tissues and milk) of Goat 2 were extracted with 0.1 M HCl/chloroform (radioactivity retained in the organic layer was <24 %) followed by two ion exchange column chromatography steps for the aqueous phase. Results of the acidified aqueous extraction and of the entire multi-stage extraction procedure are summarised in the table below. Portions of ca. 60 %, ca. 63 %, ca. 39 % and ca. 45 % of the TRR were extractable in urine (24 – 48 h), faeces (24 – 48 h), liver and kidney, respectively, by the multi-stage extraction procedure. The levels of radioactive residues recovered after the multi-stage extraction procedure in the cases of milk, fat and muscle were too low to quantify. It has been assumed in the report that losses incurred during the extraction procedure were not associated with one or more specific components and the final extracts represented the residues in the original samples. The remaining non-extractable residues were not further examined.

Table 6.2.3-5: Extraction of radioactive residues from urine, faeces, milk and tissues of the lactating Goat 2 following repeated oral administration of ^{14}C-glyphosate at a nominal dose level of 200 mg/kg feed

Matrix / tissue	Extraction efficiency[1]	
	Acidified aqueous extraction	Multi-stage extraction procedure
Urine (24 – 48 h)	ca. 90 %	ca. 60 %
Faeces (24 – 48 h)	>78 %	ca. 63 %
Milk (24 – 48 h)		Levels of radioactivity recovered too low to quantify, but glyphosate visualised on TLC (system 2, autoradiography)
Fat	>100 %	Levels of radioactivity recovered too low to quantify, but glyphosate visualised on TLC (system 1, autoradiography)
Liver	ca. 78 %	ca. 39 % TRR (ca. 0.088 mg eq/kg)
Kidney	ca. 76 %	ca. 45 % TRR (ca. 5.468 mg eq/kg)
Muscle		Levels of radioactivity recovered too low to quantify, but glyphosate visualised on TLC (autoradiography)

[1] Multi-stage complex extraction procedure starting with extraction with chloroform and 0.1 M HCl (further workup of the aqueous phase); In the cases of muscle, milk and fat, extraction efficiencies could not be quantified due to the very low levels of radioactivity present;
It has been assumed in the report that losses incurred during the extraction procedure were not associated with one or more specific components and the final extract represented the residue in the original sample

Aliquots of the concentrated extracts prepared by the multi-stage extraction procedure were derivatised with FMOC reagent and analysed by HPLC. Further aliquots of the extracts were analysed by TLC using two solvent systems. The results of the chromatographic analyses are summarised in the table below, and the concentrations of the components of the radioactive residues in mg eq/kg are calculated in the table below.

In the extract of liver after the multi-stage extraction procedure, portions of more than 95 % of the total area detected in the analysed samples ("% Total", corresponding to "% of the TRR" under the assumption of the report that the final extract represented the residues in the original sample (no specific components lost); results of calculations of % TRR values for all matrices taking into consideration the extraction efficiencies are provided in the right column of Table 6.2.3-6) were identified as glyphosate using three different analytical methods (HPLC and TLC) and comparison with the reference item. In the final extracts of faeces and kidney, portions of more than 91 % Total were identified as glyphosate using HPLC and TLC. Evidence for the occurrence of the metabolite AMPA from minor TLC regions after developing with solvent system 1 and solvent system 2 (approximately 4 % or 2 % Total, respectively, in faeces and approximately 8 % or 5 % Total, respectively, in kidney extract) was supposed to be a likely artefact of the chromatographic procedure (e. g. formation of AMPA during TLC or AMPA region resulting from tailing of the glyphosate band) as HPLC data supporting this assignment were lacking. An unknown component was detected in addition in the faeces extract (below 4 % Total in each analysis). In the case of the TLC analyses of the faeces extract, minor regions were located at the origin and attributed to non-specific binding. In the extract of urine after the multi-stage extraction procedure, a portion of approximately 96 % Total was identified as glyphosate by HPLC, and the presence of glyphosate was confirmed by both TLC systems (comparison with reference items). The assignment of minor portions of radioactive residues in the urine extract (5 – 6 % Total) as AMPA according to TLC was not substantiated by HPLC. Portions of radioactive residues in the extract of urine designated as unknown components according to TLC analysis were mainly located at the origin (10 – 19 % Total) and probably resulted from non-specific

binding (e. g. due to disturbed cellulose sorbent layer; this effect was substantiated in the case of urine and faeces by over-laying sample extracts with cold glyphosate standard prior to developing in solvent system 2, which significantly reduced the binding, see Table 6.2.3-6). In the cases of the extracts of urine, faeces and kidney, the identification of glyphosate was confirmed by the FT-IR spectra of isolated main regions from semi-preparative TLC (system 1) in comparison with those of the respective reference item. The concentrations of radioactive residues in the final extracts of fat, muscle and milk were below the level of detection following HPLC analysis using both on-line and fraction collection detection. TLC analyses of the fat extract using solvent system 1, of the muscle extract (solvent systems 1 and 2) and of the milk extract using solvent system 2 yielded only one radioactive region each, which corresponded to the glyphosate standard.
The concentrations of glyphosate calculated using the "% Total" values accounted for 0.215 – 0.217 mg eq/kg in the extracts of liver and 11.128 – 11.777 mg eq/kg in the extracts of kidney (worst-case calculations from the chromatography results without considering the extraction efficiency, reflecting the assumption of the report that losses during extraction were not specific to particular metabolites and the final extracts represented the residues in the initial samples).

Table 6.2.3-6: Identification of radioactive residues in urine, faeces, liver and kidney following repeated oral administration of ^{14}C-glyphosate to the lactating Goat 2 at a nominal dose level of 200 mg/kg feed

Sample / analysis	% Total[1]				% TRR[1]
	Glyphosate	AMPA	Unknown	Total (allocated)	Total (recovered and allocated)
Urine (24 – 48 h), radio-HPLC	95.86	-	-	95.86	*57.52*
Urine (24 – 48 h), TLC system 1	71.74	5.55	22.34[2]	99.63	*59.78*
Urine (24 – 48 h), TLC system 2, sample + reference item[3]	84.66	5.09	9.93[4]	99.68	*59.81*
Urine (24 – 48 h), TLC system 2, pure sample	79.74	4.68	15.46[4]	99.88	*59.93*
Faeces (24 – 48 h), radio-HPLC	94.06	-	3.23[5]	97.29	*61.29*
Faeces (24 – 48 h), TLC system 1	92.68	3.95	2.12[4]	98.74	*62.21*
Faeces (24 – 48 h), TLC system 2	94.00	2.23	3.55[4]	99.78	*62.86*
Liver, radio-HPLC	95.52	-	-	95.52	*37.25*
Liver, TLC system 1	96.64	-	-	96.64	*37.69*
Liver, TLC system 2	95.89	-	-	95.89	*37.40*
Kidney, radio-HPLC	96.93	-	-	96.93	*43.62*
Kidney, TLC system 1	91.59	8.01	-	99.60	*44.82*
Kidney, TLC system 2	94.08	4.90	-	98.97	*44.54*

Values in *italics* (% TRR) were calculated upon dossier compilation

[1] % Total = percent of total area detected in analysed sample by chromatographic analysis (radiodetection):
"% Total" = "% ROI" – "% Unallocated" (because Total Area = Region Of Interest + Unallocated);
The values in "% TRR" of the initial matrix sample could be calculated for the actually measured analytical sample (regarding the recovery after the multi-stage extraction procedure, given in Table 6.2.3-5) as
"% Total" x "% extraction efficiency" ÷ 100;
for instance, 95.52 % Total in liver (radio-HPLC) x 39 % recovery after extraction ÷ 100 = 37.25 % TRR actually measured in extract sample after extraction and sample preparation for chromatographic analysis,
91.59 % Total for glyphosate and 8.01 % Total for AMPA in kidney (TLC system 1) x 45 % recovery after extraction ÷ 100 = 41.22 % TRR for glyphosate and 3.60 % TRR for AMPA,
94.08 % Total for glyphosate and 4.90 % Total for AMPA in kidney (TLC system 2) x 45 % recovery after extraction ÷ 100 = 42.34 % TRR for glyphosate and 2.21 % TRR for AMPA actually measured in extract sample;
for the respective values calculated in mg eq/kg see subsequent Table 6.2.3-14

[2,5] Two components representing 19.43 % and 2.91 % Total, respectively

[3] Sample extract and unlabeled reference items spotted on the same locations, which reduced non-specific binding

[4] One single unknown band in each sample, located at the origin of the TLC plate
(R_f near 0; probably resulting from non-specific binding)

[5] One unknown component with a retention time of 2.22 minutes

Table 6.2.3-7: Calculated concentrations of components of the radioactive residues in liver and kidney following repeated oral administration of ^{14}C-glyphosate to the lactating Goat 2 at a nominal dose level of 200 mg/kg feed

Tissue / analysis	Concentration in mg eq/kg[1]	
	Glyphosate	AMPA
Liver, radio-HPLC	*0.215*	not detected
Liver, TLC system 1	*0.217*	not detected
Liver, TLC system 2	*0.216*	not detected
Kidney, radio-HPLC	*11.777*	not detected
Kidney, TLC system 1	*11.128*	*0.973*
Kidney, TLC system 2	*11.431*	*0.595*

All values (in *italics*) were calculated upon dossier compilation

[1] Calculated using the TRR in the respective tissue of Goat 2 (given in Table 6.2.3-2) and the portion of the component in the analysed sample in % Total (given in Table 6.2.3-13);
Since calculation with the % TRR values (right column or footnote 1 in Table 6.2.3-13) would lead to lower mg/kg values (e.g. 0.084 mg eq/kg glyphosate in liver and 5.300 mg eq/kg glyphosate in kidney according to radio-HPLC), the given values represent a worst-case calculation and reflect the assumption of the report that losses during extraction were not specific to a particular metabolite(s) and the final extracts represented the residues in the original samples (compare footnote in Table 6.2.3-12)

C. Storage stability

Tissue homogenates, urine, faeces and milk were stored at -20 °C following processing and sub-sampling for radioassay (faeces samples were stored initially at ~8 °C). The storage intervals between sample collection and start of extraction were 77 days to 78 days, and the storage intervals between end of analytical phase and date of sample collection were 108 days to 111 days for liver, muscle, milk and fat, and 207 days to 208 days in the cases of kidney, urine and faeces. Analysis of extracts showed that the parent compound glyphosate was the major component of the residue and thus degradation during storage was negligible.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) was administrated to lactating goats twice daily for five (Goat 1) or three consecutive days (Goat 2), respectively. The target dose level was 200 mg ^{14}C-labelled glyphosate per kg feed consumed, and the actual daily dose levels were 355.4 mg/animal and 399.6 mg/animal (Goat 1 and Goat 2, corresponding to 7.6 mg/kg bw and day and 6.4 mg/kg bw and day, respectively). Goat 1 was sacrificed at ca. 23.5 h after the final dose, and Goat 2 was sacrificed at plasma radioactivity c_{max} ca. 8 h after the last dosing.

Approximately 90 % of the administered dose was recovered in the case of Goat 1 in total, and approximately 58 % was recovered in the case of Goat 2 (study termination closer to the final dose). The major portions of radioactive residues were recovered in faeces (52.58 – 78.16 % of the dose), urine (4.74 – 9.44 % of the dose), cage debris and cage washings, and less than 0.1 % of the administered dose was associated in both animals with edible matrices (tissues and milk in sum). At study termination, the major radioactive residues in the relevant edible matrices were detected in liver (0.404 mg/kg for Goat 1 and 0.225 mg/kg for Goat 2) and kidney (3.852 mg eq/kg for Goat 1 and 12.15 mg/kg for Goat 2). Transfer of glyphosate or its metabolites into milk was low.

The major residue in the extracts of urine, faeces, liver and kidney was unchanged glyphosate (approximately 72 – 97 % of the total area detected in the analysed samples ("% Total"); absolute concentrations in liver: 0.215 – 0.217 mg eq/kg and in kidney: 11.128 – 11.777 mg eq/kg). Indications for the occurrence of the metabolite AMPA from minor TLC regions (up to 8 % Total) in the extracts of urine, faeces and kidney or of unknown components in the extracts of urine and faeces were not substantiated by HPLC and therefore supposed to be chromatographic artefacts. The concentrations of radioactive residues

in the final extracts of fat, muscle and milk were below the level of detection following HPLC analysis, and TLC analyses of these extracts yielded only one radioactive region, which corresponded to the glyphosate standard.

In summary, orally administered glyphosate in goat was rapidly and essentially quantitatively excreted. Chromatographic analysis of excreta, whole milk and selected tissues suggested that orally administered glyphosate was poorly absorbed and then eliminated without being extensively metabolised.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behaviour of glyphosate in lactating goats has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals 503, with major deficits (the radioactivity balance was 58 – 90 %; Radioactive residues in fat were below the limits of detection for both animals, but this limits accounted for 0.028 ppm equivalents for Goat 1 and 0.036 ppm equivalents for Goat 2, respectively, which is today above the trigger value; extractability was only moderate for all matrices, chloroform phases were not further examined (<24 %), and the non-extractable residues were nor measured neither characterised / investigated; For milk and muscle, TRR of 0.035 – 0.086 mg eq/kg were measured, but no quantitative determination or investigation of the residues was possible after extraction). The residue identification and characterisation is poor with regard to the extractability, the recovery of residues from the ion exchange columns and the fractions not further examined. However, the study contributes data on the excretion and distribution of residues, the total radioactive residues in milk and most tissues and the time course of the residue concentration in plasma, and the identified residues do not contradict the results from other livestock metabolism studies.

The study is considered to be supportive for the assessment of the metabolic behaviour of glyphosate in lactating goats.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.3/002
Report author	[redacted]
Report year	1994
Report title	The nature of residues of orally administered [Phosphonomethylene-^{14}C] glyphosate-trimesium in goat tissues and milk
Report No	RR 93-062B
Document No	[redacted] 8325, [redacted]-93-088, [redacted] 378
Guidelines followed in study	EPA nature of residues in livestock (171-4)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • The radiochemical purity of the test item was 93 % (measured within 1 week of dosing, containing approximately 2 – 3 % AMPA) and justifications are given in the report • It is not reported if the estimated relative dose was based on a dry weight basis • The radioactivity was not quantified separately in the different

	muscle and fat types • No description of duration of storage of samples, however, a storage stability investigation was performed during the course of the study
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labeled glyphosate-trimesium) was administrated twice daily for 7 consecutive days to a lactating goat. The target dose level was 90 mg/kg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg/kg phosphonomethylglycine (PMG) per kg feed consumed). The actual dose level was 92.7 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (63.8 mg PMG per kg feed consumed) or 3.9 mg ^{14}C-PMG-labeled glyphosate-trimesium/kg bw/day (2.7 mg PMG/kg bw/day), respectively. One goat was kept as control without dosing the test substance. The goats were sacrificed between 12 to 15 hours after the last dosing.

101 % of the administered dose was recovered in total. The main part was excreted (81 % of the administered dose in faeces, 9 % in urine and 1.4 % in cage wash) accounting in sum for 91.4 %. Radioactivity recovered in GI tract with contents accounted for 9.3 %. Radioactivity associated with edible portions (tissues and milk) accounted in sum for 0.15 % of the administered dose (including liver, kidney, fat, muscle, heart and milk), with highest radioactive residues found in kidney (0.09 % of the administered dose).

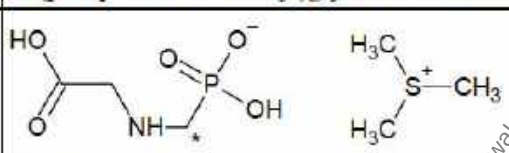
Of the relevant edible matrices of the goat, highest total radioactive residues were found in kidneys (5.58 mg/kg) and liver (0.234 mg/kg). In muscle and fat 0.0256 and 0.0175 mg/kg were found, respectively. For whole milk samples, a plateau was reached after 4 days.

Edible matrices (tissue and milk) were extracted with aqueous and organic solvents. Portions of 78 – 99.8 % TRR were extractable. In all samples, the major part of the residue was extracted with aqueous solvents (58 – 99.6 % TRR). The remaining non-extractable residues were between 0.1 and 21.1 % TRR (or <0.001 – 0.007 mg/kg).

PMG (59.4 – 91.3 % TRR) and AMPA (4.7 – 21.4 % TRR) accounted for the majority of the radioactive residue in liver, kidney, fat and muscle. In milk, PMG (0.005 mg/kg) and AMPA (0.001 mg/kg) together represented 25 % TRR. Lactose and triglycerides constituted over 45 % TRR in milk, while material associated with post-extraction milk solids comprised 21 % TRR (0.005 mg/kg), which is consistent with natural incorporation into proteins.

I. Materials and methods

A. Materials

Test material	N-(phosphono-^{14}C-methyl)glycine trimesium salt
Chemical structure:	 * Position of the radio label
Radiochemical purity:	93 % (measured within 1 week of dosing, containing approximately 2 – 3 % AMPA), justifications for use without further purification are given in the report
Specific activity:	7.5 MBq/mg (0.204 µCi/mg = 49.9 mCi/mmol)
CAS No:	81591-81-3
Log $P_{o/w}$:	-2.9

Test animals:	
Species:	Goat, *Capra aegagrus hircus*
Strain:	Mixed-breed goats
Breeding facility:	[redacted]
Gender and numbers involved:	Female, 2 animals (1 control group and 1 treatment group), identified by ear tattoo, neckband and cage card
Body weight:	50.3 kg (day 1 of dosing)
Age:	1-5.5 years
Location of the in-life phase:	[redacted]
Acclimatisation:	9 days before first treatment
Housing:	Individually housed in metabolic cages (1.8 m x 0.9 m x 1.6 m) with artificial light at a 14/10 hours light/dark cycle Temperature: 20 – 22°C, Humidity: 49 – 51 %
Feed and water:	Purina Rumilab Chow with supplementary alfalfa cubes and hay; water (Columbus Municipal Supply), *ad libitum*

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	3.9 mg N-(phosphono-^{14}C-methyl)glycine trimesium salt equiv./kg bw/day or 2.7 mg phosphonomethylglycine equiv./kg bw/day*
Feed consumption:	2140 g/day
Vehicle:	Gelatine capsules
Timing:	Twice a day by balling gun
* Calculated based on body weight of 50.3 kg, the actual dose level of 92.7 mg ^{14}C-PMG-labeled glyphosate-trimesium or 63.8 mg PMG per kg feed consumed and the actual feed consumption of 2140 g per day.	

N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labeled glyphosate-trimesium) was used to dose a single non-pregnant, lactating, mixed-breed goat. The target dose level was 90 mg/kg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg/kg phosphonomethylglycine (PMG) per kg feed consumed), based on a target feed consumption value of 2500 g per goat per day. The actual dose level was 92.7 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (63.8 mg PMG per kg feed consumed), based on actual feed consumption of 2140 g per day or 3.9 mg ^{14}C-PMG-labeled glyphosate-trimesium/kg bw/day (2.7 mg PMG/kg bw/day). One additional control goat was given capsules containing cellulose plus water.

The treated goat was given capsules containing ^{14}C-PMG-labeled glyphosate-trimesium twice a day (in the morning and in the afternoon) for seven consecutive days, at a single dose level, by the oral route of administration.

The gelatine capsules were prepared at Western Research Center (Richmond, CA, USA) and shipped to Battelle Columbus Laboratories (Columbus, OH, USA) on dry ice overnight, were the capsules were stored frozen at approximately -20°C. One capsule was extracted with 1 M HCl, diluted with water and three aliquots were radioassayed.

The goats were acclimatised for 9 days and remained in the same room during acclimatisation and dosing period. Animals were observed twice daily for mortality and moribundity. Body weights were recorded on receipt, randomisation, day 1 and at termination. Feed consumption was recorded daily and clinical observations twice daily.

2. Sampling and storage

Urine and faeces were collected prior to dosing and at 24 hours intervals after initiation of dosing until termination. Cage sides and floor as well as excreta collection pans were rinsed every 24 hours using deionised water. Milk was collected twice daily. Milk collected prior to the morning dosing were stored at 4°C and pooled with milk collected prior to afternoon dosing. A subsample of whole milk from day 5 and 6 was separated into fat and skim milk.

The treated and control goats were sacrificed by exsanguination between 12 to 15 hours after the last dosing on day 7. At termination, liver, kidney, fat (subcutaneous, renal and visceral), small intestine, large intestine and stomach contents, stomach diaphragm tissue, muscle, blood, heart, small intestine, large intestine and stomach were collected. All samples were stored frozen at approximately -20°C at the site of the in-life part (Battelle Columbus Laboratories). All tissue, milk and excreta were sent frozen on dry ice to Western Research Center (Richmond, CA, USA). The samples were stored at -20°C at Western Research Center.

3. Analytical procedures

Specimen of tissues, milk, excreta and blood were homogenised and analysed in triplicates for total radioactivity using tissue combustion and/or liquid scintillation counting (LSC). Kidney, muscle and

faeces were homogenised with water in a 1:0.5, 1:1 and 1:2 ratio, respectively, and liver was homogenised without added water. A subsample of fat was minced with scissors and directly combusted for radioassay, while milk and urine were directly mixed with scintillation cocktail and radioassayed. The radioactivity in extracts of milk and tissue samples was determined by LSC.

An aliquot of homogenised liver was extracted with 0.1 N aqueous HCl (3 x, the first extraction was followed by an extraction with dichloromethane), dichloromethane (1 x), a 0.1 N aqueous HCl dichloromethane mixture (2 x), methanol (3 x) and diethyl ether (1 x). After each extraction the homogenate was centrifuged. The combined aqueous, the combined dichloromethane and the combined methanol diethyl ether extracts as well as the residue after extraction were radioassayed. The combined aqueous extract was analysed by HPLC. The aqueous extract was further purified by Chelex resin filtration followed by anion-exchange resin filtration. The filtrate was analysed by TLC and derivatised and analysed by GC/MS.
An aliquot of homogenised kidney was extracted three times with 0.1 N aqueous HCl followed by an extraction with dichloromethane. After each extraction the homogenate was centrifuged. The aqueous extracts were combined and analysed by LSC, HPLC and TLC. The extraction residue was sonicated with methanol and the combined dichloromethane methanol extract as well as the residue after extraction were radioassayed.
The minced fat was extracted three times with water and chloroform. The aqueous and chloroform phases were separated and both combined phases were analysed by LSC. The combined aqueous phase was further analysed by HPLC. The postextracted solid was dissolved by acid hydrolysis and aliquots of the hydrosylate were analysed by LSC.
An aliquot of homogenised muscle was extracted two times with 0.1 N aqueous HCl followed by an extraction with methanol and diethyl ether. After each extraction the homogenate was centrifuged. Acetone was added to the initial aqueous muscle extract in an attempt to improve the pellet. The aqueous extracts, the combined methanol diethyl ether extract and the extraction residue were radioassayed. The combined aqueous extract was further analysed by HPLC.
An aliquot of milk was mixed with 0.6 % aqueous acetic acid and centrifuged. The residue was extracted once with chloroform and twice with a chloroform water mixture. The combined aqueous and the chloroform extracts as well as the residue after extraction were radioassayed. The combined chloroform extract was additionally analysed by TLC and the combined aqueous extract by HPLC. The aqueous extract was filtered through Chelex resin in the iron form and the filtrate was analysed by HPLC and TLC.

Peak assignment was based on co-chromatography with reference standards or comparison of retention times and R_f values with reference standards.

II. Results and discussion

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in in the table below. 101 % of the administered dose was recovered. The main part was excreted (81 % of the administered dose in faeces, 9 % in urine and 1.4 % in cage wash) accounting in sum for 91.4 %.
Radioactivity recovered in GI tract with contents accounted for 9.3 %. Radioactivity associated with edible portions (tissues and milk) accounted in sum for 0.15 % of the administered dose (including liver, kidney, fat, muscle, heart and milk), with highest radioactive residues found in kidney (0.09 % of the administered dose).

Table 6.2.3-8: Distribution of radioactive residues in tissues, milk and excreta of lactating goats after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

Matrix	% dose[1]
Liver	0.02
Kidney	0.09
Fat	0.00
Muscle	0.01
Heart	0.00
Blood	0.03
GI tract and contents[2]	9.3
Milk (whole milk)	0.03
Urine	9.0
Faeces	81.0
Cage rinse	1.4
Total	101

[1] % dose = percent of administered dose
[2] GI tract and contents include small intestine contents, large intestine contents, stomach contents and stomach diaphragm.

In the table below the total radioactive residues (TRR) are summarised for samples of lactating goat, following administration of 92.7 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed for 7 days corresponding to 3.9 mg equiv./kg bw/day. TRRs are expressed as PMG equivalents. Highest TRR values were found in kidney (5.58 mg/kg) and liver (0.234 mg/kg). In muscle and fat 0.0256 and 0.0175 mg/kg were found, respectively.
For whole milk samples, a plateau was reached after 4 days. Separation of milk collected on days 5 and 6 resulted in higher concentrations in milk fat than in skim milk by approximately two fold. Overall, approximately 80 % of the milk total radioactive residue was found in the skim milk.

Table 6.2.3-9: Total radioactive residue in samples of lactating goat after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

Matrix	TRR (mg/kg)[1]
Liver	0.234
Kidney	5.58
Fat	0.0175[2]
Muscle[3]	0.0256
Heart	0.0424
Milk (day 7)[3]	0.0222

[1] TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents; determined at Battelle Columbus Laboratories
[2] The TRR values determined by combustion analysis at Battelle Columbus Laboratories for fat were variable, ranging from 0.01 to 0.03 mg/kg with a standard deviation of 0.006 mg/kg. The TRR value(0.032 mg/kg) determined by extraction at Western Research Center was set as 100 %, as a larger sample (8 g) were analysed at Western Research Center compared to combustion analysis at Battelle Columbus Laboratories (approximately 0.1 g).
[3] For muscle and milk (day 7) the TRR was further determined by combustion at Western Research Center 0.022 and 0.024 mg/kg were found, respectively.

Table 6.2.3-10: Radioactive residues in milk of lactating goats after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

Days	TRR (mg/kg)[1]		
	Whole milk	Skim milk	Milk fat
1	0.00255	-	-
2	0.0139	-	-
3	0.0189	-	-
4	0.0217	-	-
5	0.0212	0.0191	0.0495
6	0.0211	0.0173	0.0320
7	0.0222	-	-
8	0.0225	-	-

[1] TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents

B. Extraction and characterisation of residues

Edible matrices (tissue and milk) were extracted with aqueous and organic solvents and the results are summarised in the tables below. Portions of 78 – 99.8 % of the TRR were extractable. In all samples, the major part of the residue was extracted with aqueous solvents (58 – 99.6 % TRR). The remaining non-extractable residues were between 0.1 and 21.1 % TRR (or <0.001 – 0.007 mg/kg).

Table 6.2.3-11: Extraction of the radioactive residues in liver, kidney and fat of lactating goats following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 7 days

	Liver		Kidney		Fat	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.234**	**100**	**5.58**	**100**	**0.032[1]**	**100**
ERR	***0.228***	***98***	***5.57***	***99.8***	**0.032**	**99**
Aqueous extract	0.214	92	5.56	99.6	0.032	99
Methanol/ diethyl ether extract	0.012	5	N/A	N/A	N/A	N/A
Methanol/ dichloromethane extract	N/A	N/A	0.01	0.2	N/A	N/A
Dichloromethane extract	0.002	1	N/A	N/A	N/A	N/A
Chloroform extract	N/A	N/A	N/A	N/A	<0.001	0
RRR	**0.006**	**2.7**	**0.007**	**0.1**	**<0.001**	**1.0**
Accountability[2]	106.4 %		105.1 %		100 %	

Values in *italics* were calculated upon dossier compilation. Residue values are normalised to TRR values.

[1] Value determined by extraction at Western Research Center. The TRR values determined by combustion analysis at Battelle Columbus Laboratories for fat were variable, ranging from 0.01 to 0.03 mg/kg with a standard deviation of 0.006 mg/kg. The TRR value determined by extraction at Western Research Center was set as 100 %, as a larger sample (8 g) were analysed at Western Research Center compared to combustion analysis at Battelle Columbus Laboratories (approximately 0.1 g).

[2] Accountability = recovery after extraction with aqueous and organic solvents (not normalised values).

TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents

ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents

RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents

N/A = not applicable

Table 6.2.3-12: Extraction of the radioactive residues in muscle and milk of lactating goats following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 7 days

	Muscle		Milk (day 7)	
	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.026**	**100**	**0.022**	**100**
ERR	***0.025***	***98.7***	***0.018***	***78***
Aqueous extract	0.024	95.6	0.013	58
Methanol/ diethyl ether extract	0.001	3.1	N/A	N/A
Chloroform extract	N/A	N/A	0.005	20
RRR	**0.000**	**1.2**	**0.005**	**21.1**
Accountability[1]	102.9 %		100.4 %	

Values in *italics* were calculated upon dossier compilation. Residue values are normalised to TRR values.
[1] Accountability = recovery after extraction with aqueous and organic solvents (not normalised values).
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents
N/A = not applicable

The aqueous extracts were analysed by HPLC. PMG and AMPA in the aqueous extracts accounted for more than 80 % TRR for all tissues. For kidney, fat and muscle, PMG accounted for more than 80 % TRR and AMPA accounted for less than 10 % TRR, while for liver, PMG accounted for 59 % TRR and AMPA accounted for 21 % TRR.
Two different analytical methods (TLC and HPLC) were used to identify PMG and AMPA in kidney and liver aqueous extracts by co-chromatography with reference standards or comparison of retention times and R_f values with reference standards. In addition, GC/MS spectroscopically confirmed the presence of PMG in liver aqueous extract. The GC/MS confirmatory analysis was unable to discern the corresponding volatile AMPA derivative from the background. A further peak was detected in each aqueous extract of tissue. In kidney and liver aqueous extracts, this unidentified peak comprised less than 10 % TRR and in muscle and fat less than 10 % TRR and less than 0.01 mg/kg.
The aqueous supernatant of milk (day 7) was analysed by HPLC. PMG (0.005 mg/kg) and AMPA (0.001 mg/kg) in the aqueous supernatant represented 25 % TRR. After filtering through Chelex resin in iron form lactose was identified by retention time comparison in HPLC and R_f comparison in TLC. Hydrolysis of the lactose yielded radiolabelled HPLC peaks with retention times consistent with glucose and galactose. Triglycerides were extracted with chloroform from the dilute-acetic acid precipitated protein pellet. Phosphatidylcholine and cholesterol were applied as control substances. Phosphatidylcholine remained near the origin, the Rf of cholesterol was 0.35, while triglycerides appeared at an Rf of approximately 0.75. Approximately 21 % TRR (0.005 mg/kg) remained in the residue after extracting with water and chloroform. The report referred to a literature source where the average weight percent values for protein, fat, and lactose in goat milk are quoted. The average weight percent's for protein (3.52 %), fat (4.25 %) and lactose (4.27 %) in goat milk were roughly equal, as the radioactive residues attributed to the protein pellet (0.005 mg/kg), triglycerides (0.005 mg/kg) and lactose (0.006 mg/kg). They concluded that the radioactive residue found in the milk protein pellet is consistent with the level expected from natural incorporation to the extent found in triglycerides and lactose.

Table 6.2.3-13: Identification and characterisation of the radioactive residues in liver, kidney and fat of lactating goats following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 7 days

	Liver		Kidney		Fat	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.234**	**100**	**5.58**	**100**	**0.032**	**100**
ERR	***0.228***	***98***	***5.57***	***99.8***	***0.032***	***99***
Aqueous extract	0.214	92	5.56	99.6	0.032	99
Aqueous extract analysed by HPLC						
PMG	0.139	59.4	4.816	86.3	0.029	91.3
AMPA	0.050	21.4	0.418	7.5	0.001	4.7
Unknown	0.017	7.3	0.242	4.3	0.001	3.0
Methanol/ diethyl ether extract	0.012	5	N/A	N/A	N/A	N/A
Methanol/ dichloromethane extract	N/A	N/A	0.01	0.2	N/A	N/A
Dichloromethane extract	0.002	1	N/A	N/A	N/A	N/A
Chloroform extract	N/A	N/A	N/A	N/A	<0.001	0.0
Total identified	***0.189***	***80.8***	***5.234***	***93.8***	***0.030***	***96.0***
Total characterised[1]	***0.031***	***13.3***	***0.252***	***4.5***	***0.001***	***3***
RRR	**0.006**	**2.7**	**0.007**	**0.1**	**<0.001**	**1.0**

Values in *italics* were calculated upon dossier compilation. Residue values are normalised to TRR values.
[1] Characterised by extraction and/or chromatographic behaviour.
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents
N/A = not applicable

Table 6.2.3-14: Identification and characterisation of the radioactive residues in muscle and milk (day 7) of lactating goats following treatment with ^{14}C-PMG-labeled glyphosate-trimesium for 7 days

	Muscle		Milk (day 7)	
	mg/kg	% TRR	mg/kg	% TRR
TRR	**0.026**	**100**	**0.022**	**100**
ERR	***0.025***	***98.7***	***0.018***	***78***
Aqueous extract	0.024	95.6	0.013	58
Aqueous extract analysed by HPLC				
PMG	0.022	87.1	0.005	22.3
AMPA	0.002	6.3	0.001	2.4
Unknown	0.001	2.2	0.007[2]	31.5[2]
Methanol/ diethyl ether extract	0.001	3.1	N/A	N/A
Chloroform extract	N/A	N/A	0.005	20[3]
Total identified	***0.024***	***93.4***	***0.012[4]***	***49.9[4]***
Total characterised[1]	***0.002***	***5.3***	***0.006[4]***	***26.3[4]***
RRR	**0.000**	**1.2**	**0.005**	**21.1**

Values in *italics* were calculated upon dossier compilation. Residue values are normalised to TRR values.
[1] Characterised by extraction and/or chromatographic behaviour.
[2] After filtering through Chelex resin in iron form lactose was identified by retention time comparison in HPLC and R_f comparison in TLC. Approximately 80 % of the radioactivity in unknown was assigned as lactose (<0.01 mg/kg, 25.2 % TRR).
[3] Tentatively identified as triglycerides via TLC.
[4] Milk: total identified = sum of PMG, AMPA and lactose; total characterised = sum of unknown (except lactose) and chloroform extract (as triglycerides were only tentatively identified via TLC)
TRR = total radioactive residue, expressed as ^{14}C-PMG-equivalents
ERR = extractable radioactive residue, expressed as ^{14}C-PMG-equivalents
RRR = residual radioactive residue, expressed as ^{14}C-PMG-equivalents
N/A = not applicable

C. Storage stability

All samples, stored frozen at approximately -20°C, were extracted and initially analysed within 1.6 to 3.9 months after sacrifice. Comparison of HPLC chromatograms of extracts showed that degradation of PMG or metabolites during the period of storage was negligible.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labeled glyphosate-trimesium) was administrated twice daily for 7 consecutive days to a lactating goat. The target dose level was 90 mg/kg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg/kg phosphonomethylglycine (PMG) per kg feed consumed). The actual dose level was 92.7 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (63.8 mg PMG per kg feed consumed) or 3.9 mg ^{14}C-PMG-labeled glyphosate-trimesium/kg bw/day (2.7 mg PMG/kg bw/day), respectively. One goat was kept as control without dosing the test substance. The goats were sacrificed between 12 to 15 hours after the last dosing.
The major portion of radioactive residue was recovered in faeces, urine, cage rinse and GI tract with content. 0.15 % of the administrated dose was associated with edible portions (tissue and milk).
The major residue in all tissues was PMG itself (59.4 – 91.3 % TRR or 0.022 – 4.816 mg/kg). The major metabolite was AMPA, which constituted approximately 20 % TRR in the liver (0.050 mg/kg) and less than 10 % TRR in kidney (0.418 mg/kg), fat (0.001 mg/kg), muscle (0.002 mg/kg) and milk (0.001 mg/kg). AMPA is a minor component of the residue in tissues and milk relative to PMG. Some natural incorporation of the radiolabel present in ^{14}C-PMG also occurred. As examples, lactose, triglycerides and the protein pellet accounted for the majority of the radioactive residue in milk. Presumably other tissues also contained small amounts of radioactivity incorporated into natural components.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behavior of glyphosate in lactating goats has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 503 with minor deficits (no description of duration of storage of samples, however, a storage stability investigation was performed).
Based on the dates of the in-life phase and end of analytical phase stated the maximum storage period of stored samples is 283 days. This storage duration is well covered by available storage stability studies (see MCA 6.1). Furthermore, all samples were extracted and initially analysed within 3.9 months. HPLC analysis showed that degradation of PMG during the period of storage was negligible.
Therefore, the study is considered reliable and covers the required metabolism studies in lactating goats.

Assessment and conclusion by RMS:

Studies previously submitted to the EU

1. Information on the studies

Data point:	CA 6.2.3/003
Report author	[redacted]
Report year	1988
Report title	Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part I
Report No	[redacted] 6103-113
Document No	[redacted]-7586
Guidelines followed in study	Not specified
Data point:	CA 6.2.3/004
Report author	[redacted]
Report year	1988
Report title	Metabolism study of synthetic $^{13}C/^{14}C$-labeled Glyphosate and Aminomethylphos-phonic acid in lactating goats. Part II
Report No	[redacted]-7458
Document No	Not available
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • The radioactivity was not quantified separately in the different muscle and fat types • The radioactivity balances was between 83.6 and 86.7 % • No flow chart depicting the overall extraction and fractionation strategies for each sample matrix was provided • No quantification of the residues as concentration (mg/kg, as active ingredient equivalents) in the original sample matrix analysed (re-calculation possible) • The recovery of radioactive residues after extraction of fat (test 3) was only 85.4 % (TRR was only 0.004 mg/kg) • The recovery of radioactive residues after deproteination of liver was only 82.4 – 87.5 % and the recovery of radioactive residues after concentration of muscle and fat were only 63.2 – 69.4 % and 65.4 – 67.1 %, respectively. • No description of duration of storage of samples
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

Two test with lactating goats were conducted with a 9:1 mixture of N-(phosphono-$^{13}C/^{14}C$-methyl)glycine ($^{13}C/^{14}C$-glyphosate) and amino-$^{13}C/^{14}C$-methylphosphonic acid ($^{13}C/^{14}C$-AMPA) to investigate their behaviour in goats. One additional test was performed as a control group without dosing with test substance (test 1).

The goats received a dose of 120 mg/kg feed = 300.9 mg test mixture/day ≙ 272.2 mg ($^{13}C/^{14}C$-glyphosate) disodium salt and 28.7 mg $^{13}C/^{14}C$-AMPA monosodium salt per day (2.83 – 2.95 mg/kg bw/day ≙ 2.55 – 2.65 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 0.28 – 0.29 mg $^{13}C/^{14}C$-AMPA/kg bw/day). One capsule was administered each day for 5 consecutive days. Two experiments with radioactive test substances were conducted (test 2: two goats; test 3: one goat). In test 2 the goats were sacrificed 22 to 24 hours after the last dose. In test 3, a 5-day depuration phase was added after the 5th dose after which the goat was sacrificed.

Between 83.6 and 86.7 % of the administered dose was recovered. The main part was excreted, accounting in sum (urine, faeces plus pan rinse) for 74.95 – 86.46 %. Highest TRR values were detected in kidneys (0.505 – 7.0 mg/kg) and liver (0.381 – 0.493 mg/kg). In muscle and fat 0.009 – 0.027 mg/kg and 0.004 – 0.010 mg/kg were found, respectively.

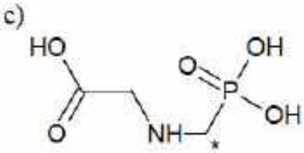
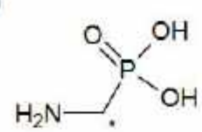
In milk the radioactive residue ranged between 0.019 and 0.060 mg/kg during the dosing period. After the 5 day depuration the radioactive residue decreased to 0.006 mg/kg.

In tissue, portions of 73.3 to 97.8 % of TRR were extractable. In all samples, the major part of the residue was extracted with water. The remaining non-extractable residues were between 2.3 and 5.5 % TRR (or 0.000 – 0.157 mg/kg) except muscle, where 11.5 – 26.7 % TRR (or 0.002 – 0.003 mg/kg) were found. For milk, 78.9 – 80.9 % TRR were found in the HCl supernatant and 19.1 – 21.1 % TRR (or 0.005 – 0.010 mg/kg) in the RRR. The RRRs were not further investigated.

Glyphosate (47.8 – 89.6 % TRR or 0.003 – 6.429 mg/kg) and AMPA (4.9 – 30.7 % TRR or 0.000 – 0.677 mg/kg) accounted for the majority of the radioactive residue in all matrices. Furthermore an unknown compound was assigned in milk (23.5 – 28.0 % TRR or 0.005 mg/kg). The unknown in milk was isolated and further analysed by gel filtration chromatography and acid hydrolysis. The ^{14}C activity appeared to be associated with small molecular weight proteins or glycoproteins of molecular weight less than 6,500 daltons.

I. Materials and methods

A. Materials

Test material	a) N-(phosphono-^{14}C-methyl)glycine b) N-(phosphono-^{13}C-methyl)glycine c) N-(phosphonomethyl)glycine d) Amino-^{14}C-methylphosphonic acid e) Amino-^{13}C-methylphosphonic acid f) Aminomethylphosphonic acid
Chemical structure:	a, b, c)  d, e, f)  * Position of the radio label
Radiochemical purity:	>98 %

Specific activity:	a, b, c) 2.18 MBq/mg (58.9 µCi/mg = 9.96 mCi/mmol) d, e, f) 3.36 MBq/mg (90.8 µCi/mg = 10.08 mCi/mmol)
CAS No:	a, b, c) 1071-83-6 d, e, f) 1066-51-9
Log $P_{o/w}$:	a, b, c) -3.2 d, e, f) -2.47

Test animals:	
Species:	Goat, *Capra aegarus hircus*
Strain:	Not reported
Breeding facility:	[redacted]
Gender and numbers involved:	Female, 4 animals (1 control group – test 1, 3 treatment groups: 2 test 2 and 1 test 3), identified by numbered neck and tag
Body weight:	49 – 58 kg (treated animals, day 12 of acclimation)
Age:	Not reported
Location of the in-life phase:	[redacted]
Acclimatisation:	12 days before first treatment
Housing:	Individually housed in metabolic cages with artificial light at a 12/12 hours light/dark cycle Temperature: 18 – 27 °C, Humidity: 60 – 78 %
Feed and water:	Alfalfa grass *ad libitum*; grain based milking ration (Sunshine Farms, Portage, Wisconsin, USA), 1.0 kg/goat/day and tap water, *ad libitum*

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	2.95 and 2.83 mg equiv./kg bw/day*
Feed consumption:	1.293 – 1.336 kg/day
Vehicle:	Gelatine capsules
Timing:	Once daily
Duration:	5 days (+ 5 days depuration phase in test 3)

* Calculated based on average body weights of the goats per test at day 12 of acclimation (54 and 55 kg for test 2 and test 3, respectively), the actual dose level of 120 mg/kg feed consumed and an average feed consumption of 1.315 and 1.299 kg feed/day for test 2 and 3, respectively.

Two test with lactating goats (test 2: two goats; test 3: one goat) were conducted with a 9:1 mixture of N-(phosphono-$^{13}C/^{14}C$-methyl)glycine ($^{13}C/^{14}C$-glyphosate) and amino-$^{13}C/^{14}C$-methylphosphonic acid ($^{13}C/^{14}C$-AMPA) to investigate their behaviour in goats. For this, the ^{14}C-labelled glyphosate and ^{14}C-labelled AMPA were each diluted with the corresponding ^{13}C-enriched and unlabelled materials so as to produce a final ^{13}C enrichment of approximately 50 % with the above mentioned specific activities.

The test mixture (272.2 mg $^{13}C/^{14}C$-glyphosate and 28.7 mg amino-$^{13}C/^{14}C$-methylphosphonic acid) was administered once a day for five consecutive days in a gelatine capsule and were administrated orally. One additional lactating goat was given capsules containing sucrose as the control group (test 1). Actual dose levels are summarised in the table below:

Table 6.2.3-15: Dose levels

	Test 2 (120 mg/kg feed)	Test 3 (120 mg/kg feed)
mg equiv./kg bw/day (glyphosate/AMPA)	2.95 (2.65/0.29)	2.83 (2.55/0.28)
Average body weight (kg)	54	55

Dose levels were calculated using average body weights of the goats per test at day 12 of acclimation and an average feed consumption of 1.315 and 1.299 kg feed/day for test 2 and 3, respectively.

Due to the low water solubility of glyphosate at neutral pH, glyphosate and AMPA were converted to their respective sodium salt forms in order to ensure complete administration. The free acid forms of the test mixtures were neutralised to pH 7.0 with standard 5 N sodium hydroxide solution. At this pH, glyphosate was converted to its disodium salt and AMPA to its monosodium salt. The neutralised solutions of the test mixtures were adsorbed onto sucrose which was then filled in gelatine capsules.
Following their preparation, the capsules were immediately frozen (-20 °C) and sent to Hazleton Laboratories America (on dry ice) for the in-life part of the study where the doses in the capsules were verified. The dosing capsules were analysed to determine the actual total radioactivity in each of the dose capsules. Two capsules were diluted with water and analysed by liquid scintillation counting (LSC).

Animals were observed twice daily for mortality and moribundity as well as once daily for general appearance and behaviour. Body weights were recorded on days 4 and 12 of acclimation and on days 6 and 10 of the test period. Feed consumption and milk production were recorded daily.

2. Sampling and storage

Urine and faeces were collected twice a day. The surface of the metabolism pans were rinsed with trisodium phosphate solutions after the goats were sacrificed. Milk was collected twice daily. On two occasions, the animal of the control group and animals of test 2 were milked twice in the morning and the milkings were combined.
Goats of tests 1 to 2 were sacrificed 22 to 24 h after last dosing and the goat of test 3 was sacrificed 5 days after last dosing with the test material using a captive-bolt pistol and exsanguination. A macroscopic examination of each goat was performed. Blood and the following tissues and organs were collected from each animal at sacrifice: kidney (both), liver, muscle, fat (approximately 1:1 renal and omental) and GI tract and contents (divided into two separated samples, one containing contents of the rumen, reticulum, omasum and abomasum, and the other containing the contents of the small and large intestine). All samples were pooled separately by treatment group.
Blood was stored refrigerated until after determination of radioactivity in the samples and was then stored below 0 °C. All other samples were stored below 0 °C at the site of the in-life part (Hazleton Laboratories). After determination of radioactivity in the samples, they were sent to Monsanto Co. (site of analysis) on dry ice via overnight freight. The samples were stored at -20 °C at Monsanto Co.

3. Analytical procedures

The total ^{14}C-activity present in samples was determined directly by combustion of homogenised samples (triplicates) of kidney, liver, muscle and fat and blood followed by LSC. Faeces, rumen contents and GI trace contents were homogenised with water and centrifuged. The residues were lyophylised and the dried residues were combusted. Triplicate aliquots of the supernatants, urine, milk and pans rinse were mixed

with Atomlight scintillation cocktail and analysed by LSC. Samples of kidney, liver, muscle, fat and blood were homogenised without added water.

Radioactive components from homogenised tissue samples were extracted using chloroform and water. The samples were extracted twice using a chloroform/water mixture (1:1; v:v) followed by a third extraction using only water. Water and chloroform phases and precipitate were separated by centrifugation. The combined chloroform extracts and the water extracts were analysed by LSC and the precipitate was combusted.
Protein was precipitated from the combined water extracts of tissue samples by treating the extract with methanol or by placing the sample in a boiled water bath for 10 minutes. After centrifugation to remove the proteins, the water extracts were concentrated to dryness. The resulting residues were solubilised in water, centrifuged and analysed by HPLC.
Radioactive residues in faeces were extracted with water, centrifuged and the water extract was analysed by LSC and HPLC.
Milk samples from each goat were pooled so that a representative milk sample from the entire collection period was obtained. Pooled milk samples were mixed with an equivalent volume of concentrated HCl. In order to precipitate proteins the mixture was shaken for 30 minutes. After centrifugation, the aqueous extract was analysed by LSC and concentrated to dryness. The resulting residues were solubilised in water, centrifuged and analysed by HPLC.

Two HPLC methods were employed to characterise the residues in the extracts: an ion pair HPLC and a cation exchange HPLC.
For determination of the distribution of radioactive residues in the samples, the results of the analyses of the water extracts with the cation exchange HPLC were used.
Kidney and milk extracts were purified using a Fe(III)-Chelex column. In addition to HPLC, a gel filtration Fast Protein Liquid Chromatography (FPLC) method was used to purify and size the unknown radioactive residue in milk. Furthermore, the milk unknown was subjected to acid digestion (6 N HCl, 110 °C, 24 h). The residue was extracted with 0.2 N citrate buffer at pH 2.2. The extract was analysed by LSC and an amino acid analyser.

For identification of residues in the extracts, HPLC fractions corresponding to glyphosate were isolated from kidney extracts using cation exchange HPLC. The desired HPLC fraction was concentrated to dryness via lyophilisation followed by treatment with trifluoroacetic anhydride and trifluoroethanol at 90 °C for 2.5 h. The resulting mixtures were analysed by GC with radioactivity detection and GC/MS. Fractions containing glyphosate and AMPA were isolated from kidney extracts and were purified using a Fe(III)-Chelex column. After derivatisation as described above, the resulting derivate of AMPA was isolated by reverse phase HPLC. The AMPA derivative in the HPLC effluent was extracted into dichloromethane, dried over anhydrous sodium sulphate, concentrated and analysed by GC/MS. Peak assignment was based on retention time comparison.

II. Results and discussion

Radioactive residues in tissue, milk, faeces and urine of the control group (test 1) were below the detection limits of the analytical methods used. Therefore only results of treated dose groups are presented.

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in the table below. For test 2 two goats were treated, in the following tables single values as well as calculated mean values are depicted. For discussion in the text passages only to the mean values is referred. Between 83.6 and 86.7 % of the administered dose was recovered. The main part was excreted, accounting in sum (urine, faeces plus pan rinse) for 74.95 (test 2) and 86.46 % (test 3). In test 3 (with depuration for 5 days), in all other matrices <0.01 % of the dose was detected except for liver and GI tract with contents, where 0.03 and 0.15 % of the dose were found, respectively. In test 2, amounts in animal matrices ranged between <0.01 and 0.09 % of the dose except for the GI tract with contents and rumen contents, where 7.41 and 1.07 % of the dose were

found, respectively.

Table 6.2.3-16: Radioactivity balances: Distribution of radioactive residues in tissues, milk and excreta of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA

Matrix	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)			Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)
	Goat 2	Goat 3	Calculated mean of goat 2 and goat 3	
	% dose[2]			
Kidney	0.13	0.05	*0.09*	<0.01
Liver	0.04	0.03	*0.04*	0.03
Muscle	<0.01	<0.01	*<0.01*	<0.01
Fat	<0.01	<0.01	*<0.01*	<0.01
Milk	<0.01	<0.01	*<0.01*	<0.01
Urine	23.6	20.3	*22.0*	20.1
Pan rinse	0.20	0.40	*0.30*	0.06
Faeces	58.2	47.2	*52.7*	66.3
Rumen contents	0.59	1.55	*1.07*	<0.01
GI tract with contents	3.32	11.5	*7.41*	0.15
Blood	N/A	N/A	*N/A*	N/A
Total	86.1	81.1	*83.6*	86.7

[1] For test 2 mean values were calculated from individual values of both goats.
[2] % dose = Percent of administered dose
N/A = not applicable
Italic figures were not part of the report, but correspond to values calculated upon figures given in the report.

In the table below the total radioactive residues are summarised for samples of lactating goats, following administration of 120 mg per kg feed for 5 days. TRRs are expressed as glyphosate equivalents. Highest TRR values were detected in kidneys (test 2: 7.0 mg/kg; test 3: 0.505 mg/kg) and liver (test 2: 0.493 mg/kg; test 3: 0.381 mg/kg). In muscle, fat and blood 0.009 – 0.027, 0.004 – 0.010 and 0.016 – 0.106 mg/kg were found, respectively.
In milk the radioactive residue ranged between 0.019 and 0.060 mg/kg during the dosing period and reached a plateau after 3 days. After the 5 day depuration the radioactive residue decreased to 0.006 mg/kg.

Table 6.2.3-17: Total radioactive residue in samples of l lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA

Matrix	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)			Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)
	Goat 2	Goat 3	Calculated mean of goat 2 and goat 3	
	TRR (mg equiv./kg)			
Kidneys	10.5	3.49	*7.0*	0.505[2]
Liver	0.529	0.457	*0.493*	0.381
Muscle	0.028	0.026	*0.027*	0.009
Fat	0.011	0.009	*0.010*	0.004
Blood	0.129	0.082	*0.106*	0.016

[1] For test 2 mean values were calculated from individual values of both goats.
[2] Average of two separately analysed portions
TRR = total radioactive residue, expressed as glyphosate-equivalents
Italic figures were not part of the report, but correspond to values calculated upon figures given in the report.

Table 6.2.3-18: Radioactive residues in milk of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA

Days	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)			Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)
	Goat 2	Goat 3	Calculated mean of goat 2 and goat 3	
	TRR (mg equiv./kg)			
Predose	n.d.	n.d.	n.d.	n.d.
1	0.020	0.049	*0.035*	0.019
2	0.033	0.076	*0.055*	0.030
3	0.034	0.086	*0.060*	0.038
4	0.035	0.080	*0.058*	0.037
5	0.031	0.078	*0.055*	0.038
6	N/A	N/A	N/A	0.025
7	N/A	N/A	N/A	0.013
8	N/A	N/A	N/A	0.007
9	N/A	N/A	N/A	0.006

[1] For test 2 mean values were calculated from individual values of both goats.
TRR = total radioactive residue, expressed as glyphosate-equivalents
n.d. = not detectable
N/A = not applicable

B. Extraction and characterisation of residues

The analysis of the radioactive residues following extraction was reported in the second part of the study (part II). Kidney, liver, fat, muscle and milk samples were investigated for their composition of glyphosate and AMPA.

For easier comprehension, values from the report were re-calculated to give amounts relative to the TRR (% of TRR). Due to rounding, discrepancies may occur when re-calculating the values. In addition to % TRR values, TRR values were calculated in mg equiv./kg. The values for the respective total water extracts were considered for calculation. The report also contains values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for calculations. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

In tissue, portions of 73.3 to 97.8 % of TRR were extractable. In all samples, the major part of the residue was extracted with water. Only low amounts were found in the chloroform extracts (0.0 – 11.4 % TRR or 0.00 – 0.002 mg/kg). The remaining non-extractable residues were between 2.3 and 5.5 % TRR (or 0.000 – 0.157 mg/kg) except muscle, where 11.5 – 26.7 % TRR (or 0.002 – 0.003 mg/kg) were found. For milk, 78.9 – 80.9 % TRR were found in the HCl supernatant and 19.1 – 21.1 % TRR (or 0.005 – 0.010 mg/kg) in the RRR.

The RRRs were not further investigated.

Table 6.2.3-19: Extraction of the radioactive residues in kidney of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Kidney	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR
TRR	**10.529**	**100**	**3.491**	**100**	***7.010***	***100***	**5.050**	**100**
ERR	***10.424***	***99.0***	***3.351***	***96.0***	***6.888***	***97.5***	***4.893***	***96.9***
Chloroform extract	*0.000*	0.0	*0.000*	0.0	*0.000*	*0.0*	*0.000*	0.0
Water extract	*10.424*	99.0	*3.351*	96.0	*6.888*	*97.5*	*4.893*	96.9
RRR	***0.105***	**1.0**	***0.140***	**4.0**	***0.122***	***2.5***	***0.157***	**3.1**
Accountability[2]	103.6		100.7		*102.2*		100.3	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.3-20: Extraction of the radioactive residues in liver of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Liver	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR
TRR	**0.529**	**100**	**0.457**	**100**	***0.493***	***100***	**0.381**	**100**
ERR	***0.507***	***95.8***	***0.426***	***93.3***	***0.467***	***94.6***	***0.362***	***95.0***
Chloroform extract	*0.001*	0.1	*0.003*	0.7	*0.002*	*0.4*	*0.001*	0.3
Water extract	*0.506*	95.7	*0.423*	92.6	*0.465*	*94.2*	*0.361*	94.7
RRR	***0.022***	**4.1**	***0.031***	**6.8**	***0.026***	***5.5***	***0.019***	**5.0**
Accountability[2]	103.9		103.4		*103.6*		103.5	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.3-21: Extraction of the radioactive residues in muscle of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Muscle	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR
TRR	**0.028**	**100**	**0.026**	**100**	***0.027***	***100***	**0.009**	**100**
ERR	***0.026***	***92.0***	***0.022***	***85.1***	***0.024***	***88.6***	***0.007***	***73.3***
Chloroform extract	*0.000*	0.4	*0.000*	0.7	*0.000*	*0.6*	*0.000*	0.0
Water extract	*0.026*	91.6	*0.022*	84.4	*0.024*	*88.0*	*0.007*	73.3
RRR	*0.002*	8.1	*0.004*	14.8	***0.003***	***11.5***	***0.002***	**26.7**
Accountability[2]	107.8		114.1		*111.0*		106.5	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.3-22: Extraction of the radioactive residues in fat of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Fat	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR
TRR	**0.011**	**100**	**0.009**	**100**	***0.010***	***100***	**0.004**	**100**
ERR	***0.011***	***99.0***	***0.009***	***96.6***	***0.010***	***97.8***	***0.004***	***96.0***
Chloroform extract	*0.000*	2.6	*0.000*	4.3	*0.000*	*3.5*	*0.000*	11.4
Water extract	*0.011*	96.4	*0.008*	92.3	*0.009*	*94.4*	*0.003*	84.6
RRR	***0.000***	**1.0**	***0.000***	**3.5**	***0.000***	***2.3***	***0.000***	**4.0**
Accountability[2]	99.4		104.8		*102.1*		85.4	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] For all samples, extraction accountability was given in the report. The values were calculated based on the amount of residues in the sample determined by combustion.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.3-23: Extraction of the radioactive residues in milk of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Milk	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR	mg equiv./ kg	% TRR
TRR	**0.028**	**100**	**0.068**	**100**	***0.048***	***100***	**0.022**	**100**
ERR	***0.023***	***82.9***	***0.054***	***78.9***	***0.038***	***80.9***	***0.017***	**78.9**
HCl supernatant	*0.023*	82.9	*0.054*	78.9	*0.038*	*80.9*	*0.017*	78.9
RRR	***0.005***	**17.1**	***0.014***	**21.1**	***0.010***	***19.1***	***0.005***	**21.1**

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Glyphosate and AMPA were identified in an isolated and derivatised fraction of kidney using spectroscopic methods (GC with radioactivity detection and GC/MS). Identification rates ranged from 94.9 to 95.8 % of TRR in kidneys, from 90.5 to 92.1 % of TRR in liver, from 62.2 to 81.9 % of TRR in muscle, from 84.7 to 93.3 % of TRR in fat and from 52.7 to 55.4 % of TRR in milk.
Two HPLC methods were employed to characterise the residues in the extracts: an ion pair HPLC and a cation exchange HPLC, both results are depicted in the following tables. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations. For discussion in the text passages only to the values of cation exchange HPLC is referred. Amounts of glyphosate (47.8 – 89.6 % TRR or 0.003 – 6.429 mg/kg) were generally higher than amounts of AMPA (4.9 – 30.7 % TRR or 0.000 – 0.677 mg/kg) in all extracts. In milk, one unknown compound was detected. The amounts ranged from 23.5 to 28.0 % of TRR (or 0.005 – 0.014 mg equiv./kg). The unknown in milk was isolated and further analysed by gel filtration chromatography and acid hydrolysis. The ^{14}C activity appeared to be associated with small molecular weight proteins or glycoproteins of molecular weight less than 6,500 daltons.

Table 6.2.3-24: Identification and characterisation of the radioactive residues in kidney of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Kidney	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR
TRR	**10.529**	**100**	**3.491**	**100**	***7.010***	***100***	**5.050**	**100**
ERR	***10.424***	***99.0***	***3.351***	***96.0***	***6.888***	***97.5***	***4.893***	***96.9***
Chloroform extract	*0.000*	0.0	*0.000*	0.0	*0.000*	*0.0*	*0.000*	0.0
Water extract	*10.424*	99.0	*3.351*	96.0	*6.888*	*97.5*	*4.893*	96.9
Water extract analysed by cation exchange HPLC:								
AMPA	*0.411*	3.9	*0.293*	8.4	*0.352*	*6.2*	*0.677*	13.4
Glyphosate	*9.876*	93.8	*2.981*	85.4	*6.429*	*89.6*	*4.116*	81.6
Water extract analysed by ion pair HPLC:								

Table 6.2.3-24: Identification and characterisation of the radioactive residues in kidney of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Kidney	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR
AMPA[2]	*0.417*	*4.0*	*0.305*	*8.7*	*0.361*	*6.3*	*0.773*	*15.3*
Glyphosate[2]	*9.944*	*94.4*	*3.030*	*86.8*	*6.487*	*90.6*	*4.071*	*80.6*
Total identified	***10.287***	***97.7***	***3.275***	***93.8***	***6.781***	***95.8***	***4.792***	***94.9***
Total characterised[3]	***0.000***	***0.0***	***0.000***	***0.0***	***0.000***	***0.0***	***0.000***	***0.0***
RRR	***0.105***	**1.0**	***0.140***	**4.0**	***0.122***	***2.5***	***0.157***	**3.1**
Recovery of the extracts (% of water extract)[4]								
Deproteination	99.5		98.7		*99.1*		98.7	
Concentration	98.0		94.4		*96.2*		96.5	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.
[3] Characterised by extraction and/or chromatographic behaviour.
[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.3-25: Identification and characterisation of the radioactive residues in liver of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Liver	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR

Table 6.2.3-25: Identification and characterisation of the radioactive residues in liver of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Liver	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days – 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR
TRR	**0.529**	**100**	**0.457**	**100**	***0.493***	***100***	**0.381**	**100**
ERR	***0.507***	***95.8***	***0.426***	***93.3***	***0.467***	***94.6***	***0.362***	***95.0***
Chloroform extract	*0.001*	0.1	*0.003*	0.7	*0.002*	*0.4*	*0.001*	0.3
Water extract	*0.506*	95.7	*0.423*	92.6	*0.465*	*94.3*	*0.361*	94.7
Water extract analysed by cation exchange HPLC:								
AMPA	*0.078*	14.8	*0.048*	10.6	*0.063*	*12.7*	*0.117*	30.7
Glyphosate	*0.417*	78.9	*0.351*	76.7	*0.384*	*77.8*	*0.234*	61.4
Water extract analysed by ion pair HPLC:								
AMPA[2]	*0.095*	*18.0*	*0.081*	*17.8*	*0.088*	*17.9*	*0.130*	*34.1*
Glyphosate[2]	*0.402*	*76.0*	*0.333*	*72.8*	*0.367*	*74.4*	*0.224*	*58.7*
Total identified	***0.496***	***93.7***	***0.399***	***87.3***	***0.447***	***90.5***	***0.351***	***92.1***
Total characterised[3]	***0.001***	***0.1***	***0.003***	***0.7***	***0.002***	***0.4***	***0.001***	***0.3***
RRR	***0.022***	**4.1**	***0.031***	**6.8**	***0.026***	**5.5**	***0.019***	**5.0**
Recovery of the extracts (% of water extract)[4]								
Deproteination	85.3		79.5		*82.4*		87.5	
Concentration	90.6		96.6		*93.6*		92.5	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] For test 2 mean values were calculated from individual values of both goats.

[2] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[3] Characterised by extraction and/or chromatographic behaviour.

[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

Table 6.2.3-26: Identification and characterisation of the radioactive residues in muscle of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Muscle	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR
TRR	**0.028**	**100**	**0.026**	**100**	**0.027**	**100**	**0.009**	**100**
ERR	***0.026***	***92.0***	***0.022***	***85.1***	***0.024***	***88.6***	***0.007***	***73.3***
Chloroform extract	*0.000*	0.4	*0.000*	0.7	*0.000*	*0.6*	*0.000*	0.0
Water extract	*0.026*	91.6	*0.022*	84.4	*0.024*	*88.0*	*0.007*	73.3
Water extract analysed by cation exchange HPLC:								
AMPA	*0.001*	4.3	*0.003*	10.6	*0.002*	*7.5*	*0.001*	10.4
Glyphosate	*0.023*	83.2	*0.017*	65.7	*0.020*	*74.5*	*0.005*	51.8
Water extract analysed by ion pair HPLC:								
AMPA[2]	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Glyphosate[2]	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Total identified	***0.025***	***87.5***	***0.020***	***76.3***	***0.022***	***81.9***	***0.006***	***62.2***
Total characterised[3]	***0.000***	***0.4***	***0.000***	***0.7***	***0.000***	***0.6***	***0.000***	***0.0***
RRR	***0.002***	**8.1**	***0.004***	**14.8**	***0.003***	***11.5***	***0.002***	**26.7**
Recovery of the extracts (% of water extract)[4]								
Deproteination	100.4		98.0		*99.2*		99.7	
Concentration	67.9		70.8		*69.4*		63.2	

Values in *italics* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.
[3] Characterised by extraction and/or chromatographic behaviour.
[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue
N/A = not applicable

Table 6.2.3-27: Identification and characterisation of the radioactive residues in fat of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Fat	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days + 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR
TRR	**0.011**	**100**	**0.009**	**100**	**0.010**	**100**	**0.004**	**100**
ERR	***0.011***	***99.0***	***0.009***	***96.6***	***0.010***	***97.8***	***0.004***	***96.0***
Chloroform extract	*0.000*	2.6	*0.000*	4.3	*0.000*	*3.5*	*0.000*	11.4
Water extract	*0.011*	96.4	*0.008*	92.3	*0.009*	*94.4*	*0.003*	84.6
Water extract analysed by cation exchange HPLC:								

AMPA	*0.001*	8.7	*0.001*	10.5	*0.001*	*9.6*	*0.000*	8.9
Glyphosate	*0.010*	87.0	*0.007*	80.4	*0.008*	*83.7*	*0.003*	75.8
Water extract analysed by ion pair HPLC:								
AMPA[2]	*0.001*	*9.1*	*0.001*	*15.5*	*0.001*	*12.3*	*0.000*	*12.3*
Glyphosate[2]	*0.009*	*82.7*	*0.007*	*76.2*	*0.008*	*79.5*	*0.003*	*70.0*
Total identified	***0.011***	***95.7***	***0.008***	***90.9***	***0.009***	***93.3***	***0.003***	***84.7***
Total characterised[3]	***0.000***	***2.6***	***0.000***	***4.3***	***0.000***	***3.5***	***0.000***	***11.4***
RRR	***0.000***	**1.0**	***0.000***	**3.5**	***0.000***	***2.3***	***0.000***	**4.0**
Recovery of the extracts (% of water extract)[4]								
Deproteination	100.1		103.8		*102.0*		93.5	
Concentration	93.5		89.6		*91.6*		111.4	

Values in *italics* were calculated upon dossier compilation. The % TRR values shown are normalised values.

[1] For test 2 mean values were calculated from individual values of both goats.

[2] The extract was additionally analysed by ion pair HPLC. Since the values of ion pair and cation exchange HPLC analyses were rather similar, only the results of the cation exchange HPLC were used for further calculations.

[3] Characterised by extraction and/or chromatographic behaviour.

[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.

TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue
N/A = not applicable

Table 6.2.3-28: Identification and characterisation of the radioactive residues in milk of lactating goats after treatment with a 9:1 mixture of $^{13}C/^{14}C$-glyphosate and $^{13}C/^{14}C$-AMPA for 5 days

Milk	Test 2[1] (2.65 mg glyphosate and 0.29 mg AMPA per kg bw and day, 5 days)						Test 3 (2.55 mg glyphosate and 0.28 mg AMPA per kg bw and day, 5 days – 5 days depuration)	
	Goat 2		Goat 3		Calculated mean of goat 2 and goat 3			
	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR	mg equiv./kg	% TRR
TRR	**0.028**	**100**	**0.068**	**100**	***0.048***	***100***	**0.022**	**100**
ERR	***0.023***	***82.9***	***0.054***	***78.9***	***0.038***	***80.9***	***0.017***	***78.9***
HCl supernatant	*0.023*	82.9	*0.054*	78.9	*0.038*	*80.9*	*0.017*	78.9
Water extract analysed by cation exchange HPLC:								
AMPA	*0.002*	5.6	*0.003*	4.3	*0.002*	*4.9*	*0.002*	7.4
Glyphosate	*0.015*	53.1	*0.029*	42.4	*0.022*	*47.8*	*0.011*	48.0
Unknown[2]	*0.007*	24.3	*0.021*	31.6	*0.014*	*28.0*	*0.005*	23.5
Total identified	***0.016***	***58.7***	***0.032***	***46.7***	***0.024***	***52.7***	***0.012***	***55.4***
Total characterised[3]	***0.007***	***24.3***	***0.021***	***31.6***	***0.014***	***28.0***	***0.005***	***23.5***
RRR	***0.005***	**17.1**	***0.014***	**21.1**	***0.010***	***19.1***	***0.005***	**21.1**
Recovery of the extracts (% of water extract)[4]								
Concentration	65.9		68.3		*67.1*		65.4	

Values in *italic* were calculated upon dossier compilation. The % TRR values shown are normalised values.
[1] For test 2 mean values were calculated from individual values of both goats.
[2] The unknown in milk was isolated and further analysed by gel filtration chromatography and acid hydrolysis. The ^{14}C activity appeared to be associated with small molecular weight proteins or glycoproteins of molecular weight less than 6,500 daltons.
[3] Characterised by extraction and/or chromatographic behaviour.
[4] The report gave values for recovery of radioactivity during sample preparation for analysis. These recovery values were not considered for further calculations and are given here only for information. The residues lost during sample preparation are regarded as equivalent to the total amount in the water extract.
TRR = total radioactive residue; given in mg free glyphosate acid equivalents per kg sample
ERR = extractable radioactive residue
RRR = residual radioactive residue

C. Storage stability

All samples, stored frozen at -20 °C. Storage stability was determined using one of the faeces samples from test 3. This faecal sample was first analysed at the beginning of the study and was reanalysed at its conclusion. The faeces sample was extracted with water and the aqueous extract and the residue were analysed by LSC and combustion analysis, respectively. In the initial analysis, greater than 87 % of the ^{14}C activity was extracted into water and was analysed by cation exchange HPLC. The distribution of glyphosate and AMPA was 80.9 % and 17.4 %, respectively. The analysis of the same faeces sample at the end of the study recovered 92.5 % of the activity in the water extract. Cation exchange HPLC analysis of the water extract resulted in a distribution of glyphosate and AMPA of 79.6 % and 18.4 %, respectively. These results demonstrated that no appreciable changes in the nature or distribution of the ^{14}C residues had occurred during storage.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

Two test with lactating goats were conducted with a 9:1 mixture of N-(phosphono-$^{13}C/^{14}C$-methyl)glycine ($^{13}C/^{14}C$-glyphosate) and amino-$^{13}C/^{14}C$-methylphosphonic acid ($^{13}C/^{14}C$-AMPA) to investigate their

behaviour in goats. One additional test was performed as a control group without dosing with test substance (test 1).
The goats received a dose of 120 mg/kg feed = 300.9 mg test mixture/day ≙ 272.2 mg $^{13}C/^{14}C$-glyphosate disodium salt and 28.7 mg $^{13}C/^{14}C$-AMPA monosodium salt per day (2.83 – 2.95 mg/kg bw/day ≙ 2.55 – 2.65 mg $^{13}C/^{14}C$-glyphosate/kg bw/day and 0.28 – 0.29 mg $^{13}C/^{14}C$-AMPA/kg bw/day). One capsule was administered each day for 5 consecutive days. Two experiments with radioactive test substances were conducted (test 2: two goats; test 3: one goat). In test 2 the goats were sacrificed 22 to 24 hours after the last dose. In test 3, a 5-day depuration phase was added after the 5th dose after which the goat was sacrificed.
The major portion of radioactive residue was recovered in urine, faeces and pan rinse. Highest TRR values were detected in kidneys (0.505 – 7.0 mg/kg) and liver (0.381 – 0.493 mg/kg). In muscle and fat 0.009 – 0.027 mg/kg and 0.004 – 0.010 mg/kg were found, respectively.
In milk the radioactive residue ranged between 0.019 and 0.060 mg/kg during the dosing period. After the 5 day depuration the radioactive residue decreased to 0.006 mg/kg.
In tissue, portions of 73.3 to 97.8 % of TRR were extractable. In all samples, the major part of the residue was extracted with water. The remaining non-extractable residues were between 2.3 and 5.5 % TRR (or 0.000 – 0.157 mg/kg) except muscle, where 11.5 – 26.7 % TRR (or 0.002 – 0.003 mg/kg) were found. For milk, 78.9 – 80.9 % TRR were found in the HCl supernatant and 19.1 – 21.1 % TRR (or 0.005 – 0.010 mg/kg) in the RRR. The RRRs were not further investigated.
Glyphosate (47.8 – 89.6 % TRR or 0.003 – 6.429 mg/kg) and AMPA (4.9 – 30.7 % TRR or 0.000 – 0.677 mg/kg) accounted for the majority of the radioactive residue in all matrices. Furthermore an unknown compound was assigned in milk (23.5 – 28.0 % TRR or 0.005 – 0.014 mg/kg). The unknown in milk was isolated and further analysed by gel filtration chromatography and acid hydrolysis. The ^{14}C activity appeared to be associated with small molecular weight proteins or glycoproteins of molecular weight less than 6,500 daltons.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behavior of glyphosate in lactating goats has been previously evaluated at EU level. It was performed under GLP. The study does not entirely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 503 with major deficits (the radioactivity balances was between 83.6 and 86.7 %, the recovery of radioactive residues after extraction of fat (test 3) was only 85.4 (TRR was only 0.004 mg/kg), the recovery of radioactive residues after deproteination of liver was only 82.4 – 87.5 % and the recovery of radioactive residues after concentration of muscle and fat were only 63.2 – 69.4 % and 65.4 – 67.1 %, respectively, no description of duration of storage of samples).
For fat (test 3), the recovery of radioactive residues after extraction was only 85.4 %. However, a low absolute residue level was found (0.004 mg/kg) and therefore a further characterisation is not necessary.
The recovery of radioactive residues after deproteination of liver was also only moderate (82.4 – 87.5 %). The residues after deproteination were, however, not radioanalysed and therefore no complete recovery was determined within the report.
The duration of storage of samples is not reported within the report. Nevertheless, a storage stability analysis of faeces samples at the end of the study demonstrated that no appreciable changes in the nature or distribution of the ^{14}C residues had occurred during storage.
The study is considered to be supportive for the metabolism in lactating goats.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.3/005
Report author	[redacted]
Report year	2007
Report title	Metabolism of [^{14}C]-*N*-Acetylglyphosate (IN-MCX20) in the lactating goat
Report No	28130
Document No	[redacted]-19796
Guidelines followed in study	Residue Test Guideline, OPPTS 860.1300, Nature of the Residue – Livestock, U.S. Environmental Protection Agency, August 1996 and FAO Guidelines as Recommended by EU Commission Directive 96/68/EC Annex 1, Section 6.2, (21 October 1996)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 503: • Urine and faeces were collected only once daily • Radioactivity was not quantified separately in the different muscle types • Identification of components by "co-chromatography" with one method and confirmation of compounds by LC-MS/MS only in faeces • It is not reported if the estimated relative dose was based on a feed dry weight basis. • The radioactivity balance is 87.8 % (GIT and its contents and carcasses were not measured) • Balance of components in matrices (liver, kidney, milk, muscle, omental fat, reneal fat and subcutaneous fat) miss portions of up to 29.93 % TRR or up to 0.338 mg/kg (recovery or calculation issue).
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The purpose of this study was to investigate the extent to which residues may be transferred to food products destined for human consumption and establish the nature of any transferred residues.
A dose solution was prepared using ^{14}C-*N*-acetylglyphosate (in aqueous solution) and non-radiolabelled *N*-acetylglyphosate. The mixture was administered twice daily to a lactating goat as an oral dose of ^{14}C-*N*-acetylglyphosate for 5 consecutive days. The nominal dose level was 200 mg/kg of feed consumed per day. The actual dose level based on feed consumption was 205.42 mg *N*-acetylglyphosate equivalent/kg (8.42 mg *N*-acetylglyphosate equivalent/kg bw/day). Faeces and urine were collected once daily and milk collected twice daily. The goat was sacrificed approximately 12 hours after the last dose and the total radioactive residues (TRR) in bile, liver, kidney, muscle, omental fat, renal fat, and subcutaneous fat determined.

Recovery of the total administered dose in excreta, milk and tissues was 87.83 %. Faeces, urine, and cage wash contained 74.17 %, 11.45 %, and 2.12 %, of the total administered dose, respectively. Milk, liver, and kidney each contained 0.03 % of the administered dose. The TRR in muscle, kidney, and liver was 0.047, 4.689, and 0.715 mg/kg *N*-acetylglyphosate equivalents, respectively. The TRR in omental, renal, and subcutaneous fat was 0.065, 0.093, and 0.108 mg/kg *N*-acetylglyphosate equivalents, respectively.

Composite (Study Day 1-5) samples of faeces, urine, and milk were analysed. Composite urine was not extracted, but was centrifuged to remove particulates prior to analysis. Composite faeces and milk, liver, kidney, and muscle samples were extracted with 0.2 N hydrochloric acid. Fat (subcutaneous, omental, and renal) samples were extracted with 0.2 N hydrochloric acid and dichloromethane. Approximately 35 – 97 % TRR was extracted from the tissues. The TRR remaining in the post extracted solids of liver, kidneys, muscle, and omental fat were subjected to sequential treatment with pepsin and protease enzymes, which released additional radioactivity (up to 28 % TRR). Metabolites were reported to be identified by HPLC co-chromatography with authentic radiolabelled and unlabelled reference standards, and then later confirmed in selected samples using mass spectrometry.

Four radiolabelled components were detected in faeces, the most abundant was *N*-acetylglyphosate accounting 53.16 % of the dose. AMPA, glyphosate, and *N*-acetyl AMPA were also detected and accounted for 0.81 %, 3.27 % and 16.41 % of the dose, respectively.

In urine, *N*-acetylglyphosate was the only detected component accounting for 11.41 % of the dose.

Individual daily milk concentrations ranged from 0.030 to 0.036 mg/kg *N*-acetylglyphosate equivalents. Three radiolabelled components were detected in the composite milk extract. The most abundant component, *N*-acetylglyphosate, accounted for 39.98 % TRR (0.011 mg/kg N-acetylglyphosate equivalents). Two components, AMPA and glyphosate, accounted for 3.35 % TRR (0.001 mg/kg N-acetylglyphosate equivalents) and 3.59 % TRR (0.001 mg/kg N-acetylglyphosate equivalents), respectively.

Four radiolabelled components were detected in liver, the most abundant was *N*-acetylglyphosate accounting for 55.51 % TRR (0.446 mg/kg). AMPA and glyphosate were also detected and accounted for 8.45 % TRR (0.068 mg/kg N-acetylglyphosate equivalents) and 14.71 % TRR (0.118 mg/kg N-acetylglyphosate equivalents), respectively. A single minor unknown component accounted for 0.52 % TRR (0.004 mg/kg *N*-acetylglyphosate equivalents).

Nine radiolabelled components were detected in kidney, the most abundant was *N*-acetylglyphosate and accounted for 77.12 % TRR (3.742 mg/kg). Glyphosate was also detected and accounted for 4.98 % TRR (0.242 mg/kg N-acetylglyphosate equivalents). Seven additional unknown components were detected, all of which accounted for *ca* 1-2 % TRR (0.040-0.103 mg/kg *N*-acetylglyphosate equivalents).

Four radiolabelled components were detected in muscle, the most abundant was *N*-acetylglyphosate and accounted for 16.70 % TRR (0.014 mg/kg N-acetylglyphosate equivalents). The remaining three components were minor unknowns and accounted for a maximum of 6.00 % TRR (0.006 mg/kg *N*-acetylglyphosate equivalents).

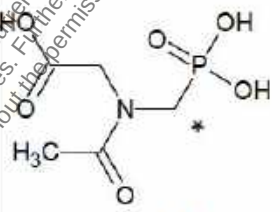
The most abundant component in all fat samples was *N*-acetylglyphosate and accounted for 21.43 % TRR (0.040 mg/kg N-acetylglyphosate equivalents), 73.19 % TRR (0.078 mg/kg N-acetylglyphosate equivalents), and 64.73 % TRR (0.090 mg/kg N-acetylglyphosate equivalents) in omental, renal, and subcutaneous fat, respectively. AMPA, glyphosate, and *N*-acetyl AMPA in all fat samples accounted for a maximum of 4.77 % TRR (0.007 mg/kg N-acetylglyphosate equivalents), 6.03 % TRR (0.011 mg/kg N-acetylglyphosate equivalents), and 14.86 % TRR (0.021 mg/kg N-acetylglyphosate equivalents), respectively. Several minor unknown components were detected in omental and renal fat samples, none of which accounted for more than 2.49 % TRR (0.003 mg/kg *N*-acetylglyphosate equivalents).

N-acetylglyphosate was metabolised in the goat by de-acetylation to form glyphosate. *N*-acetylglyphosate and glyphosate were metabolised to form *N*-acetyl AMPA and AMPA, respectively. *N*-acetyl AMPA may also have undergone de-acetylation to form AMPA.

N-acetylglyphosate and its metabolites were eliminated rapidly primarily in the excreta (87.74 % of the dose). There was not a significant transfer of residues of *N*-acetylglyphosate and its metabolites to milk or edible tissues (liver, kidney, muscle, and fat). Milk and edible tissues contained <1 % of the administered total dose.

I. Materials and methods

A. Materials

Test material	[^{14}C]*N*-Acetylglyphosate
Chemical structure:	 * position of radiolabel
Radiochemical purity:	>99 % (assay conducted by Charles River Laboratories)
Specific activity:	0.51 MBq/mg (13.83 µCi/mg)
Lot number CAS No:	3562-059 129660-96-4 (non-radiolabeled)
Log $P_{o/w}$:	Log Pow = -6.29 at 25 °C (at pH 5) Log Pow = -6.26 at 25 °C (at pH 7) Log Pow = -6.86 at 25 °C (at pH 9)
Test animals:	
Species:	Goat, *Capra aegarus hircus*
Strain:	British Saanen variety
Breeding facility:	[redacted]
Gender and numbers involved:	Female (lactating), 1 animal, identified by a numbered ear tag
Body weight:	31.5 kg (Study Day 1) to 31.0 kg (Study Day 6)

Age:	Not reported
Location of the in-life phase:	[redacted]
Acclimatisation:	16 days prior to the start of dosing; transfer to metabolism cage 1 day prior to the start of dosing
Housing:	Housed in a metabolism cage. Throughout the acclimation and dosing periods, the test goat was kept on a 16 hr light/8 hr dark cycle. Temperature and humidity during acclimation and dosing periods were recorded daily with ranges of 12-25 °C and 22-78 %, respectively.
Feed and water:	Protein concentrate (Dodson and Horrell Limited Goat Mix, Batch No. (17) 56B 023104) offered at *ca* 400 g twice daily (at each milking), i.e. a total of *ca* 800 g/day. Hay offered at *ca* 1.2 kg/day. Water (Mains tap water), *ad libitum*.

B. Study design

1. In-life phase including sacrifice

Dosing regime

Administration:	Oral
Dose rate:	205.42 mg *N*-acetylglyphosate equivalent/kg feed or 8.42 mg *N*-acetylglyphosate equivalent /kg bw/day[1] If expressed as glyphosate equivalent, 164.34 mg glyphosate equivalent/kg feed or 6.74 mg glyphosate equivalent/kg bw/day[2]
Feed consumption:	1296 g/day (average for the 5-day dosing period)
Vehicle:	Gelatine capsules
Timing:	Twice per day (administered orally with balling gun)

[1] Dose level in diet / feed calculated as an average from total daily feed consumption and daily dose administered. Dose level expressed on basis of animal bodyweight calculated based on average daily dose of 263.19 mg *N*-acetylglyphosate equivalent/day and 31.25 kg bodyweight (average during dosing period).
[2] Dose level was also expressed as glyphosate equivalent, derived from calculation using a conversion factor of 0.8 based on *N*-acetylglyphosate and glyphosate molecular weight of 211.11 and 169.07, respectively.

^{14}C-*N*-acetylglyphosate (sodium salt) was used to dose a single lactating goat. The target dose level based on feed consumption was 200 mg *N*-acetylglyphosate equivalent /kg in the diet (or 160 mg/kg in the diet if expressed as glyphosate equivalents). The actual dose level based on feed consumption was 205.42 mg *N*-acetylglyphosate equivalent/kg feed based on average daily feed consumption during the 5-day dosing interval of 1.296 kg/day. If expressed as glyphosate equivalent in the diet, the actual dose was 164.34 mg glyphosate equivalent/kg feed. Based on an average bodyweight of 31.25 kg during the dosing phase of the study, the actual dose administered was 8.42 mg *N*-acetylglyphosate equivalent /kg bw/day (or if expressed as glyphosate equivalent was 6.74 mg glyphosate equivalent/kg bw/day).

The dose was administered orally twice daily in gelatin capsules. A dose solution was prepared using ^{14}C-*N*-acetylglyphosate (in aqueous solution) and non-radiolabelled *N*-acetylglyphosate. The radioactive content of the dosing solution and homogeneity were confirmed at the time of preparation, following storage, and during the dosing period. The specific activity of the test item in the dose solution was determined as 5.95 µCi/mg (0.22 MBq/mg). The dose solution was stored at *ca* 4 °C during the dosing period.

Dosing solution was dispensed into a gelatine capsule containing feed immediately prior to dose administration. Dose solution was dispensed into small gelatine capsules, which were subsequently placed inside larger capsules to ensure no loss of dose prior to administration. The test goat received a single oral dose of [^{14}C]-*N*-acetylglyphosate via a gelatine capsule twice daily for five consecutive days. The capsules were placed in a balling gun, which was subsequently placed over the back of the animal's mouth and then released. The dose was administered twice *a* day, immediately after morning and evening milking and immediately prior to feeding.

Animals were observed twice daily for mortality and morbidity. Body weights were recorded on arrival, at the start of acclimatisation, on the first day of dosing and at necropsy.

2. Sampling and storage

Urine and faeces were collected prior to dosing and at 24 hour intervals after initiation of dosing until the time of sacrifice. A composite urine sample was prepared using approximately 10 % of each daily urine sample from Study Day 1-5. Faeces samples were processed (homogenised) on the day of collection. Following processing, a subsample (ca 5 %) was collected. After acceptance of total radioactivity analysis, the subsamples collected during dosing (Study Days 1 5) were combined to produce a composite faeces sample, which was homogenised prior to storage. The cage was rinsed with water after each faeces collection and the rinses retained for analysis.

Milk samples were collected prior to beginning of dosing and twice daily until sacrifice. The afternoon milk (PM) for each sampling timepoint was retained and combined with the milk collected the following morning (AM). Additionally, a composite milk sample was prepared by combining *ca* 10 % of each AM/PM combined milk sample from Study Day 1-5.

Approximately 12 hours after administration of the tenth and final dose, the goat was sacrificed using a captive bolt, followed by pithing and exsanguination. Bile was collected from the gall bladder by syringe. Tissues were removed and retained for analysis. Tissues collected included the whole liver, both kidneys, muscle (composite of loin, hind, and fore quarter muscle in approximately equal proportions), and fat (individual omental, renal, and subcutaneous fat samples). The gastrointestinal tract and its contents were collected separately, but not analysed further as good mass balance was achieved following analysis of all other samples.

Samples not analysed immediately for levels of TRR were stored frozen at *ca* -20 °C until taken for analysis, with the exception of cage wash samples (which were stored at ambient temperature for at least 24 hours prior to analysis) and milk collected at PM milking occasions (which was stored at *ca* 4 °C overnight). All samples removed from frozen storage for analysis were returned to storage at *ca* -20 °C after analysis.

3. Analytical procedures

Specimen of faeces, urine, cage wash, milk, bile, and tissues were analysed in triplicate to quantify total radioactivity. Faeces samples were initially soaked in ca 3 vol/g water to soften the pellets prior to homogenisation and aliquots of the resultant slurry were taken for combustion analysis before radioactivity was quantified using liquid scintillation counting (LSC). Urine and milk samples were analysed on the day of collection while cage wash samples were analysed ca 24 h after collection. Urine, cage wash, and milk samples were stirred to ensure homogeneity and aliquots were taken and mixed with scintillation fluid before radioactivity was quantified using LSC. Bile was analysed on the day of collection. Aliquots were taken for combustion analysis before radioactivity was quantified LSC. The bile sample was not analysed further. Tissue samples were processed frozen with dry ice (grated/chopped, followed by pulverisation) after which the samples were held in frozen storage for at least 24 hours before analysis to allow any remaining dry ice to dissipate. Aliquots of fat samples were taken for direct LSC analysis following sonication with scintillation fluid. Aliquots of liver, kidney and muscle samples were taken for combustion analysis followed by LSC to quantify total radioactivity

Concentration and homogeneity checks were carried out for composite samples prior to chromatographic analysis. The samples were thawed and then either homogenised and subjected to combustion analysis (faeces) or stirred and then mixed with scintillation fluid (urine and milk) before LCS analysis.

The composite faeces sample was extracted three times with 0.2 N HCl. On each occasion, samples were homogenised then centrifuged and the supernatant decanted. The extracts were combined and aliquots removed for radioassay. The extract was concentrated to dryness by rotary evaporation then reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v). The extracted radioactive residues were determined by assaying triplicate aliquots of each extract by LSC. The Post Extracted Solid (PES) was assayed by combustion followed by LSC analysis.

The composite urine sample was not extracted prior to HPLC analysis, but was centrifuged to remove particulate material. The radioactive content of the urine was determined by LSC analysis before and after centrifugation.

The composite milk sample was extracted using 0.2 N HCl. Dichloromethane (equivalent to *ca* 66 % of the milk volume) was added to precipitate milk solids from the extract. The sample was shaken, centrifuged, and the aqueous extract decanted. The extraction process was repeated an additional two times, the aqueous extracts were combined and the radioactive content determined by LSC analysis. The extract was partitioned two times against an equal volume of hexane to remove fatty material. The radioactive content of the hexane fraction was determined by LSC and found to be negligible and as such was not processed further. The cleaned aqueous extract remaining was then concentrated to dryness by rotary evaporation and reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v), and extracted radioactive residues were determined by LSC.

Liver, kidney and muscle samples were extracted three times with 0.2 N HCl. On each occasion, the sample was macerated followed by centrifugation and decanting of the extract. The extracts were combined and radioactivity was determined using LSC. The kidney and muscle extracts were partitioned against an equal volume of hexane to remove fatty material. The radioactive content of the hexane fraction was determined by LSC analysis and found to be negligible and as such was not processed further. The aqueous extract was then concentrated to dryness by rotary evaporation and reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v). The extracted radioactive residues were determined by LSC. Subsamples of the PES were collected, combusted, and radioactive residues determined by LSC.

Omental, renal, and subcutaneous fat samples were extracted with 0.2 N HCl and dichloromethane was added to dissolve fatty material. The extract was centrifuged and the aqueous extract decanted. The extraction process was repeated an additional two times, the aqueous extracts were combined and the radioactive content determined by LSC analysis. The dichloromethane fraction and PES were placed under a gentle stream of nitrogen to remove solvent. The aqueous extract was partitioned against hexane to remove fatty material and the radioactive content of the hexane fraction determined by LSC, found to be negligible, and as such was not processed further. The aqueous extract was then concentrated to dryness by rotary evaporation and reconstituted in 0.1 % trifluoroacetic acid:methanol (96:4, v/v). The extracted radioactive residues were determined by LSC. Subsamples of the PES were collected, combusted, and radioactive residues determined by LSC.

Residues in liver, kidney, muscle, and omental fat PES were all in excess of 0.01 mg/kg and as such these residues were further characterised by enzyme hydrolysis (pepsin and protease).

The PES from liver, kidney, muscle, and omental fat were mixed with pepsin and 0.1 N hydrochloric acid. Samples were incubated (37 °C) in a shaking water bath for approximately 24 hours. Following incubation, the radioactive content of the samples was determined by LSC prior to and post filtration. The used filter paper was combined with the PES returned and protease enzyme added along with 100 mM phosphate buffer (pH 7.5). Samples were incubated in a shaking water bath for approximately 24 hours and the radioactive content of both samples was measured prior to and post filtration.

Attempts were made to clean up the enzyme digests using iron loaded Chelex 100 ligand exchange resin followed by AG1X8 resin columns. The radioactive content of the column eluent for both samples was determined by LSC analysis. Procedural recovery following column clean up was low (*ca* 17-37 %). As a consequence of the low levels of recovered radioactivity, it was not possible to profile these cleaned up samples.

Levels of radioactivity were determined in each extract by Liquid Scintillation Counting (LSC) or oxidative combustion followed by LSC. The TRR was calculated as the sum of extractable and unextracted residues.

Faeces, urine, milk, and tissue extracts were analysed using HPLC. Peak assignment by HPLC retention comparison with authenticated standards in one system by HPLC was based on co-chromatography with authentic radiolabelled and un-labelled reference standards. LC-MS/MS experiments were performed on reference standards and selected (faeces) extracts to confirm assignments made by co-chromatography.

II. Results and discussion

A. Recovery of radioactivity and total radioactive residues (TRRS)

The overall recovery of the radioactive dose applied is provided in in the table below. A total of 87.83 % of the administered dose was recovered. The majority of the administered dose was excreted via the faeces (74.17 %) and urine (11.45 %). A further 2.12 % of the administered dose was recovered from cage wash samples. Milk samples only accounted for a total of 0.03 % of the administered dose. The elimination of [^{14}C]-*N*-acetylglyphosate residue was rapid as the overall recovery of radioactivity from faeces, urine, milk, and cage wash samples was 87.77 % of the applied dose by the end of the dosing period. Distribution of radioactive residues in tissues (liver, kidney, muscle, omental fat, renal fat, and subcutaneous fat) and bile was low, accounting for ≤ 0.03 % of the applied dose in each of these matrices.

Listed in the table below are concentrations of radioactive residues in bile, milk, and tissues, expressed as *N*-acetylglyphosate equivalents. The highest concentration of total radioactive residues (TRR) was observed in kidneys (4.689 mg/kg), followed by liver (0.715 mg/kg). Muscle samples had the lowest concentration of TRR (0.047 mg/kg) out of all tissue samples, while the three fat samples (omental, renal, and subcutaneous) had concentrations ranging from 0.065-0.108 mg/kg. By Study Day 5 (end of the dosing period), the concentration of TRR in milk was 0.036 mg/kg. The TRR in bile was found at a concentration of 0.013 mg/kg.

In addition to the concentration of TRR expressed as *N*-acetylglyphosate equivalents, TRR concentration is also displayed in the table below (and in other tables that follow) in glyphosate equivalents. The glyphosate equivalent values were not included in the study report, but were calculated from TRR expressed as *N*-acetylglyphosate equivalents and a conversion factor of 0.8, based on the molecular weights of glyphosate and *N*-acetylglyphosate.

Table 6.2.3-29: Distribution and concentration of radioactive residues in excreta, milk, and tissues of a lactating goat after oral administration of ^{14}C-*N*-acetylglyphosate for 5 consecutive days

Matrix[1]	% Administered dose	TRR (mg *N*-acetylglyphosate equivalents/kg)[2]	*TRR (mg glyphosate equivalents/kg)*[3]
Faeces	74.17	NA	*NA*
Urine	11.45	NA	*NA*
Cage wash	2.12	NA	*NA*
Bile	NA	0.013	*0.010*
Milk	0.03	0.036[4]	*0.029*[5]
Liver	0.03	0.715	*0.572*
Kidney	0.03	4.689	*3.751*
Muscle	NA	0.047	*0.038*
Omental fat	NA	0.065	*0.052*
Renal fat	NA	0.093	*0.074*
Subcutaneous fat	NA	0.108	*0.086*
Total recovery	87.83	NA	*NA*

1 The gastrointestinal tract and its contents were collected separately, but not analysed further as good mass balance was achieved following analysis of all other samples.
2 TRR = total radioactive residue, expressed as *N*-acetylglyphosate equivalents.
3 Total radioactive residues, expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.
4 Milk TRR concentrations are the results for the sample collected at the end of the dosing period (on Study Day 5). The % administered dose value is the cumulative dose collected across all 5 dosing days.
NA = not applicable.

In the table below, the concentration of total radioactive residues (TRR), expressed as *N*-acetylglyphosate equivalents, are summarised for daily milk samples collected over the dosing period (Study Days 1-5). Residue levels over the 5-day period remained relatively constant, ranging from 0.030 mg/kg to 0.036 mg/kg, indicating that a plateau level of residues was attained by at least Study Day 2. In addition to the concentration of TRR expressed as *N*-acetylglyphosate equivalents, TRR concentration is also displayed in the table below in glyphosate equivalents (calculated value added during dossier compilation).

Table 6.2.3-30: Radioactive residues in milk of a lactating goat during oral administration ^{14}C-*N*-acetylglyphosate over a period of 5 consecutive days

Study Day	TRR (mg/kg)	
	N-Acetylglyphosate equivalents[1]	*Glyphosate equivalents*[2]
1	0.030	*0.024*
2	0.033	*0.026*
3	0.032	*0.026*
4	0.033	*0.026*
5	0.036	*0.029*

1 TRR = total radioactive residue, expressed as *N*-acetylglyphosate equivalents.
2 TRR = Total radioactive residues, expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetylglyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetylglyphosate of 211.11 and molecular weight of glyphosate of 169.07.

B. Extraction and characterisation of residues

Results of extraction and characterisation/identification of residues in excreta (faeces and urine), milk, and edible tissues (liver, kidney, muscle, and fat) are described and summarised below.

A summary of the results of extraction and characterisation / identification of residues in faeces and urine is shown the table below. Extraction of the composite faeces sample from Study Days 1-5 recovered 73.64 % of the administered dose. Subsequent processing of the extract resulted in no loss of radioactivity. Four radiolabelled components were detected in the faeces, the most abundant was *N*-acetylglyphosate and

accounted for 53.16 % of the administered dose. AMPA, glyphosate, and *N*-acetyl AMPA were also detected and accounted for 0.81 %, 3.27 %, and 16.41 % of the administered dose, respectively. Unextracted residues accounted for 0.53 % of the administered dose. *N*-acetylglyphosate was also detected in unextracted urine and accounted for 11.41 % of the administered dose .

Table 6.2.3-31: Extraction and identification of the radioactive residues in composite faeces and urine from a lactating goat dosed with ^{14}C-*N*-acetylglyphosate for 5 consecutive days

Fraction / Component	% Administered dose	
	Faeces	Urine
TRR	**74.17**	**11.45**
ERR	**73.65**	**11.41**
Concentrated extract (faeces) / Centrifuged urine	73.65	11.41
AMPA	0.81	-
Glyphosate	3.27	
N-acetyl AMPA	16.41	-
N-acetylglyphosate	53.16	11.41
Total identified	**73.65**	**11.41**
Total characterised	**-**	**-**
RRR	**0.53**	**<0.01**
Differences during processing[1]	<0.01	<0.01

1 Differences during processing reflect any loss incurred during concentration and/or sample clean up for HPLC analysis. The concentrated extract was assumed to be representative of the initial extract and metabolite concentrations were calculated as such.

TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue

A summary of the results of extraction and identification of residues in liver and kidney is shown the table below.

Extraction of liver recovered 83.21 % TRR (0.669 mg/kg *N*-acetylglyphosate equivalents). Subsequent concentration of the liver extract resulted in minor losses of radioactivity; however, levels of radioactivity in the initial extract were too low to accurately determine the extent of lost radioactivity. The concentrated extract was assumed to be representative of the initial extract and metabolite concentrations were calculated as such. Pepsin digestion of the remaining liver PES released a further 6.90 % TRR (0.055 mg/kg *N*-acetylglyphosate equivalents). Attempts to concentrate and clean the pepsin digest resulted in significant losses such that the cleaned sample contained low (negligible) levels of radioactivity. The pepsin digest was not analysed further. Protease digestion of the liver pellet released insignificant levels of radioactivity. Four radiolabelled components were detected in the liver extract, the most abundant was *N*-acetylglyphosate accounting for 55.51 % TRR (0.446 mg/kg *N*-acetylglyphosate equivalents). AMPA and glyphosate were also detected and accounted for 8.45 % TRR (0.068 mg/kg *N*-acetylglyphosate equivalents) and 14.71 % TRR (0.118 mg/kg *N*-acetylglyphosate equivalents), respectively. A single minor unknown component, accounted for 0.52 % TRR (0.004 mg/kg *N*-acetylglyphosate equivalents). Unextracted residues accounted for 9.89 % TRR (0.080 mg/kg *N*-acetylglyphosate equivalents) in liver.

Extraction of kidney recovered 97.03 % TRR (4.708 mg/kg *N*-acetylglyphosate equivalents). Subsequent concentration of the kidney extracts resulted in minor losses of radioactivity. Pepsin digestion of the remaining PES released a further 4.64 % TRR (0.225 mg/kg *N*-acetylglyphosate equivalents). It was not possible to further process the pepsin digest due to significant losses on concentration. Protease digestion of the kidney residue released insignificant levels of radioactivity. The most abundant was *N*-acetylglyphosate accounting for 77.12 % TRR (3.742 mg/kg). Glyphosate was also detected, accounting for 4.98 % TRR (0.242 mg/kg). Seven minor unknown components (each *ca* 1-2 % TRR) were also

detected in the kidney extract. Unextracted residues accounted for <0.01 % TRR (<0.001 mg/kg *N*-acetylglyphosate equivalents) in kidney.

Table 6.2.3-32: Extraction and identification of the radioactive residues in liver and kidney from a lactating goat dosed with ^{14}C-*N*-acetylglyphosate for 5 consecutive days

Fraction / Component	Liver			Kidney		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
TRR	**100**	**0.804**	*0.643*	**100**	**4.852**	*3.882*
ERR	**83.21**	**0.669**	*0.535*	**97.03**	**4.708**	*3.766*
Concentrated extract	83.21	0.669	*0.535*	97.03	4.708	*3.766*
AMPA	8.45	0.068	*0.054*	-	-	-
Glyphosate	14.71	0.118	*0.095*	4.98	0.242	*0.194*
N-acetylglyphosate	55.51	0.446	*0.357*	77.12	3.742	*2.994*
Minor unknown(s)	0.52[3]	0.004[3]	*0.003*[3]	7.97[4]	0.386[4]	*0.309*[4]
RRR	**16.79**	**0.135**	***0.108***	**2.97**[6]	**0.144**[6]	***0.115***
Pepsin digest	6.90	0.055	*0.044*	4.64[5]	0.225[6]	*0.180*
Protease digest	<0.01	<0.001	*<0.001*	<0.01	<0.001	*<0.001*
Total identified	***78.67***	***0.632***	***0.506***	***82.1***	***3.984***	***3.187***
Total characterised	***0.52***	***0.004***	***0.003***	***7.97***	***0.386***	***0.309***
Final residue	**9.89**	**0.080**	*0.064*	**<0.01**	**<0.001**	*<0.001*
Differences during processing[5]	<0.01	<0.001	*<0.001*	0.01	<0.001	*<0.001*

1 Values expressed as *N*-acetylglyphosate equivalents.
2 Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the listed *N*-acetylglyphosate equivalents using a conversion factor of 0.80, based on the molecular weight of *N*-acetylglyphosate of 211.11 and the molecular weight of glyphosate of 169.07.
3 Single minor unknown component.
4 Seven minor unknown components, each *ca* 1-2 % TRR.
5 Differences during processing reflect any loss incurred during processing.
6 Although 4.64 % of the TRR (0.255 mg/kg) was reported to be extracted of the PES by pepsin, the PES before extraction accounted for only 2.97 % of the TRR (0.144 mg/kg).

Numbers in italic were calculated upon dossier compilation.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue

A summary of the results of extraction and identification of residues in milk and muscle is shown in the table below.

Extraction of the composite milk sample (Study Days 1-5) recovered 76.85 % TRR (0.021 mg/kg *N*-acetylglyphosate equivalents). Subsequent processing of the extract resulted in no loss of radioactivity. The most abundant component was *N*-acetylglyphosate accounting for 39.98 % TRR (0.011 mg/kg *N*-acetylglyphosate equivalents). AMPA and glyphosate were also detected and accounted for 3.35 % TRR (0.001 mg/kg *N*-acetylglyphosate equivalents) and 3.59 % TRR (0.001 mg/kg *N*-acetylglyphosate equivalents), respectively. The non-extractable (RRR) residue was determined as 0.006 mg/kg N-acetylglyphosate equivalents (23.15 % TRR), which was not investigated further.

Extraction of muscle recovered 42.03 % TRR (0.036 mg/kg *N*-acetylglyphosate equivalents). Concentration of the muscle extract resulted in minor losses of radioactivity. Pepsin and protease digestion of the PES residue released insignificant levels of radioactivity. The most abundant component in muscle was N-acetylglyphosate accounting for 16.70 % TRR (0.014 mg/kg *N*-acetylglyphosate equivalents). Three

unknown components accounted for a total of 11.13 % TRR (0.009 mg/kg *N*-acetylglyphosate equivalents); no individual unknown component accounted for greater than 6.00 % TRR. Low levels of characterised metabolites in muscle were a result of low solvent extractability. Unextracted residues accounted for 57.97 % TRR (0.050 mg/kg *N*-acetylglyphosate equivalents) in muscle.

Table 6.2.3-33: Extraction and identification of the radioactive residues in milk and muscle from a lactating goat dosed with ^{14}C-*N*-acetylglyphosate for five consecutive days

Fraction / Component	Milk			Muscle		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
TRR	**100**	**0.027**	*0.022*	**100**	**0.086**	*0.069*
ERR	**76.85**	**0.021**	*0.017*	**42.03**	**0.036**	*0.029*
Concentrated extract	76.85	0.021	*0.017*	42.03	0.036	*0.029*
AMPA	3.35	0.001	*≤0.001*	-	-	-
Glyphosate	3.59	0.001	*≤0.001*	-	-	-
N-acetylglyphosate	39.98	0.011	*0.009*	16.70	0.014	*0.011*
Minor unknowns	-	-	-	11.13[3]	0.009[3]	*0.007[3]*
RRR	**23.15**	**0.006**	***0.005***	**57.97**	**0.050**	***0.040***
Pepsin digest	NA	NA	*NA*	<0.01	<0.001	*<0.001*
Protease digest	NA	NA	*NA*	<0.01	<0.001	*<0.001*
Total identified	***46.92***	***0.013***	***0.011***	***16.7***	***0.014***	***0.011***
Total characterised	**-**	**-**	**-**	***11.13***	***0.009***	***0.007***
Final residue	**23.15**	**0.006**	***0.005***	**57.97**	**0.050**	***0.040***
Differences during processing[4]	<0.01	<0.001	*<0.001*	<0.01	<0.001	*<0.001*

1 Values expressed as *N*-acetylglyphosate equivalents.
2 Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the listed *N*-acetylglyphosate equivalents using a conversion factor of 0.80, based on the molecular weight of *N*-acetylglyphosate of 211.11 and the molecular weight of glyphosate of 169.07.
3 Three minor unknown components each > 6.00 % TRR.
4 Differences during processing reflect any loss incurred during processing.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
N/A = not applicable

A summary of the results of extraction and identification of residues in fat (omental, renal, and subcutaneous fat) is shown in the two tables below.

Extraction of omental, renal and subcutaneous fat recovered 34.81 % TRR (0.065 mg/kg *N*-acetylglyphosate equivalents), 93.57 % TRR (0.100 mg/kg *N*-acetylglyphosate equivalents), and 92.41 % TRR (0.128 mg/kg *N*-acetylglyphosate equivalents), respectively. Subsequent processing of the extracts resulted in minor losses. Pepsin digestion of the omental fat PES released a further 28.45 % TRR (0.053 mg/kg *N*-acetylglyphosate equivalents). Attempts to concentrate and clean the pepsin digest resulted in significant losses such that the cleaned sample contained low (negligible) levels of radioactivity. The pepsin digest was not analysed further. Protease digestion of the remaining residue released insignificant levels of radioactivity. The most abundant component in all fat samples was *N*-acetylglyphosate accounting for 21.43 % TRR (0.040 mg/kg *N*-acetylglyphosate equivalents), 73.19 % TRR (0.078 mg/kg *N*-acetylglyphosate equivalents), and 64.73 % TRR (0.090 mg/kg *N*-acetylglyphosate equivalents) in omental, renal, and subcutaneous fat, respectively. AMPA, glyphosate, and *N*-acetyl AMPA were also detected in all fat samples, accounting for a maximum of 4.77 % TRR (0.007 mg/kg *N*-acetylglyphosate equivalents), 6.03 % TRR (0.011 mg/kg *N*-acetylglyphosate equivalents), and 14.86 % TRR (0.021 mg/kg *N*-acetylglyphosate equivalents), respectively. Several

minor unknown components were detected in omental and renal fat samples, none of which accounted for more than 2.49 % TRR (0.003 mg/kg *N*-acetylglyphosate equivalents). Unextracted residues accounted for 65.19 % TRR (0.122 mg/kg *N*-acetylglyphosate equivalents), 6.43 % TRR (0.007 mg/kg *N*-acetylglyphosate equivalents), and 7.59 % TTR (0.011 mg/kg *N*-acetylglyphosate equivalents) in omental, renal, and subcutaneous fat, respectively.

Table 6.2.3-34: Extraction and identification of the radioactive residues in omental and renal fat from a lactating goat dosed with ^{14}C-*N*-acetylglyphosate for 5 consecutive days

Fraction / Component	Omental fat			Renal fat		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
TRR	**100**	**0.187**	*0.150*	**100**	**0.107**	*0.086*
ERR	**34.81**	**0.065**	*0.052*	**93.57**	**0.100**	*0.080*
Concentrated extract	34.81	0.065	*0.052*	93.57	0.100	*0.080*
AMPA	0.50	0.001	*≤0.001*	1.20	0.001	*≤0.001*
Glyphosate	6.03	0.011	*0.009*	5.02	0.005	*0.004*
N-acetyl AMPA	4.31	0.007	*0.006*	0.59	0.001	*≤0.001*
N-acetylglyphosate	21.43	0.040	*0.032*	73.19	0.078	*0.062*
Minor unknowns	1.86[3]	<0.001[3]	*<0.001*[3]	8.31[3]	0.009[3]	*0.007*[3]
RRR	**65.19**	**0.122**	***0.098***	**6.43**	**0.007**	***0.006***
Pepsin digest	28.45	0.053	*0.042*	NA	NA	*NA*
Protease digest	<0.01	<0.001	*<0.001*	NA	NA	*NA*
Total identified	***32.27***	***0.059***	***0.047***	***80.00***	***0.085***	***0.068***
Total characterised	**1.86**	**<0.001**	***<0.001***	**8.31**	**0.009**	***0.007***
Final residue	***36.74[5]***	***0.069[5]***	***0.056[5]***	**6.43**	**0.007**	***0.006***
Differences during processing[4]	<0.01	<0.001	*<0.001*	<0.01	<0.001	*<0.001*

1 Values expressed as *N*-acetylglyphosate equivalents.
2 Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the listed *N*-acetylglyphosate equivalents using a conversion factor of 0.80, based on the molecular weight of *N*-acetylglyphosate of 211.11 and the molecular weight of glyphosate of 169.07.
3 Consists of several minor unknown components, none of which accounted for more than 2.49 % TRR (0.003 mg/kg *N*-acetylglyphosate equivalents.
4 Differences during processing reflect any loss incurred during processing.
5 For omental fat, the final residue was calculated by subtraction of pepsin and protease digest from the RRR
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
N/A = not applicable

Table 6.2.3-35: Extraction and identification of the radioactive residues in subcutaneous fat from a lactating goat dosed with ^{14}C-*N*-acetylglyphosate for 5 consecutive days

Fraction / Component	Subcutaneous fat		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
TRR	**100**	**0.139**	*0.111*
ERR	**92.41**	**0.128**	*0.102*

Table 6.2.3-35: Extraction and identification of the radioactive residues in subcutaneous fat from a lactating goat dosed with ^{14}C-*N*-acetylglyphosate for 5 consecutive days

Fraction / Component	Subcutaneous fat		
	% TRR	mg/kg (*N*-acetyl-glyphosate equivalents[1])	*mg/kg (glyphosate equivalents[2])*
Concentrated extract	92.41	0.128	*0.102*
AMPA	4.77	0.007	*0.006*
Glyphosate	2.65	0.004	*0.003*
N-acetyl AMPA	14.86	0.021	*0.017*
N-acetylglyphosate	64.73	0.090	*0.072*
RRR	**7.59**	**0.011**	***0.009***
Pepsin digest	NA	NA	*NA*
Protease digest	NA	NA	*NA*
Total identified	***87.01***	***0.122***	***0.098***
Total characterised	-	-	-
Final residue	**7.59**	**0.011**	***0.009***
Differences during processing[3]	<0.01	<0.001	*<0.001*

1 Values expressed as *N*-acetylglyphosate equivalents.
2 Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the listed *N*-acetylglyphosate equivalents using a conversion factor of 0.80, based on the molecular weight of *N*-acetylglyphosate of 211.11 and the molecular weight of glyphosate of 169.07.
3 Differences during processing reflect any loss incurred during processing.
TRR = total radioactive residue
ERR = extractable radioactive residue
RRR = residual radioactive residue
N/A = not applicable

C. Storage stability

All samples were stored at *ca* -20 °C until taken for analysis and returned to storage as soon as possible after analysis. An analysis of storage stability was not conducted as part of this study since samples were analysed within two months of collection.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in livestock at the end of this chapter.

III. Conclusions

N-acetylglyphosate was administered twice daily to a lactating goat as an oral dose (via gelatine capsule) of ^{14}C-*N*-acetylglyphosate for five consecutive days. The mean daily dose level achieved was 205.42 mg *N*-acetylglyphosate/kg of feed consumed (8.42 mg *N*-acetylglyphosate equivalent/kg bw/day).

N-acetylglyphosate and its metabolites were eliminated rapidly by the goat, primarily in the excreta, accounting for 87.74 % of the dose. Total radioactive recovery was 87.83 % of the dose not including the radioactivity in the gastrointestinal contents.

N-acetylglyphosate (53.16 % of dose), glyphosate (3.27 % of dose), *N*-acetyl AMPA (16.41 % of dose), and AMPA (0.81 % of dose) were detected in the faeces. *N*-acetylglyphosate (11.4 % of dose) was the only radiolabeled component in the urine.

The levels of total radioactive residues in milk were between 0.030 and 0.036 mg/kg. *N*-acetylglyphosate, AMPA, and glyphosate were detected at trace levels (<0.01 mg/kg) in milk.

The total radioactive residues in the edible tissues ranged from 0.047 mg/kg (muscle) to 4.689 mg/kg (kidney). *N*-acetylglyphosate was the predominant residue found in all tissues. Glyphosate, *N*-acetyl AMPA, and AMPA were also detected in the tissues.

N-acetylglyphosate was metabolised in the goat by de-acetylation to form glyphosate. *N*-acetylglyphosate and glyphosate were metabolised to form *N*-acetyl AMPA and AMPA, respectively. *N*-acetyl AMPA may also have undergone de-acetylation to form AMPA.

Based on these results, it is concluded that there is not a significant transfer of residues of *N*-acetylglyphosate and its metabolites into fat, meat, and milk. The administered dose was eliminated rapidly primarily in the excreta. Milk and edible tissues contained <1 % of the administered total dose. The metabolism of *N*-acetylglyphosate in ruminants (lactating goat) is adequately understood and is consistent with that seen in laying hen.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the metabolic behavior of *N*-acetylglyphosate in lactating goats has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 503 with minor deficits: Radioactivity was not quantified separately in the different muscle types or separately in fat and aqueous milk fractions; the radioactivity balance is 87.8 % (GIT and its contents and carcasses were not measured); Urine and faeces were collected only once daily; identification of components by "co-chromatography" with one method and confirmation of compounds by LC-MS/MS only in faeces; Balance of components in matrices (liver, kidney, milk, muscle, omental fat, renal fat and subcutaneous fat) miss portions of up to 29.93 % TRR or up to 0.338 mg/kg (recovery or calculation issue).

Data available in the study report allowed calculation and expression of the dose on a basis of mg/kg bodyweight. Additionally, the diet was composed of low moisture feed items and the potential impact of moisture level would likely be low. The levels of radioactive residue in milk and muscle samples were relatively low (0.021 mg/kg extracted residue in milk, in *N*-acetylglyphosate equivalents; 0.036 mg/kg extracted residue in muscle, in *N*-acetylglyphosate equivalents). Therefore, the potential impact of not analysing fat and aqueous milk fractions separately or muscle types separately is expected to be small.

The study is considered supportive and covers the required metabolism study in lactating ruminant (goat) for *N*-acetylglyphosate.

Assessment and conclusion by RMS:

Overall assessment and conclusion on metabolism studies

Overall summaries are given in the following. Data are summarised also in Appendix G, and those studies where the nature of residues was investigated are considered for the definition of residues (see CA 6.7.1).

Several metabolism studies are available investigating the fate and nature of glyphosate-derived residues in **lactating ruminants**. An overview on the studies is given in the following table:

Table 6.2.3-36: Overview over available lactating goat metabolism studies

Ruminant	Application	Application dose	Reference
Lactating goat	5 daily applications (twice daily) via gavage	*N*-(phosphono-^{14}C-methyl)glycine 7.6 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.3/001: [redacted] 1994, (^{14}C)-Glyphosate: Absorption, distribution, metabolism and excretion following repeated oral administration to the dairy goat, Report No. 676/9-1011
	3 daily applications (twice daily) via gavage	*N*-(phosphono-^{14}C-methyl)glycine 6.4 mg/kg bw/day or 200 mg/kg feed (expressed in glyphosate equiv.)	
Lactating goat	7 daily applications (twice daily) via gelatine capsules	*N*-(phosphono-^{14}C-methyl)glycine (as trimesium salt) 2.7 mg/kg bw/day or 63.8 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.3/002: [redacted] 1994, The nature of residues of orally administered [Phosphonomethylene-^{14}C] glyphosate-trimesium in goat tissues and milk, Report No. RR 93-062B
Lactating goat	5 daily application (once daily) via gelatine capsules	*N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (9:1 mixture) 2.95 mg/kg bw/day (2.65 mg glyphosate/kg bw/day and 0.29 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	CA 6.2.3/003: [redacted] 1988, Metabolism study of synthetic ^{13}C/^{14}C-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part I, Report No. [redacted]-7586 and CA 6.2.3/004: [redacted] 1988, Metabolism study of synthetic ^{13}C/^{14}C-labeled Glyphosate and Aminomethylphosphonic acid in lactating goats. Part II, Report No. [redacted]-7458
	5 daily application (once daily) via gelatine capsules + 5 days depuration	*N*-(phosphono-^{13}C/^{14}C-methyl)glycine and amino-^{13}C/^{14}C-methylphosphonic acid (9:1 mixture) 2.83 mg/kg bw/day (2.55 mg glyphosate/kg bw/day and 0.28 mg AMPA/kg bw/day) or 120 mg/kg feed (expressed in glyphosate equiv.)	
Lactating goat	5 daily applications (twice daily) via gelatine capsules	*N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine 6.74/kg bw/day or 164.34 mg/kg feed (expressed in glyphosate equiv.) 8.42 bw/day or 205.42 mg/kg feed (expressed in *N*-acetyl-glyphosate equiv.)	CA 6.2.3/005: [redacted] 2007, Metabolism of [^{14}C]-*N*-Acetylglyphosate (IN-MCX20) in the lactating goat, Report No. [redacted]19796

In [redacted] 1994, *N*-(phosphono-^{14}C-methyl)glycine as administrated to lactating goats twice daily for five (Goat 1) or three consecutive days (Goat 2), respectively. Goat 1 was sacrificed at ca. 23.5 h after the final dose, and Goat 2 was sacrificed at plasma radioactivity c_{max} ca. 8 h after the last dosing.
Approximately 90 % of the administered dose was recovered in the case of Goat 1 in total, and approximately 58 % was recovered in the case of Goat 2 (study termination closer to the final dose). The major portions of radioactive residues were recovered in faeces (52.58 – 78.16 % of the administrated dose), urine (4.74 – 9.44 % of the dose), cage debris (up to 1.74 % of the administrated dose) and cage washings (0.29 – 0.48 % of the administrated dose). Less than 0.1 % of the administered dose was associated in both animals with edible matrices (tissues and milk in sum). At study termination, the major radioactive residues in the relevant edible matrices were detected in liver (0.404 mg/kg for Goat 1 and 0.225 mg/kg for Goat 2) and kidney (3.852 mg eq/kg for Goat 1 and 12.15 mg/kg for Goat 2). Transfer of glyphosate or its metabolites into milk was low.
Only urine, faeces and edible matrices (tissues and milk) of Goat 2 were extracted and the extracts were further analysed for metabolite identification. The main residue in the extracts of liver and kidney of Goat 2 was unchanged **glyphosate** (approximately 92 – 97 % of the total area detected in the analysed samples ("% Total"); % Total are presented and not % TRR as these values represent a worst case). Indications for the occurrence of the metabolite **AMPA** from minor TLC regions (up to 8 % Total) in the extract of kidney was not substantiated by HPLC and therefore supposed to be chromatographic artefacts. The concentrations of radioactive residues in the final extracts of fat, muscle and milk were below the level of detection following HPLC analysis, and TLC analyses of these extracts yielded only one radioactive region, which corresponded to the glyphosate standard.

N-(phosphono-^{14}C-methyl)glycine (^{14}C-PMG-labeled glyphosate) was administrated twice daily for 7 consecutive days to a lactating goat as its trimesium salt ([redacted] **1994**). The goats were sacrificed between 12 to 15 hours after the last dosing.
101 % of the administered dose was recovered in total. The main part was excreted (81 % of the administered dose in faeces, 9 % in urine and 1.4 % in cage wash) accounting in sum for 91.4 %. Radioactivity recovered in GI tract with contents accounted for 9.3 %. Radioactivity associated with edible portions (tissues and milk) accounted in sum for 0.15 % of the administered dose (including liver, kidney, fat, muscle, heart and milk), with highest radioactive residues found in kidney (0.09 % of the administered dose).
Of the relevant edible matrices of the goat, highest total radioactive residues were found in kidneys (5.58 mg/kg) and liver (0.234 mg/kg). In muscle and fat 0.0256 and 0.0175 mg/kg were found, respectively. For whole milk samples, a plateau was reached after 4 days.
PMG (glyphosate-anion) (59.4 – 91.3 % TRR) and **AMPA** (4.7 – 21.4 % TRR) accounted for the majority of the radioactive residue in liver, kidney, fat and muscle. In milk, PMG (22.3 % TRR) and AMPA (2.4 % TRR) together represented 25 % TRR. **Lactose** and **triglycerides** constituted over 45 % TRR in milk, while material associated with post-extraction milk solids comprised 21 % TRR, which is consistent with natural incorporation into **proteins**. Presumably other tissues also contained small amounts of radioactivity incorporated into **natural components**.

Two test with lactating goats were conducted with a 9:1 mixture of *N*-(phosphono-^{13}C/^{14}C-methyl)glycine (^{13}C/^{14}C-glyphosate) and amino-^{13}C/^{14}C-methylphosphonic acid (^{13}C/^{14}C-AMPA) to investigate their behaviour in goats ([redacted] **1988** and [redacted] **1988**). One capsule was administered each day for 5 consecutive days. In one test goats were sacrificed 22 to 24 hours after the last dose. In the second test, a 5-day depuration phase was added after the 5th dose after which the goat was sacrificed.

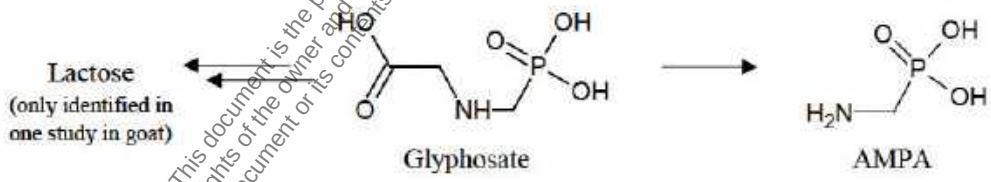
Between 83.6 and 86.7 % of the administered dose was recovered. The main part was excreted, accounting in sum (urine, faeces plus pan rinse) for 74.95 – 86.46 %. Highest TRR values were detected in kidneys (0.505 – 7.0 mg/kg) and liver (0.381 – 0.493 mg/kg). In muscle and fat 0.009 – 0.027 mg/kg and 0.004 – 0.010 mg/kg were found, respectively.

In milk the radioactive residue ranged between 0.019 and 0.060 mg/kg during the dosing period. After the 5 day depuration the radioactive residue decreased to 0.006 mg/kg.
Glyphosate (47.8 – 89.6 % TRR or 0.003 – 6.429 mg/kg) and **AMPA** (4.9 – 30.7 % TRR or 0.000 – 0.677 mg/kg) accounted for the majority of the radioactive residue in all matrices. Furthermore, an unknown compound was assigned in milk (23.5 – 28.0 % TRR or 0.005 – 0.014 mg/kg). The unknown in milk was isolated and further analysed by gel filtration chromatography and acid hydrolysis. The ^{14}C activity appeared to be associated with small molecular weight **proteins** or **glycoproteins** of molecular weight less than 6,500 daltons.

In [redacted] **2007** [^{14}C]-*N*-acetyl-*N*-(phosphomethyl)glycine) ([^{14}C]-*N*-acetylglyphosate) was administered to one lactating goat (twice daily) for 5 consecutive days. The goat was sacrificed approximately 12 hours after the last dose.
N-acetylglyphosate and its metabolites were eliminated rapidly by the goat, primarily in the excreta, accounting for 87.74 % of the dose. Total radioactive recovery was 87.83 % of the dose not including the radioactivity in the gastrointestinal contents.
The levels of total radioactive residues in milk were between 0.024 and 0.029 mg/kg. These values, as well as the other values are expressed as glyphosate equivalent (calculated based on a conversion factor of 0.8). ***N*-acetylglyphosate**, **AMPA**, and **glyphosate** were detected at trace levels (<0.01 mg/kg) in milk.
The total radioactive residues in the edible tissues ranged from 0.038 mg/kg (muscle) to 3.751 mg/kg (kidney). ***N*-acetylglyphosate** (16.70 – 77.12 % TRR) was the predominant residue found in all tissues. **Glyphosate** (up to 14.71 % TRR or up to 0.194 mg/kg), ***N*-acetyl AMPA** (up to 14.86 % TRR or up to 0.017 mg/kg, only found in fat), and **AMPA** (up to 8.45 % TRR or up to 0.054 mg/kg) were also observed in the tissues.

Overall conclusion on metabolism in ruminants
Within all studies investigating the metabolism of ^{14}C-glyphosate in ruminants it was shown that elimination of radioactivity via excreta was the primary elimination route.
After administration of glyphosate, a mixture of glyphosate and AMPA or PMG-labelled glyphosate-(as trimesium salt) to lactating goats a similar picture on metabolism was found. **Glyphosate** accounted for the main part of radioactive residues in all studies. In addition, **AMPA** was identified as a major metabolite in kidney and liver. In one study, **lactose** was also determined in milk. Furthermore radioactivity was also detected in other **natural products** (e.g. triglycerides, proteins and glyoproteins).

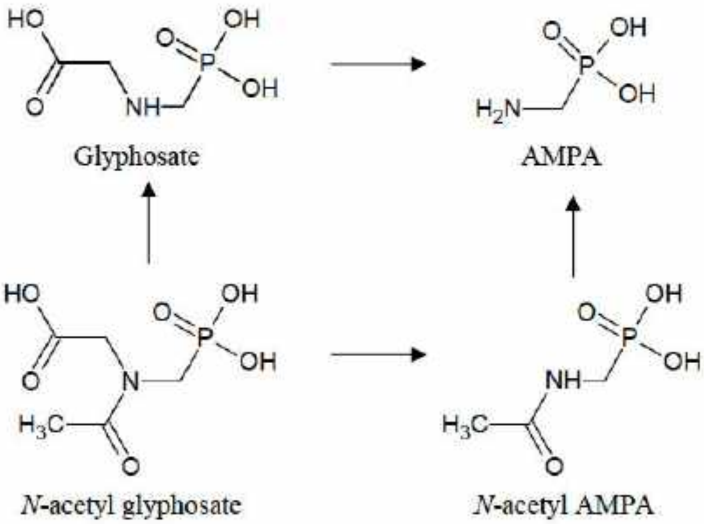
Pathway for livestock (feeding with glyphosate) - ruminants



AMPA: Major metabolite in kidney and liver. Minor metabolite in muscle, fat and milk (up to 0.002 mg/kg).
Lactose: Indicated in milk (25.2 % TRR).

After administration of *N*-acetyl-glyphosate to one lactating goat ***N*-acetylglyphosate** accounted for the main part of radioactive residues. **Glyphosate** was identified as major metabolite in liver and kidney, while **AMPA** and ***N*-acetyl AMPA** were major metabolites in liver and fat, respectively.

Pathway for livestock (feeding with *N*-acetyl glyphosate) - ruminants



Glyphosate:	Major metabolite in liver and kidney. Minor metabolite in milk and fat (up to 0.009 mg/kg).
AMPA:	Major metabolite in liver. Minor metabolite in milk and fat (up to 0.006 mg/kg).
***N*-acetyl AMPA**:	Major metabolite in fat.

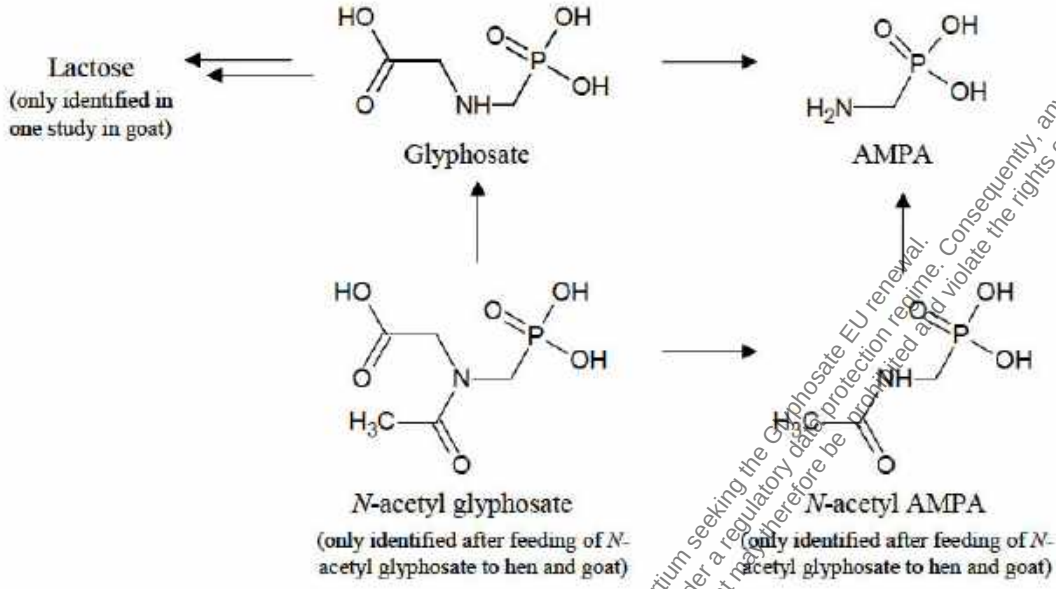
CA 6.2.4 Pigs

Studies in rabbits, lactating goats and laying hen demonstrated a similar pattern of toxicokinetics and metabolism pathways. Therefore, the findings in ruminants can therefore be extrapolated to pigs.

Overall conclusion on relevant metabolites for livestock

The results of the different metabolism studies in livestock are very consistent.
After administration of glyphosate, a mixture of glyphosate and AMPA or PMG-labelled glyphosate (as trimesium salt) to laying hens and lactating goats **glyphosate** was the main ^{14}C-component in all edible matrices. **AMPA** was additionally identified as a major metabolite in egg yolk, kidney, liver and muscle (only for poultry). The numerous metabolism studies in livestock demonstrate that glyphosate is slowly metabolised to AMPA.
In one goat study, lactose was also determined in milk. Furthermore radioactivity was also detected in other natural products (e.g. triglycerides, cholesterol and proteins).
After administration of *N*-acetyl-glyphosate to laying hens and lactating goats ***N*-acetylglyphosate** was the main ^{14}C-**component** in all edible matrices. **Glyphosate** was identified as major metabolite in liver, kidney (only ruminants) and fat (only poultry). In addition **AMPA** and ***N*-acetyl AMPA** were major metabolites in liver and fat of lactating goats, while there were only minor metabolites in poultry. *N*-acetylglyphosate was metabolised by de-acetylation to form glyphosate. *N*-acetylglyphosate and glyphosate were metabolised to form *N*-acetyl AMPA and AMPA, respectively. *N*-acetyl AMPA may also have undergone de-acetylation to form AMPA.

Overall pathway for livestock



Glyphosate: Major metabolite in liver (hen and goat), kidney (goat) and abdominal fat (hen). Minor metabolite in egg white (hen), egg yolk (hen), muscle (hen), milk (goat) and fat (goat) (up to 0.010 mg/kg). *Only relevant metabolite for N-acteyl-glyphosate feeding.*

AMPA: Major metabolite in egg yolk (hen), kidney (hen and goat), liver (hen and goat) and muscle (hen). Minor metabolite in egg white (hen), muscle (goat), fat (hen and goat), milk (goat) and skin (hen) (up to 0.009 mg/kg).

***N*-acetyl AMPA**: Major metabolite in fat (goat). Minor metabolite in egg white, egg yolk, liver, muscle and fat (up to 0.016 mg/kg) in hen.

Lactose: Indicated in milk (25.2 % TRR in goat).

CA 6.2.5 Fish

According to Commission Regulation (EU) No 283/2013 and working document SANCO/11187/2013 rev. 3, metabolism studies on fish may be required where a fat-soluble active substance (log Po/w ≥ 3) is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications.
Glyphosate and its metabolite AMPA relevant for conventional crops, as well as its metabolites *N*-acetyl AMPA and *N*-acetyl glyphosate only relevant for tolerant crops are all four no fat-soluble substances:

Log Po/w:
- Glyphosate: -3.2
- AMPA: -2.47
- *N*-acetyl glyphosate: -6.26
- *N*-acetyl AMPA: -2.53 (calculated with EPIsuite tool)

Therefore based on the very low fat solubility no fish metabolism studies are required.

In addition based on the intended uses supported in the EU no relevant fish feed items are included and therefore no fish metabolism study is triggered.

CA 6.3 Magnitude of Residues Trials in Plants

CA 6.3.1 Orchard use

For the use of glyphosate in orchards and vines sufficient data to support the representative GAP are available. The critical GAP is presented in the table below.

Table 6.3.1-1:Critical GAP for the use of glyphosate in orchards and vines

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Application			Application rate			PHI (days)
		Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product/ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	
Orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, banana, and table olives)	F	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 - 2 (28 days) b) 1 - 2 (28 days)	a) 3 - 4 L/ha b) 8 L/ha	a) 1.08 – 1.44 kg as/ha b) 2.88 kg as/ha	100 - 400	7
Vines (table and wine grape, leaves not intended for human consumption)	F	Ground directed, shielded spray, band application	Post-emergence of weeds	a) 1 - 2 (28 days) b) 1 - 2 (28 days)	a) 3 - 4 L/ha b) 8 L/ha	a) 1.08 – 1.44 kg as/ha b) 2.88 kg as/ha	100 - 400	7

The magnitude of residues of glyphosate and its metabolite AMPA was investigated in 15 studies with glyphosate which are considered valid to address the data point. Furthermore, 3 studies provide supportive information.

For the use in orchard, residues of glyphosate and AMPA were always below the LOQ of 0.05 mg/kg in all trials (7 trials in Northern Europe and 24 trials in Southern Europe). The data set is sufficient to derive an MRL for citrus, tree nuts, pome fruit, stone fruit, kiwi and banana.

For the use in grapes, residues of glyphosate and AMPA were always below the LOQ of 0.05 mg/kg in all trials (9 trials in Northern Europe and 8 trials in Southern Europe). The data set is sufficient to derive an MRL for wine and table grapes.

For the use in olives, residues of glyphosate and AMPA were always below the LOQ of 0.05 mg/kg in all trials (4 trials in Southern Europe). The data set is sufficient to derive an MRL for olives.

Table 6.3.1-2:Overview on residue studies in orchards

Data Point	Crop, commodities	Analyte(s)	Number of trials	Reference	Status
CA 6.3.1/001	Mandarine, peel and pulp	Glyphosate AMPA	Southern Europe: 2	[redacted] 2014 Report No. S13-02531	Valid
CA 6.3.1/002	Tree nuts, nutmeat	Glyphosate AMPA	Southern Europe: 2	[redacted] 2016 Report No. S15-00018	Valid
CA 6.3.1/003	Apple, fruit	Glyphosate AMPA	Northern Europe: 2	[redacted] 2014 Report No. S13-03425	Valid

Table 6.3.1-2:Overview on residue studies in orchards

Data Point	Crop, commodities	Analyte(s)	Number of trials	Reference	Status
CA 6.3.1/004	Apple, fruit	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2014 Report No. S13-03426	Valid
CA 6.3.1/005	Apple, fruit Pear, fruit	Glyphosate AMPA	Northern Europe: 35	[redacted] 1976 Report No. A9	Invalid
CA 6.3.1/006	Apricot, fruit	Glyphosate AMPA	Southern Europe: 4	[redacted] 2016 Report No. S15-00019	Valid
CA 6.3.1/007	Cherry, fruit	Glyphosate AMPA	Southern Europe: 2	[redacted] 2014 Report No. S13-03427	Valid
CA 6.3.1/008	Plum, fruit	Glyphosate AMPA	Southern Europe: 2	[redacted] 2014 Report No. S13-03233	Valid
CA 6.3.1/009	Plum, peach, and apricot, fruit	Glyphosate AMPA	Northern Europe: 3 Southern Europe: 4	[redacted] 2013 Report No. S12-03071	Valid
CA 6.3.1/010	Grape, fruit	Glyphosate AMPA	Northern Europe: 2	[redacted] 2016 Report No. S15-00491	Valid
CA 6.3.1/011	Grape, fruit	Glyphosate AMPA	Northern Europe: 4 Southern Europe: 2	[redacted] 2015 Report No. S14-04157	Valid
CA 6.3.1/012	Grape, fruit	Glyphosate AMPA	Northern Europe: 3 Southern Europe: 2	[redacted] 2015 Report No. S14-04158	Valid
CA 6.3.1/013	Grape, fruit	Glyphosate AMPA	Southern Europe: 4	[redacted] 2015 Report No. S14-04226	Valid
CA 6.3.1/014	Grape, fruit	Glyphosate AMPA	Northern Europe: 6	[redacted] 1989 Report No. MLL 30227	Invalid
CA 6.3.1/015	Olive, fruit	Glyphosate AMPA	Southern Europe: 4	[redacted] 1996 Report No. MLL 30469	Valid
CA 6.3.1/016	Olive, fruit	Glyphosate	Southern Europe: 2	[redacted] 1996 Report No. RJ2217B	Supportive
CA 6.3.1/017	Olive, fruit	Glyphosate	Southern Europe: 2	[redacted], 1996 Report No. RJ2218B	Supportive
CA 6.3.1/018	Olive, fruit	Glyphosate	Southern Europe: 1	[redacted] 1991 Report No. M5354B	Supportive
CA 6.3.1/019	Kiwi, peel and pulp	Glyphosate AMPA	Southern Europe: 2	[redacted] 2016 Report No. S15-00469	Valid
CA 6.3.1/020	Banana, whole fruit, peel and pulp	Glyphosate AMPA	Southern Europe: 4	[redacted] 2015 Report No. S14-04159	Valid

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/001
Report author	[redacted]
Report year	2014
Report title	Glyphosate-Residue study on mandarin oranges in Spain in 2013
Report No	S13-02531
Document No	A12798QA_10348
Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in mandarin (peel and pulp) after one application of A12798QA, an SL formulation containing 360 g/L of glyphosate (present as the acid).

The study included two trials conducted in Spain in 2013. The application was performed on the ground at normal commercial harvest (0-day PHI) and at a target rate of 2.88 kg glyphosate per hectare. Samples of mandarin peel and pulp were analysed for glyphosate and AMPA. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	A12798QA
Batch number:	BSN0J0451
EAS test item code:	None
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	359.98 g/L
Content of a.s. analysed:	373 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	10.03.2011
Expiry date:	31.01.2015

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S13-02531-01	Mandarin	*Citrus reticulate*	Okitsu	Fruit	≥ 2 kg / ≥ 30 units
S13-02531-02	Mandarin	*Citrus reticulate*	Okitsu	Fruit	≥ 2 kg / ≥ 35 units

Test facilities	
Study directory:	[redacted]
Field phase (S13-02531-01):	[redacted]
Field phase (S13-02531-02):	[redacted]ain
Analytical phase:	[redacted]

B. Methods

1. Field phase

Two residue trials were conducted on mandarins (outdoor) during 2013 in Spain (S13-02531-01 and S13-02531-02). One application of A12798QA (360 g/L glyphosate) was performed to the strip of ground area at the base of a row of mandarin trees (8 trees per plot) at 6.91 to 7.55 L product/ha, diluted with water immediately prior to application to a spray volume of 266 to 291 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-3: Application information

Trial no.	Application code	Timing (BBCH)	Application rate kg a.s./ha	Water volume L/ha
S13-02531-01	A1	83	2.49	266
S13-02531-02	A1	83	2.72	291

Regions, varieties and cultivation were typical for the cultivation of mandarins.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand on the same day as application. Each field specimen was taken using a suitable distributive pattern. Crop was healthy throughout the duration of the trial with samples collected at harvest being of a commercially acceptable standard. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). Specimens were stored deep-frozen (at or below ≤ - 18 °C) after arrival at the test sites.

Table 6.3.1-4: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S13-02531-01	Mandarin	Fruit	0	83	≥ 2 kg / ≥ 30 units	26.09.2013
S13-02531-02	Mandarin	Fruit	0	83	≥ 2 kg / ≥ 35 units	26.09.2013

1 Days after last application

2. Sample preparation

The stalks/stems were removed from the specimens. Peel was removed while the specimens were frozen. Peel and pulp were homogenised separately while frozen.

3. Analytical phase

Glyphosate and AMPA (Syngenta method GRM067.01A) were extracted from crop matrices by maceration using deionised water and dichloromethane. Following centrifugation, derivatisation of an aliquot of the aqueous phase extraction was performed with 9-fluorenylmethyl chloroformate (FMOC). Samples were purified by partition with dichloromethane. The analytes were determined by LC-MS/MS and quantified using an external standardisation procedure and single point calibration. .
Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA.

Treated and untreated specimens were maintained in a deep frozen condition and kept separate during storage and shipment. The maximum sample storage interval from harvest to extraction was 4 months, and the maximum interval from extraction to analysis was 7 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

For glyphosate and AMPA in mandarins (peel and pulp), the limit of quantitation (LOQ) was 0.05 mg/kg each.

During analysis of mandarin (peel and pulp) specimens, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) were performed. The results are summarised in the table below.

Table 6.3.1-5: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Mandarin fruits, peel	Glyphosate	0.05	107	107	-	1
		0.5	106	106	-	1
		Overall	106-107	107	-	2
	AMPA	0.05	87	87	-	1
		0.5	82	82	-	1
		Overall	82-87	85	-	2
Mandarin fruits, pulp	Glyphosate	0.05	116	116	-	1
		0.5	96	96	-	1
		Overall	96-116	106	-	2
	AMPA	0.05	90	90	-	1
		0.5	87	87	-	1
		Overall	87-90	89	-	2

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of A12798QA when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of mandarin peel and pulp. Additionally, residue values for whole fruit were calculated based on quantity of residue in peel and pulp along with corresponding weight of the whole fruit sample.

Detailed residue levels are shown in the table below.

Table 6.3.1-6: Residue levels of glyphosate and AMPA in mandarin after one application of A12798QA (359.88 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2] (mg/kg)		DALA[3] (days)	Date of analysis
				Glypho-sate	AMPA		
S13-02531-01, 46691 Vallada, Valencia, Spain / SEU / 2013	Mandarin / Okitsu	83	Peel	<0.05	<0.05	0	10.12.2013-08.01.2014
			Pulp	<0.05	<0.05	0	10.12.2013-08.01.2014
			Whole fruit[4]	<0.05	<0.05	0	N/A – calculated value

Table 6.3.1-6: Residue levels of glyphosate and AMPA in mandarin after one application of A12798QA (359.88 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2] (mg/kg)		DALA[3] (days)	Date of analysis
				Glypho-sate	AMPA		
S13-02531-01 / 46270 Castelló de la Ribera, Valencia, Spain / SEU / 2013	Mandarin / Okitsu	83	Peel	<0.05	<0.05	0	10.12.2013-08.01.2014
			Pulp	<0.05	<0.05	0	10.12.2013-08.01.2014
			Whole fruit[4]	<0.05	<0.05	0	N/A – calculated value

1 Growth stage at last application
2 LOQ (limit of quantification): 0.05 mg/kg (glyphosate expressed as glyphosate and AMPA expressed as AMPA)
3 Days after last application
4 Residue in whole fruit (peel + pulp) was calculated based on quantity of residue in peel and pulp along with the weight of the corresponding whole fruit specimen sample. However, since residue in both peel and pulp were <LOQ (<0.05 mg/kg), residue calculated for whole fruit was also reported as <LOQ (<0.05 mg/kg).

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of mandarin peel and pulp sampled at BBCH 83 (normal commercial harvest).

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 509. The tested application rates (2.49 and 2.72 kg a.s./ha) are 13.5 % and 5.6 % lower than the critical maximum seasonal application rate of 2.88 kg a.s./ha. The samples were taken directly after the application. This is a worst case with regard to the proposed PHI of 7 days in the GAP table. The metabolism studies show that there is no uptake of glyphosate from the soil into the trees. Consequently, no higher residues are expected in the tree fruit at a longer PHI. Therefore, the study adequately supports the representative use for glyphosate in plantations of citrus trees in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/002
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in tree nuts (outdoor) at 2 sites in Southern Europe 2015
Report No	S15-00018
Document No	MSL0027487
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in tree nuts (nutmeat) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 2 field trials (with hazelnut and pistachio, respectively) in the southern zone. The tree plantations were treated once. The application was directed to the soil under the trees and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of tree nuts were taken for analysis at normal harvest, which was 7 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L

Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00018-01	Tree nut / Hazelnut	*Corylus avellana*	Pavelet	Nutmeat	≥ 0.19 kg[1] / > 50 units
S15-00018-02	Tree nut / Pistachio	*Pistacia vera*	Napoletana	Nutmeat	≥ 1.8 kg / > 50 units

1 Sample sizes were below the protocol minimum of 1 kg, however a sufficient number of nuts were available and the overall sample amount was sufficient for analysis. The total weight of sampled nuts (before preparation of nutmeat) was 0.73 kg.

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00018-01):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S15-00018-02):	Eurofins Agroscience Services SRL, 95121 Catania, Italy
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Two residue trials were conducted on tree nuts (hazelnut and pistachio) during the 2015 season. One trial was conducted on hazelnut in Spain (S15-00018-01) and one trial was conducted on pistachio in Italy (S15-00018-02). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the trees at the nominal rate of 7.5 L product/ha 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 300-324 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-7: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S15-00018-01	2	87 BBCH	3.601	300
S15-00018-02	2	87 BBCH	3.888	324

Regions, varieties and cultivation were typical for the cultivation of tree nuts.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of whole nuts were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 89), which was 7 days after application. Each field sample was taken from at least 4 trees from all segments of the tree, high and low, exposed and protected by foliage, avoiding the ends of the

row. At least 12 sampling locations were chosen. The nutmeat was separated manually from the shell of the nuts at the trial sites. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the samples for analysis were taken in duplicate. In the trial S15-00018-02 a further field sample was taken as retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).
In trial S15-00018-01, the weight of nutmeat analytical samples collected was less than the 1 kg minimum specified in the study plan. However, an adequate number of nuts was collected to provide a representative field sample (0.73 kg) and the quantities of nutmeat were sufficient for analysis.

Table 6.3.1-8: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00018-01	Tree nuts / Hazelnut	Nutmeat	7	89	≥ 0.19 kg / > 50 units	03.09.2015
S15-00018-02	Tree nuts / Pistachio	Nutmeat	7	89	≥ 1.8 kg / > 50 units	31.08.2015

1 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in plant commodities with a high oil content (EAS Chem study S14-05172). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 91 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in hazelnut (3 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. Furthermore, concurrent recoveries were also determined for glyphosate and AMPA in pistachio at fortification levels of 0.05 mg/kg and 0.50 mg/kg. The results were satisfactory, as shown in the table below.

Table 6.3.1-9: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Nutmeat (Hazelnut)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	100, 98, 100	99	-	1.2	3
		0.5	91, 98, 97	95	-	4.0	3
		Overall	91-100	97	-	3.4	6
		Confirmation transition 168 > 79 m/z					
		0.05	89, 101, 100	97	-	6.9	3
		0.5	102, 103, 99	101	-	2.1	3
		Overall	89-103	99	-	5.2	6
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	92, 90, 98	93	-	4.5	3
		0.5	101, 96, 99	99	-	2.6	3
		Overall	90-101	99	-	4.4	6
		Confirmation transition 110 > 79 m/z					
		0.05	106, 97, 100	101	-	4.5	3
		0.5	101, 95, 98	98	-	3.1	3
		Overall	95-106	100	-	3.9	6
Nutmeat (Pistachio)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	94	-	-	-	1
		0.5	96	-	-	-	1
		Overall	94-96	95	-	-	2
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	89	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-89	88	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of tree nuts (nutmeat).

Detailed residue levels are shown in the table below.

Table 6.3.1-10: Residue levels of glyphosate and AMPA in tree nuts after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00018-01 / 43143 El Milá, Tarragona, Spain / SEU / 2015	Tree nuts / Hazelnut / Pavelet	89	Nutmeat	n.d. n.d.	n.d. n.d.	7	17.11.2015
S15-00018-02 / 95034 Bronte, Sicily, Italy / SEU / 2015	Tree nuts / Pistachio / Napoletana	89	Nutmeat	n.d. n.d.	n.d. n.d.	7	01.12.2015

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of tree nuts (nutmeat) sampled at BBCH 89 (commercial maturity), 7 days after soil application of glyphosate in the tree row at the rate of 3.60-3.89 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. In the trial S15-00018-01 the weight of nutmeat samples was less than according to Guideline, but the samples may nevertheless be considered representative. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application at a rate of 3.6-3.9 kg a.s./ha. This is 25 % to 35 % higher than the critical GAP maximum seasonal application rate, which is acceptable since the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in plantations of nut trees in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/003
Report author	[redacted]
Report year	2014
Report title	Glyphosate - Residue study on apple in the United Kingdom and Germany in 2013
Report No	S13-03425

Document No	A12798QA_10340
Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in apple (fruit) after one application of A12798QA, an SL formulation containing 360 g/L of glyphosate (present as the acid).

The study included two trials conducted in United Kingdom and Germany in 2013. The application was performed on the ground at normal commercial harvest (0-day PHI) and at a target rate of 2.88 kg glyphosate per hectare. Samples of apple fruit were analysed for glyphosate and AMPA. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	A12798QA
Batch number:	BSN0J0451
EAS test item code:	None
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	359.98 g/L
Content of a.s. analysed:	373 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	10.03.2011
Expiry date:	31.01.2015

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S13-03425-01	Apple	*Malus domestica*	Jonagold	Fruit	≥ 2 kg / > 18 units
S13-03425-02	Apple	*Malus domestica*	Golden Delicious	Fruit	≥ 2 kg / 14 units

Test facilities	
Study directory:	Eurofins Agroscience Services Ltd, DE73 8AG Wilson, Derbyshire, United Kingdom
Field phase (S13-03425-01):	Eurofins Agroscience Services Ltd, CO7 8SD Colchester, Essex, United Kingdom
Field phase (S13-03425-02):	Eurofins Agroscience Services GmbH, D-21684 Stade, Germany
Analytical phase:	Eurofins Agroscience Services Chem, DE73 8AG Wilson, Derbyshire, United Kingdom

B. Methods

1. Field phase

Two residue trials were conducted on apple (outdoor) during 2013 in the United Kingdom (S13-03425-01) and Germany (S13-03425-02). One application of A12798QA (360 g/L glyphosate) was performed to the strip of ground area around the base of a row of apple trees (6 - 8 trees per plot) at 7.98 to 8 L product/ha, diluted with water immediately prior to application to a spray volume of 300 to 399 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-11: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S13-03425-01	A1	87-89 BBCH	2.880	300
S13-03425-02	A1	87-89 BBCH	2.871	399

The actual application rate across the two trials ranged from 2.87 to 2.88 kg a.s./ha.

Regions, varieties and cultivation were typical for the cultivation of apples. Weather data were taken from the regions relevant weather stations of official weather services.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand on the same day as application. Each field specimen was taken using a suitably distributive pattern. Crop was healthy throughout the duration of the trial with samples collected at harvest being of a commercially acceptable standard. Stalks were removed from the fruit prior to storage. Duplicate specimens were taken as cover. Specimens were deep-frozen after arrival at the field test sites. In the trial S13-03425-01 the storage temperature was ≤ -17°C. The storage duration at > -18°C is not specified in the report. But since the temperature deviation was minimal and since the samples were analysed within 2 months of sampling (see below) no impact on the reliability of the study results is expected. In the trial S13-03425-02 the storage temperature at the field test site was ≤ -18°C.

Table 6.3.1-12: Crop sampling information

Trial	Crop	Commodity	Timing[1]	Growth stage (BBCH)	Quantity	Date of sampling
S13-03425-01	Apple	Fruit	NCH	87-89	≥ 2 kg / ≥ 18 units	08.10.2013
S13-03425-02	Apple	Fruit	NCH	87-89	≥ 2 kg / 14 units	07.10.2013

1 NCH = Normal Commercial Harvest.

2. Sample preparation

The stalks/stems were removed from the specimens. The remaining apple fruit were homogenised while frozen.

3. Analytical phase

Glyphosate and AMPA (Syngenta method GRM067.01A) were extracted from crop matrices by maceration using deionised water and dichloromethane. Following centrifugation, derivatisation of an aliquot of the aqueous phase extraction was performed with 9-fluorenylmethyl chloroformate (FMOC). Samples were purified by partition with dichloromethane. The analytes were determined by LC-MS/MS and quantified using an external standardisation procedure and single point calibration.
Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA.

Treated and untreated specimens were maintained in a deep frozen condition and kept separate during storage and shipment. The maximum sample storage interval from harvest to extraction was 2 months, and the maximum interval from extraction to analysis was 6 days. Samples were stored frozen at ≤ -18 °C at the analytical facility prior to analysis.

For glyphosate and AMPA in apple (fruit), the limit of quantitation (LOQ) was 0.05 mg/kg each.

During analysis of apple (fruit) specimens, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) each were performed. The results are summarised in the table below.

Table 6.3.1-13: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Apple, fruit	Glyphosate	0.05	98	98	-	1
		0.5	91	91	-	1
		Overall	91-98	95	-	2
	AMPA	0.05	95	95	-	1
		0.5	89	89	-	1
		Overall	89-95	92	-	2

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of A12798QA when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of apple (fruit).

Detailed residue levels are shown in the table below.

Table 6.3.1-14: Residue levels of glyphosate and AMPA in apple after one application of A12798QA (359.88 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2] (mg/kg)		DALA[3] (days)	Date of analysis
				Glypho-sate	AMPA		
S13-03425-01 / CO105LW Assington, Essex, United Kingdom / NEU / 2013	Apple / Jonagold	87-89	Fruit	<0.05	<0.05	0	10.-16.12.2013
S13-03425-02 / 21635 Jork, Germany / NEU / 2013	Apple / Golden Delicious	87-89	Fruit	<0.05	<0.05	0	10.-16.12.2013

1 Growth stage at last application
2 LOQ (limit of quantification): 0.05 mg/kg (glyphosate expressed as glyphosate and AMPA expressed as AMPA)
3 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of apple (fruit) sampled at BBCH 87-89 (normal commercial harvest).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 509. The samples were taken directly after the application. This is a worst case with regard to the proposed PHI of 7 days in the GAP table. The metabolism studies show that there is no uptake of glyphosate from the soil into the trees. Consequently, no higher residues are expected in the tree fruit at a longer PHI. Therefore, the study adequately supports the representative use for glyphosate in plantations of pome fruit trees in Northern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/004
Report author	[redacted]
Report year	2014
Report title	Glyphosate - Residue study on apple in Spain and Italy in 2013
Report No	S13-03426
Document No	A12798QA_10343
Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4

Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in raw agricultural commodity specimens of apple (RAC fruit) after one application of A12798QA, an SL formulation containing 360 g/L of glyphosate (present as the acid).

The study included two trials in Italy and Spain in 2013. The application was performed on the ground at normal commercial harvest (0-day PHI) and at a target rate of 2.88 kg glyphosate per hectare. Samples of apple fruit were analysed for glyphosate and AMPA. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	A12798QA
Batch number:	BSN0J0451
EAS test item code:	None
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	359.98 g/L
Content of a.s. analysed:	373 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	10.03.2011
Expiry date:	31.01.2015

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S13-03426-02	Apple	*Malus domestica*	Golden	Fruit	≥ 2 kg / 12 units
S13-03426-03	Apple	*Malus domestica*	Golden Delicious	Fruit	≥ 2 kg / 14 units

Test facilities	
Study directory:	Eurofins Agroscience Services Ltd, DE73 8AG Wilson, Derbyshire, United Kingdom
Field phase (S13-03426-02):	Eurofins Agroscience Services SRL, 40016 San Giorgio Di Piano, Bologna, Italy
Field phase (S13-03426-03):	Eurofins Agroscience Services SL, E-50016 Zaragoza, Spain
Analytical phase:	Eurofins Agroscience Services Chem, DE73 8AG Wilson, Derbyshire, United Kingdom

B. Methods

1. Field phase

Two residue trials were conducted on apple (outdoor) during 2013 in Italy (S13-03426-02) and Spain (S13-03426-03). One application of A12798QA (360 g/L glyphosate) was performed to the strip of ground area around the base of a row of apple trees (6 – 8 trees per plot) at 7.9 to 8.4 L product/ha, diluted with water immediately prior to application to a spray volume of 210 to 397 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-15: Application schedule

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S13-03426-02	A1	89 BBCH	2.860	397
S13-03426-03	A1	89 BBCH	3.024	210

The actual application rate across the two trials ranged from 2.86 to 3.02 kg a.s./ha.

Regions, varieties and cultivation were typical for the cultivation of apples. Weather data were taken from the regions relevant weather stations of official weather services.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand on the same day as application. Each field specimen was taken using a suitably distributive pattern. Crop was healthy throughout the duration of the trial with samples collected at harvest being of a commercially acceptable standard. Stalks were removed from the fruit prior to storage. Duplicate specimens were taken as cover. Specimens were deep-frozen (at or below ≤ - 18 °C) after arrival at the test sites. In the trial S13-03426-02 the temperature in the freezer truck during shipment to the analytical facility exceeded -18°C for about 97.5 hours. However, since the maximum temperature was -15.2°C this does not impact the reliability of the study results.

Table 6.3.1-16: Crop sampling information

Trial	Crop	Commodity	Timing[1]	Growth stage (BBCH)	Quantity	Date of sampling
S13-03426-02	Apple	Fruit	NCH	89	≥ 2 kg / 12 units	11.09.2013
S13-03426-03	Apple	Fruit	NCH	89	≥ 2 kg / 14 units	07.11.2013

1 NCH = Normal Commercial Harvest.

2. Sample preparation

The stalks/stems were removed from the specimens. The remaining apple fruit were homogenised while frozen.

3. Analytical phase

Glyphosate and AMPA (Syngenta method GRM067.01A) were extracted from crop matrices by maceration using deionised water and dichloromethane. Following centrifugation, derivatisation of an aliquot of the aqueous phase extraction was performed with 9-fluorenylmethyl chloroformate (FMOC). Samples were purified by partition with dichloromethane. The analytes were determined by LC-MS/MS and quantified using an external standardisation procedure and single point calibration.
Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA.

Treated and untreated specimens were maintained in a deep frozen condition and kept separate during storage and shipment. The maximum sample storage interval from harvest to extraction was 3 months, and the maximum interval from extraction to analysis was 7 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

For glyphosate and AMPA in apple (fruit), the limit of quantitation (LOQ) was 0.05 mg/kg each.

During analysis of apple (fruit) specimens, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) each were performed. The results are summarised in the table below.

Table 6.3.1-17: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Apple, fruit	Glyphosate	0.05	89	89	-	1
		0.5	92	92	-	1
		Overall	89-92	91	-	2
	AMPA	0.05	86	86	-	1
		0.5	91	91	-	1
		Overall	86-91	89	-	2

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of A12798QA when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of apple (fruit).

Detailed residue levels are shown in the table below.

Table 6.3.1-18: Residue levels of glyphosate and AMPA in apple after one application of A12798QA (359.88 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2] (mg/kg)		DALA[3] (days)	Date of analysis
				Glypho-sate	AMPA		
S13-03426-02 / 40051 Altedo, Bologna, Italy / SEU / 2013	Apple / Golden	89	Fruit	<0.05	<0.05	0	10.-17.12.2013
S13-03426-03 / 50340 Maluenda, Aragon, Spain / SEU / 2013	Apple / Golden Delicious	89	Fruit	<0.05	<0.05	0	10.-17.12.2013

1 Growth stage at last application
2 LOQ (limit of quantification): 0.05 mg/kg (glyphosate expressed as glyphosate and AMPA expressed as AMPA)
3 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of apple (fruit) sampled at BBCH 87-89 (normal commercial harvest).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 509. The samples were taken directly after the application. This is a worst case with regard to the proposed PHI of 7 days in the GAP table. The metabolism studies show that there is no uptake of glyphosate from the soil into the trees. Consequently, no higher residues are expected in the tree fruit at a longer PHI. Therefore, the study adequately supports the representative use for glyphosate in plantations of pome fruit trees in Southern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

Data point:	CA 6.3.1/005
Report author	[redacted]
Report year	1976
Report title	CP 67573 : Determination of crop residues in apples and pears
Report No	A9
Document No	
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Glyphosate Monograph (1998)
Short description of	Thirty-five trials were conducted on apples between 1973 and

study design and observations:	1976 in Germany, United Kingdom, Sweden, Netherlands, France, and Belgium with application directed to the ground. Thirty-one of the trials were conducted with a single application of Roundup (MON 2139) at a rate ranging from 1.44 to 9.0 kg a.s./ha. Samples of apple fruit were collected between 26 and 355 days after treatment and analysed for glyphosate and AMPA. Four of the trials involved two to three applications of Roundup (MON 2139) distributed over two to four years preceding sampling. The total rate ranged from 3.6 to 8.1 kg a.s./ha. Samples of apple fruit were collected and analysed for glyphosate and AMPA. Three trials were conducted on pears between 1973 and 1976 in France and Italy with application directed to the ground. Two of the trials were conducted with a single application of Roundup (MON 2139) at a rate ranging from 2.7 to 8.64 kg a.s./ha. Samples of pear fruit were collected between 40 and 84 days after treatment and analysed for glyphosate and AMPA. One of the trials involved three applications of Roundup (MON 2139) distributed over the four years preceding sampling. The total rate was 11.7 kg a.s./ha. Samples of pear fruit were collected and analysed for glyphosate and AMPA. Residues of glyphosate and AMPA in apple and pear samples were analysed by partition-extraction, ion-exchange chromatography, derivatisation to the N-trifluoroacetyl methyl esters and determination by GLC using a phosphorus specific flame photometric detector. Percent recovery in apples averaged at 61 % for glyphosate (range 32 to 104 %) and 58 % for AMPA (range 40 to 87 %). Percent recovery in pears averaged at 50 % for glyphosate and 57 % for AMPA.
Short description of results:	No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated apple or pear samples., 69.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted to GLP. Furthermore the average recoveries are very low with 50 % and 61 % (apple and pear, respectively) for glyphosate and 57 % and 58 % (apple and pear, respectively) for AMPA.
Category study in AIR 5 dossier (L docs)	Category 3b

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/006
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in apricots (outdoor) at 4 sites in Southern Europe 2015
Report No	S15-00019
Document No	MSL0027488
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in apricot (fruit) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials in the southern zone. The tree plantations were treated once. The application was directed to the soil under the trees and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of apricot fruit were taken for analysis at normal harvest, which was 7 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L

Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00019-01	Apricot	*Prunus armeniaca*	Royal Roussillon	Fruit	≥ 2 kg / ≥ 48 units
S15-00019-02	Apricot	*Prunus armeniaca*	Foubaly	Fruit	≥ 2.4 kg / 45 units
S15-00019-03	Apricot	*Prunus armeniaca*	Precoce D'Imola	Fruit	≥ 2 kg / ≥ 30 units
S15-00019-04	Apricot	*Prunus armeniaca*	Reale D'Imola	Fruit	≥ 2 kg / ≥ 20 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S15-00019-01 and S15-00019-02):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S15-00019-03 and S15-00019-04):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on apricot (outdoor) during the 2015 season in Southern France (S15-00019-01 and S15-00019-02) and Italy (S15-00019-03 and S15-00019-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the trees at the nominal rate of 7.5 L product/ha 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 283-323 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-19: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S15-00019-01	2	81-85 BBCH	3.862	322
S15-00019-02	2	85 BBCH	3.870	323
S15-00019-03	2	85 BBCH	3.617	301
S15-00019-04	2	85 BBCH	3.400	283

Regions, varieties and cultivation were typical for the cultivation of apricots.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of fruits were taken by hand from the untreated and treated plots 7 days after application. Each field sample was taken from at least 4 trees from all segments of the tree or plant, high and low, exposed and protected by foliage, avoiding the ends of the row. At least 12 sampling locations were chosen. The stones were separated from the flesh of the fruits before freezing. The stones were discarded afterwards. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 6.5 hours of sampling in the field).

Table 6.3.1-20: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00019-01	Apricot	Fruit	7	87-89	≥ 2 kg / ≥ 48 units	30.06.2015
S15-00019-02	Apricot	Fruit	7	87	≥ 2.4 kg / 45 units	07.07.2015
S15-00019-03	Apricot	Fruit	7	87	≥ 2 kg / ≥ 30 units	24.06.2015
S15-00019-04	Apricot	Fruit	7	89	≥ 2 kg / ≥ 20 units	23.06.2015

1 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant commodities with a high water content (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 147 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in apricots (3 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.1-21: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1] Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Apricot, fruit (without stones)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	81, 89, 87	86	-	4.9	3
		0.5	82, 76, 86	81	-	6.2	3
		Overall	76-89	84	-	5.7	6
		Confirmation transition 168 > 79 m/z					
		0.05	82, 83, 79	81	-	2.6	3
		0.5	75, 89, 85	83	-	8.7	3
		Overall	75-89	82	-	5.9	6
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	89, 87, 87	88	-	1.3	3
		0.5	86, 88, 82	85	-	3.6	3
		Overall	82-89	87	-	2.8	6
		Confirmation transition 110 > 79 m/z					
		0.05	77, 95, 83	85	-	11	3
		0.5	84, 78, 90	84	-	7.1	3
		Overall	77-95	85	-	8.2	6

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of apricot (fruit without stone).

Detailed residue levels are shown in the table below.

Table 6.3.1-22: Residue levels of glyphosate and AMPA in apricot after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg) Glypho-sate	AMPA	DALA[4] (days)	Date of analysis
S15-00019-01 / 66600 Rivesaltes, Pyrenées-Orientales, France / SEU / 2015	Apricot / Royal Roussillon	87-89	Fruit without stone	n.d. n.d.	n.d. n.d.	7	17.-18.11.2015

Table 6.3.1-22: Residue levels of glyphosate and AMPA in apricot after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00019-02 / 66670 Bages, Pyrenées-Orientales, France / SEU / 2015	Apricot / Foubaly	87	Fruit without stone	n.d. n.d.	n.d. n.d.	7	17.-18.11.2015
S15-00019-03 / 40051 Altedo, Emilia Romagna, Italy / SEU / 2015	Apricot / Precoce D'Imola	87	Fruit without stone	n.d. n.d.	n.d. n.d.	7	17.-18.11.2015
S15-00019-03 / 40069 Zola Pedrosa, Emilia Romagna, Italy / SEU / 2015	Apricot / Reale D'Imola	89	Fruit without stone	n.d. n.d.	n.d. n.d.	7	17.-18.11.2015

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of apricot (fruit without stone) sampled at BBCH 87-89 (commercial maturity), 7 days after band application of glyphosate in the tree row at the rate of 3.40-3.87 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application at a rate of 3.4-3.9 kg a.s./ha. This application rate is 18 % to 34 % higher than the critical GAP maximum seasonal application rate, which is acceptable since the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in plantations of fruit trees (and especially stone fruit trees) in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/007
Report author	[redacted]
Report year	2014
Report title	Glyphosate - Residue study on cherry in Spain and Italy in 2013
Report No	S13-03427
Document No	A12798QA_10349
Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in raw agricultural commodity specimens of sweet cherry (fruit) after one application of A12798QA, an SL formulation containing 360 g/L of glyphosate (present as the acid).

The study included two trials conducted in Italy and Spain in 2013. The application was performed on the ground at normal commercial harvest (0-day PHI) and at normal commercial harvest (0-day PHI) at a target rate of 2.88 kg glyphosate per hectare. Samples of cherry fruit were analysed for glyphosate and AMPA. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	A12798QA
Batch number:	BSN0J0451
EAS test item code:	None
Active ingredient(s):	Glyphosate

CAS number:	1071-83-6
Content of a.s. nominal:	359.98 g/L
Content of a.s. analysed:	373 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	10.03.2011
Expiry date:	31.01.2015

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S13-03427-01	Sweet cherry	*Prunus avium*	Sweet Heart	Fruit	≥ 1 kg / 12 units
S13-03427-02	Sweet cherry	*Prunus avium*	Lapins	Fruit	≥ 1 kg / > 50 units

Test facilities	
Study directory:	Eurofins Agroscience Services Ltd, DE73 8AG Wilson, Derbyshire, United Kingdom
Field phase (S13-03427-01):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S13-03427-02):	Eurofins Agroscience Services SRL, 40016 San Giorgio Di Piano, Bologna, Italy
Analytical phase:	Eurofins Agroscience Services Chem, DE73 8AG Wilson, Derbyshire, United Kingdom

B. Methods

1. Field phase

Two residue trials were conducted on sweet cherry (outdoor) during 2013 in Spain (S13-03427-01) and Italy (S13-03427-02). One application of A12798QA (360 g/L glyphosate) was performed to the strip of ground area around the base of a row of sweet cherry trees (6 – 8 trees per plot) at 7.66 to 7.91 L product/ha, diluted with water immediately prior to application to a spray volume of 287 to 304 L/ha. The application schedule is outlined in the table below.

Table 6.3.1-23: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S13-03427-01	A1	87-89 BBCH	2.757	287
S13-03427-02	A1	87-89 BBCH	2.847	304

The actual application rate across the two trials ranged from 2.76 to 2.85 kg a.s./ha.

Regions, varieties and cultivation were typical for the cultivation of sweet cherry. Weather data were taken from the regions relevant weather stations of official weather services.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand on the same day as application. Each field specimen was taken using a suitably distributive pattern. Crop was healthy throughout the duration of the trial with samples collected at harvest being of a commercially acceptable

standard. Stalks were removed from the fruit prior to storage. Specimens were taken in duplicate (with one of the duplicates serving as retain sample) and were deep-frozen (at or below ≤ - 18 °C) after arrival at the test sites, except in the trial S13-03427-02, in which the storage temperature at the test site exceeded -18°C for less than 5 hours with a maximum of -16.1°C. Furthermore, in the trial S13-03427-01 the temperature in the freezer truck during shipment to the analytical facility exceeded -18°C for about 44.5 hours. However, since the maximum temperature was -10.7°C and the samples remained frozen this does not impact the reliability of the study results.

Table 6.3.1-24: Crop sampling information

Trial	Crop	Commodity	Timing[1]	Growth stage (BBCH)	Quantity	Date of sampling
S13-03427-01	Sweet cherry	Fruit	NCH	89	≥ 1 kg / 12 units	25.07.2013
S13-03427-02	Sweet cherry	Fruit	NCH	87-89	≥ 1 kg / > 50 units	20.06.2013

1 NCH = Normal Commercial Harvest.

2. Sample preparation

The stones were removed while the specimens were frozen. Specimens were homogenised with dry ice.

3. Analytical phase

Glyphosate and AMPA (Syngenta method GRM067.01A) were extracted from crop matrices by maceration using deionised water and dichloromethane. Following centrifugation, derivatisation of an aliquot of the aqueous phase extraction was performed with 9-fluorenylmethyl chloroformate (FMOC). Samples were purified by partition with dichloromethane. The analytes were determined by LC-MS/MS and quantified using an external standardisation procedure and single point calibration.
Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA.

Treated and untreated specimens were maintained in a deep frozen condition and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 7 months, and the maximum interval from extraction to analysis was 5 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

For glyphosate and AMPA in sweet cherry (fruit), the limit of quantitation (LOQ) was 0.05 mg/kg each.

The method GRM067.01A was validated for the determination of glyphosate and AMPA in cherry as part of this study (5 fortification trials at each 0.05 mg/kg and 0.50 mg/kg). The results are summarised in the table below.

Table 6.3.1-25: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Sweet cherry, fruit	Glyphosate	Primary transition 392 > 170 m/z				
		0.05	97, 99, 98, 96, 102	98	2.1	5
		0.5	105, 105, 99, 102, 102	102	2.5	5
		Overall	96-105	100	3.0	10
	Glyphosate	Confirmatory transition 392 > 179 m/z				
		0.05	98, 98, 96, 99, 100	98	1.6	5
		0.5	101, 102, 96, 100, 99	100	2.1	5
		Overall	96-102	99	1.9	10
	Glyphosate	Confirmatory transition 392 > 88 m/z				
		0.05	101, 98, 104, 96, 103	100	3.6	5
		0.5	100, 102, 99, 99, 102	100	1.6	5
		Overall	96-104	100	2.6	10
	AMPA	Primary transition 334 > 156 m/z				
		0.05	85, 84, 87, 81, 81	83	3.2	5
		0.5	84, 87, 82, 86, 82	84	2.7	5
		Overall	81-87	84	2.9	10
	AMPA	Confirmatory transition 334 > 179 m/z				
		0.05	81, 82, 84, 78, 78	80	3.4	5
		0.5	84, 86, 80, 85, 82	84	2.8	5
		Overall	78-86	82	3.6	10
	AMPA	Confirmatory transition 334 > 112 m/z				
		0.05	81, 84, 80, 86, 80	82	3.1	5
		0.5	85, 87, 82, 81, 83	83	2.7	5
		Overall	80-87	83	2.8	10

1 Residues of glyphosate and AMPA in blank / control matrix were less than 30 % of the limit of quantitation.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of A12798QA when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of sweet cherry (fruit). Analysis was conducted on fruit without stone. Residue values for whole fruit (including stones) were calculated based on weight of the whole fruit in the samples. However, since residue values in fruit (without stone) were <LOQ (<0.05 mg/kg), residues in whole fruit are also reported as <LOQ (<0.05 mg/kg).

Detailed residue levels are shown in the table below.

Table 6.3.1-26: Residue levels of glyphosate and AMPA in sweet cherry after one application of A12798QA (359.88 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S13-03427-01 / 41927 Mairena del Aljarafe, Seville, Spain / SEU / 2013	Sweet cherry / Sweet Heart	89	Fruit	<0.05	<0.05	0	03.-08.01.2014
S13-03427-02 / 48018 Faenza, Italy / SEU / 2013	Sweet cherry / Lapins	87-89	Fruit	<0.05	<0.05	0	03.-08.01.2014

1 Growth stage at last application
2 LOQ (limit of quantification): 0.05 mg/kg (glyphosate expressed as glyphosate and AMPA expressed as AMPA)
3 Residue in RAC, whole fruit (fruit + stone). Residue found in fruit without stone was <LOQ (<0.05 mg/kg), therefore residue calculated for whole fruit (including stone) was also reported as <LOQ (<0.05 mg/kg).
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of sweet cherry (fruit) sampled at BBCH 87-89 (normal commercial harvest).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 509. The samples were taken directly after the application. This is a worst case with regard to the proposed PHI of 7 days in the GAP table. The metabolism studies show that there is no uptake of glyphosate from the soil into the trees. Consequently, no higher residues are expected in the tree fruit at a longer PHI. Therefore, the study adequately supports the representative use for glyphosate in plantations of stone fruit trees in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/008
Report author	[redacted]
Report year	2014
Report title	Glyphosate - Residue study on plum in Italy in 2013
Report No	S13-03233
Document No	A12798QA_10347

Guidelines followed in study	7209/VI/95 rev.5 SANCO/3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in raw agricultural commodity specimens of plum (RAC fruit) after one application of A12798QA, an SL formulation containing 360 g/L of glyphosate (present as the acid).

The study included two trials conducted in Italy in 2013. The application was performed on the ground at normal commercial harvest (0-day PHI) and at normal commercial harvest (0-day PHI) at a target rate of 2.88 kg glyphosate per hectare. Samples of plum fruit were analysed for glyphosate and AMPA. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	A12798QA
Batch number:	BSN0J0451
EAS test item code:	None
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	359.98 g/L
Content of a.s. analysed:	373 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	10.03.2011
Expiry date:	31.01.2015

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S13-03233-01	Plum	*Prunus domestica*	Ersinger	Fruit	≥ 2 kg / 64 units
S13-03233-02	Plum	*Prunus domestica*	Angeleno	Fruit	≥ 2 kg / 36 units

Test facilities	
Study directory:	Eurofins Agroscience Services Ltd, DE73 8AG Wilson, Derbyshire, United Kingdom
Field phase (S13-03233-01):	Eurofins Agroscience Services SRL, 40016 San Giorgio Di Piano, Bologna, Italy
Field phase (S13-03233-02):	Eurofins Agroscience Services SRL, 40016 San Giorgio Di Piano, Bologna, Italy
Analytical phase:	Eurofins Agroscience Services Chem, DE73 8AG Wilson, Derbyshire, United Kingdom

B. Methods

1. Field phase

Two residue trials were conducted on plums (outdoor) during 2013 in Italy (S13-03233-01 and S13-03233-02). One application of A12798QA (360 g/L glyphosate) was performed to the strip of ground area around the base of a row of plum trees (6 – 8 trees per plot) at 7.63 to 8.25 L product/ha, diluted with water immediately prior to application to a spray volume of 286 to 421 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-27: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S13-03233-01	A1	87-89 BBCH	2.971	421
S13-03233-02	A1	87-89 BBCH	2747	286

The actual application rate across the two trials ranged from 2.75 to 2.97 kg a.s./ha.

Regions, varieties and cultivation were typical for the cultivation of plums. Weather data were taken from the regions relevant weather stations of official weather services.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand on the same day as application. Each field specimen was taken using a suitably distributive pattern. Crop was healthy throughout the duration of the trials with samples collected at harvest being of a commercially acceptable standard. Leaves and stalks were removed prior to storage. Samples were taken in duplicate (with one of the duplicates serving as retain sample). Specimens were stored deep-frozen (at or below ≤ - 18 °C) after arrival at the test sites.

Table 6.3.1-28: Crop sampling information

Trial	Crop	Commodity	Timing[1]	Growth stage (BBCH)	Quantity	Date of sampling
S13-03233-01	Plum	Fruit	NCH	87-89	≥ 2 kg / 64 units	03.09.2013
S13-03233-02	Plum	Fruit	NCH	87-89	≥ 2 kg / 36 units	29.08.2013

1 NCH = Normal Commercial Harvest.

2. Sample preparation

The stones were removed while the specimens were frozen. Specimens were homogenised in the presence of dry ice.

3. Analytical phase

Glyphosate and AMPA (Syngenta method GRM067.01A) were extracted from crop matrices by maceration using deionised water and dichloromethane. Following centrifugation, derivatisation of an aliquot of the aqueous phase extraction was performed with 9-fluorenylmethyl chloroformate (FMOC). Samples were purified by partition with dichloromethane. The analytes were determined by LC-MS/MS and quantified using an external standardisation procedure and single point calibration.
Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA.

Treated and untreated specimens were maintained in a deep frozen condition and kept separate during storage and shipment. The maximum sample storage interval from harvest to extraction was 4 months, and the extract solutions were analysed straight after extraction completion. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

For glyphosate and AMPA in plum (fruit), the limit of quantitation (LOQ) was 0.05 mg/kg each.

The method GRM067.01A was validated for the determination of glyphosate and AMPA in plum as part of this study (5 fortification trials at each 0.05 mg/kg and 0.50 mg/kg). The results are summarised in the table below.

Table 6.3.1-29: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Plum, fruit	Glyphosate	Primary transition 392 > 170 m/z				
		0.05	102, 103, 104, 103, 109	104	2.6	5
		0.5	100, 103, 100, 97, 101	100	2.2	5
		Overall	97-109	102	3.0	10
	Glyphosate	Confirmatory transition 392 > 179 m/z				
		0.05	101, 96, 106, 101, 97	100	3.8	5
		0.5	100, 103, 101, 101, 100	101	1.2	5
		Overall	96-106	101	2.7	10
	Glyphosate	Confirmatory transition 392 > 88 m/z				
		0.05	101, 104, 112, 106, 98	104	5.3	5
		0.5	100, 104, 100, 99, 99	100	1.9	5
		Overall	98-112	102	4.3	10
	AMPA	Primary transition 334 > 156 m/z				
		0.05	93, 96, 99, 95, 93	95	2.4	5
		0.5	92, 94, 96, 93, 92	93	1.9	5
		Overall	92-99	94	2.3	10
	AMPA	Confirmatory transition 334 > 179 m/z				
		0.05	92, 97, 102, 94, 94	96	3.9	5

Table 6.3.1-29: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
		0.5	93, 94, 96, 92, 92	93	1.7	5
		Overall	92-102	95	3.2	10
	AMPA	Confirmatory transition 334 > 112 m/z				
		0.05	100, 99, 103, 95, 91	98	4.8	5
		0.5	94, 94, 96, 93, 94	94	0.8	5
		Overall	91-103	96	3.8	10

1 Residues of glyphosate and AMPA in blank matrix were less than 30 % of the limit of quantitation.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of A12798QA when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of plum (fruit). Analysis was conducted on fruit without stone. Residue values for whole fruit (including stones) were calculated based on weight of the whole fruit in the samples. However, since residue values in fruit (without stone) were <LOQ (<0.05 mg/kg), residues in whole fruit are also reported as <LOQ (<0.05 mg/kg).

Detailed residue levels are shown in the table below.

Table 6.3.1-30: Residue levels of glyphosate and AMPA in plum after one application of A12798QA (359.88 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop / Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S13-03233-01 / 40051 Altedo, Emilia Romagna, Italy / SEU / 2013	Plum / Ersinger	87-89	Fruit	<0.05	<0.05	0	17.-19.12.2013
S13-03233-02 / 48010 Barbiano, Emilia Romagna, Italy / SEU / 2013	Plum / Angeleno	87-89	Fruit	<0.05	<0.05	0	17.-19.12.2013

1 Growth stage at last application
2 LOQ (limit of quantification): 0.05 mg/kg (glyphosate expressed as glyphosate and AMPA expressed as AMPA)
3 Residue in RAC, whole fruit (fruit + stone). Residue found in fruit without stone was <LOQ (<0.05 mg/kg), therefore residue calculated for whole fruit (including stone) was also reported as <LOQ (<0.05 mg/kg).
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of plum (fruit) sampled at BBCH 87-89 (normal commercial harvest).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 509. The samples were taken directly after the application. This is a worst case with regard to the proposed PHI of 7 days in the GAP table. The metabolism studies show that there is no uptake of glyphosate from the soil into the trees. Consequently, no higher residues are expected in the tree fruit at a longer PHI. Therefore, the study adequately supports the representative use for glyphosate in plantations of stone fruit trees in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/009
Report author	Klimmek, S., Schultz, D.
Report year	2013
Report title	Determination of Residue of Glyphosate in Stone Fruits Following One Application of Glyphosate SL 360g/L (CA2705) in Northern and Southern France, in 2012
Report No	S12-03071
Document No	NUA 1201
Guidelines followed in study	EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in stone fruit (plum, peach, and apricot) (fruit) after one application of CA2705, an SL formulation containing 360 g/L of glyphosate acid equivalents (as the isopropylammonium salt).

The study included 7 field trials (3 trials in the northern zone and 4 trials in the southern zone). The stone fruit plantations were treated once. The application was directed to the ground and the target rate was 2.88

kg glyphosate acid equivalents per hectare. Samples of fruit were taken for analysis at normal harvest, which was 21 days after application. In two trials (S12-03071-01 and S12-03071-05), samples were also harvested 0, 7, and 14 days after the application. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	CA2705
Batch number:	RD-894-160
EAS test item code:	Not provided
Active ingredient(s):	Glyphosate (s/f IPA)
CAS number:	38641-94-0
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	356.3 g/L
Formulation type:	SL
Appearance/colour:	Pale yellow brownish liquid
Certificate of analysis:	04.05.2012
Expiry date:	29.03.2014

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S12-03071-01	Plum	*Prunus domestica*	Elena	Fruit	≥ 1 kg / ≥ 24 units
S12-03071-02	Plum	*Prunus domestica*	Mirabelle	Fruit	≥ 2 kg / ≥ 24 units
S12-03071-03	Plum	*Prunus domestica*	Mirabelle	Fruit	≥ 2 kg / ≥ 24 units
S12-03071-05	Peach	*Prunus persica*	Maillardiva	Fruit	≥ 1 kg / ≥ 24 units
S12-03071-06	Peach	*Prunus persica*	Brareg	Fruit	≥ 2 kg / ≥ 24 units
S12-03071-07	Apricot	*Prunus armeniaca*	Farbaly	Fruit	≥ 2 kg / ≥ 24 units
S12-03071-08	Plum	*Prunus domestica*	Mirabelle	Fruit	≥ 2 kg / ≥ 24 units

Test facilities	
Study directory:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany
Field phase (S12-03071-01 and S12-03071-02):	Eurofins Agroscience Services France SAS, 67140 Saint Pierre, France
Field phase (S12-03071-03):	Eurofins Agroscience Services SAS, 45300 Rouvres St Jean, France
Field phase (S12-03071-05 and S12-03071-07):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S12-03071-06 and S12-03071-08):	Eurofins Agroscience Services SAS, 82290 Meauzac, France
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Seven residue trials were conducted on stone fruit (plum, peach, and apricot) (outdoor) during the 2012 season in Northern France (S12-03071-01, S12-03071-02, and S12-03071-03) and Southern France (S12-03071-05, S12-03071-06, S12-03071-07, and S12-03071-08). One application of CA2705 (360 g/L glyphosate acid equivalents) was performed to the soil under the trees (6 plants per plot) at a target rate of 8 L product/ha, at 21 days before harvest. The volume of water used to prepare the spray solution was in the range of 190-371 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-31: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S12-03071-01	2	81 BBCH	2.708	190
S12-03071-02	2	81-85 BBCH	2.890	203
S12-03071-03	2	78 BBCH	2.750	194
S12-03071-05	2	81 BBCH	2.764	193
S12-03071-06	2	81 BBCH	5.285	371
S12-03071-07	2	78-79 BBCH	2.922	205
S12-03071-08	2	81-85 BBCH	3.029	213

In trial S12-03071-06 the application rate of glyphosate was 5.285 kg a.s./ha due to an error in the calculation of the plot size in this trial.

Regions, varieties and cultivation were typical for the cultivation of stone fruits.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of fruits were taken by hand from the untreated and treated plots 20 to 21 days after the application. In the two decline trials (S12 03071 01 and S12 03071 05), samples were also harvested 0, 7, and 14 days after the application. Stone fruit specimens (at least 24 fruits) were collected from at least 4 trees. Fruits were selected from all parts, top and bottom, exposed and covered by foliage. The quantity of fruits picked was based on the amount of fruit on the tree or bush, i.e. more fruit were picked from heavily laden parts of the tree or bush. Sampling was avoided at the ends of the rows and at the edges of the test plot. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 4 hours of sampling in the field).

Table 6.3.1-32: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S12-03071-01	Plum	Fruit	0	81	≥ 1 kg / ≥ 24 units	02.08.2012
	Plum	Fruit	7	81	≥ 1 kg / ≥ 24 units	09.08.2012
	Plum	Fruit	14	85-87	≥ 1 kg / ≥ 24 units	16.08.2012
	Plum	Fruit	21	87	≥ 2 kg / ≥ 24 units	23.08.2012
S12-03071-02	Plum	Fruit	21	87-89	≥ 2 kg / ≥ 24 units	16.08.2012
S12-03071-03	Plum	Fruit	21	89	≥ 2 kg / ≥ 24 units	13.08.2012
S12-03071-05	Peach	Fruit	0	81	≥ 1 kg / ≥ 24 units	14.08.2012
	Peach	Fruit	7	81-83	≥ 1 kg / ≥ 24 units	21.08.2012
	Peach	Fruit	14	87-89	≥ 1 kg / ≥ 24 units	28.08.2012
	Peach	Fruit	20	87-89	≥ 2 kg / ≥ 24 units	03.09.2012
S12-03071-06	Peach	Fruit	21	89	≥ 2 kg / ≥ 24 units	27.08.2012
S12-03071-07	Apricot	Fruit	21	87-89	≥ 2 kg / ≥ 24 units	02.08.2012
S12-03071-08	Plum	Fruit	21	87-89	≥ 2 kg / ≥ 24 units	09.08.2012

1 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant commodities with a high water content (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 91 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A method validation for the determination of glyphosate and AMPA in stone fruit (5 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.1-33: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Stone fruit, fruit	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	81, 80, 86, 80, 83	82	2.5	3.1	5
		0.5	80, 82, 80, 85, 100	85	8.4	9.9	5

Table 6.3.1-33: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	80-100	84	6.1	7.3	10
		Confirmatory transition 168 > 150 m/z					
		0.05	86, 85, 85, 80, 96	86	5.9	6.8	5
		0.5	88, 86, 87, 85, 101	89	6.6	7.4	5
		Overall	80-101	88	6.1	6.9	10
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	85, 84, 83, 79, 90	84	4.0	4.7	5
		0.5	84, 84, 83, 83, 100	87	7.4	8.5	5
		Overall	79-100	86	5.8	6.7	10
		Confirmatory transition 110 > 79 m/z					
		0.05	80, 77, 80, 73, 84	79	4.1	5.2	5
		0.5	80, 81, 82, 80, 97	84	7.3	8.7	5
		Overall	73-97	81	6.2	7.6	10

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of CA2705 when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of stone fruit samples.

Detailed residue levels are shown in the table below.

Table 6.3.1-34: Residue levels of glyphosate and AMPA in carrot after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S12-03071-01 / 67140 Stotzheim, Bas-Rhin, France / NEU / 2012	Plum / Elena	81	Fruit	< 0.05	n.d.	0	01.11.2012
		81	Fruit	< 0.05	n.d.	7	
		85-87	Fruit	< 0.05	n.d.	14	
		87	Fruit	< 0.05	n.d.	21	
S12-03071-02 / 67880 Innenheim, Alsace, France / NEU / 2012	Plum / Mirabelle	87-89	Fruit	n.d.	n.d.	21	08.11.2012

Table 6.3.1-34: Residue levels of glyphosate and AMPA in carrot after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S12-03071-03 / 41290 Oucques, Loir et Cher, France / NEU / 2012	Plum / Mirabelle	89	Fruit	n.d.	n.d.	21	08.11.2012
S12-03071-05 / 66130 Corbere, Pyrenees-Orientales, France / SEU / 2012	Peach / Maillardiva	81	Fruit	n.d.	n.d.	0	19.10.2012
		81-83	Fruit	n.d.	n.d.	7	
		87-89	Fruit	n.d.	n.d.	14	
		87-89	Fruit	n.d.	n.d.	20	
S12-03071-06 / 82400 Golfech, Tarn et Garonne, France / SEU / 2012	Peach / Brareg	89	Fruit	n.d.	n.d.	21	19.10.2012
S12-03071-07 / 66670 Bages, Pyrenees-Orientales, France / SEU / 2012	Apricot / Farbaly	87-89	Fruit	n.d.	n.d.	21	19.10.2012
S12-03071-08 / 82130 Lafrancaise, Tarn et Garonne, France / SEU / 2012	Plum / Mirabelle	87-89	Fruit	n.d.	n.d.	21	08.11.2012

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of stone fruit sampled at BBCH 81-89 (commercial maturity), 0-21 days after ground application of glyphosate in the tree row at the rate of 2.71-5.29 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application at a rate within +/-25 % of the critical GAP maximum seasonal application rate, except in one trial in which the application rate was overdosed by 84 % compared to the critical GAP maximum seasonal application rate. Since in all trials and all samples the residues of both glyphosate and AMPA were below the limit of quantification of 0.05 mg/kg, the study adequately supports the representative use for glyphosate in plantations of fruit trees (and especially stone fruit trees) in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/010
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 2 sites in Germany 2015
Report No	S15-00491
Document No	MSL0027503
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in grapes (bunch of grapes) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 2 field trials in the northern zone. The grape plantations were treated once. The application was directed to the soil under the vine grapes and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of bunches of grapes were taken for analysis at normal harvest, which

was 7 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00491-01	Grape	*Vitis vinifera*	Trollinger (red)	Bunches	≥ 1.7 kg / 12 units
S15-00491-02	Grape	*Vitis vinifera*	Kerner (white)	Bunches	≥ 1.2 kg / 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00491-01 and S15-00491-02):	Eurofins Agroscience Services GmbH, 71706 Markgröningen, Germany
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Two residue trials were conducted on grapes (outdoor) during the 2015 season in Germany (S15-00491-01 and S15-00491-02). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the vine grapes (7-30 plants per plot) at 7.5 L product/ha 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 319-320 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-35: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S15-00491-01	2	88-89 BBCH	3.840	320
S15-00491-02	2	87 BBCH	3.829	319

Regions, varieties and cultivation were typical for the cultivation of grapes.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of bunches were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 89), which was 7 days after application. Each field sample was taken from at least 4 plants from both sides of the rows and consists of at least 12 bunches. The first and last 1 m at the edges of the plots was not harvested. Separate samples were taken from the upper and lower bunch level. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 1.5 hours of sampling in the field).

Table 6.3.1-36: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00491-01	Grape	Bunches	7	89	≥ 1.7 kg / 12 units	20.10.2015
S15-00491-02	Grape	Bunches	7	89	≥ 1.2 kg / 12 units	15.10.2015

1 Separate samples were taken from the upper and lower bunch levels, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in bunches of grapes (EAS Chem study S14-05172). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 140 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of grape (bunches) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results were satisfactory, as shown in the table below.

Table 6.3.1-37: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Grape, bunches	Glyphosate	0.05	71	-	-	-	1
		0.5	71	-	-	-	1
		Overall	71	71	-	-	2
	AMPA	0.05	82	-	-	-	1
		0.5	83	-	-	-	1
		Overall	82-83	83	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of grape (bunches).

Detailed residue levels are shown in the table below.

Table 6.3.1-38: Residue levels of glyphosate and AMPA in grape after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00491-01 / 74382 Neckarwestheim, Baden-Württemberg, Germany / NEU /2015	Grape / Trollinger (red)	88-89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	03.03.2016
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S15-00491-02 / 71706 Markgröningen, Baden-Württemberg, Germany / NEU /2015	Grape / Kerner (white)	87	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	03.03.2016
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of grape (bunches) sampled at BBCH 89 (commercial maturity), 7 days after ground application of glyphosate in the vine row at the rate of 3.83-3.84 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application with an application rate of 3.8 kg a.s./ha. This application rate is 33 % higher than the critical GAP maximum seasonal application rate, which is acceptable since in all samples the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in grape plantations in Northern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/011
Report author	[redacted]
Report year	2015
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 4 sites in Northern France and 2 sites in Southern France 2014
Report No	S14-04157
Document No	MSL0027071
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in grapes (bunch of grapes) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents.

The study included 6 field trials (4 trials in the northern zone and 2 trials in the southern zone). The grape plantations were treated once. The application was directed to the soil under the vine grapes and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of bunches of grapes were taken for analysis at normal harvest, which was 7 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S14-04157-01	Grape	*Vitis vinifera*	Pinot noir (red)	Bunches	≥ 1 kg / 12 units
S14-04157-02	Grape	*Vitis vinifera*	Chardonnay (white)	Bunches	≥ 1 kg / > 12 units
S14-04157-03	Grape	*Vitis vinifera*	Cabernet Franc (red)	Bunches	≥ 1.6 kg / 12 units
S14-04157-04	Grape	*Vitis vinifera*	Chenin (white)	Bunches	≥ 1 kg / > 12 units
S14-04157-05	Grape	*Vitis vinifera*	Viognier (white)	Bunches	≥ 1 kg / 12 units
S14-04157-06	Grapes	*Vitis vinifera*	Cabernet (red)	Bunches	≥ 1 kg / 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S14-04157-01 and S14-04157-02):	Eurofins Agroscience Services SAS, 71700 Uchizy, France
Field phase (S14-04157-03 and S14-04157-04):	Eurofins Agroscience Services SAS, 49350 Gennes, France
Field phase (S14-04157-05 and S14-04157-06):	Eurofins Agroscience Services SAS, 66200 Elne, France
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Six residue trials were conducted on grapes (outdoor) during the 2014 season in Northern France (S14-04157-01, S14 04157-02, S14-04157-03, and S14-04157-04) and two trials in Southern France (S14-04157-05 and S14-04157-06). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the vine grapes (6-40 plants per plot) at 7.5 L product/ha 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 280-328 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-39: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S14-04157-01	2	85 BBCH	3.940	328
S14-04157-02	2	85 BBCH	3.789	316
S14-04157-03	2	89 BBCH	3.369	281
S14-04157-04	2	89 BBCH	3.405	284
S14-04157-05	2	89 BBCH	3.549	296
S14-04157-06	2	89 BBCH	3.380	280

Regions, varieties and cultivation were typical for the cultivation of grapes.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of bunches were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 89), which was 7-8 days after application. Each field sample was taken from at least 4 plants from both sides of the rows and consists of at least 12 bunches. The first and last 1 m at the edges of the plots was not harvested. Separate samples were taken from the upper and lower bunch level. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample (except in the trial S14-04157-05, in which no retain samples were taken). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.1-40: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S14-04157-01	Grape	Bunches	7	89	≥ 1 kg / 12 units	08.09.2014
S14-04157-02	Grape	Bunches	7	89	≥ 1 kg / > 12 units	08.09.2014
S14-04157-03	Grape	Bunches	7	89	≥ 1.6 kg / 12 units	07.10.2014
S14-04157-04	Grape	Bunches	7	89	≥ 1 kg / > 12 units	03.10.2014
S14-04157-05	Grape	Bunches	8	89	≥ 1 kg / 12 units	20.08.2014

Table 6.3.1-40: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S14-04157-06	Grape	Bunches	7	89	≥ 1 kg / 12 units	11.09.2014

1 Separate samples were taken from the upper and lower bunch levels, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in bunches of grapes (EAS Chem study S14-05172). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. In the trials S14-04157-05 and S14-04157-06 the temperature during sample shipment exceeded -18°C for 32 hours and 19 hours, respectively (max temperature of -13°C and -15°C, respectively). Since the temperature deviation was limited, shipment and the samples remained frozen, this does not impact the validity of the trial results. The maximum sample storage interval from harvest to extraction was 240 days, and the maximum interval from extraction to analysis was 2 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of grape (bunches) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results were satisfactory, as shown in the table below.

Table 6.3.1-41: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Grape, bunches	Glyphosate	0.05	84	-	-	-	1
		0.5	86, 84, 85	85	-	1.2	3
		Overall	84-86	85	-	1.1	4
	AMPA	0.05	91	-	-	-	1
		0.5	89, 89, 93	90	-	2.6	3
		Overall	89-93	91	-	2.1	4

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

H. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of grape (bunches).

Detailed residue levels are shown in the table below.

Table 6.3.1-42: Residue levels of glyphosate and AMPA in grape after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S14-04157-01 / 71150 Rully, Sanoe et Loire, France / NEU / 2014	Grape / Pinot noir (red)	85	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	18.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04157-02 / 71260 Viré, Saone et Loire, France / NEU / 2014	Grape / Chardonnay (white)	85	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	18.04.2015 – 19.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04157-03 / 49560 Passavant-sur-Layon, Maine et Loire, France / NEU / 2014	Grape / Cabernet Franc (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	18.04.2015 – 19.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04157-04 / 49320 Blaison-Gohier, Maine et Loire, France / NEU / 2014	Grape / Chenin (white)	89	Bunches / upper plant level	<0.05 n.d.	n.d. n.d.	7	18.04.2015 – 19.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04157-05 / 66470 Sainte-Marie de la Mer Pyreneés-Orientales, France / SEU / 2014	Grape / Viognier (white)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	8	18.04.2015 – 19.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04157-06 / 66200 Elne, Pyreneés-Orientales, France / SEU / 2014	Grape / Cabernet (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	18.04.2015 – 19.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of grape (bunches) sampled at BBCH 89 (commercial maturity), 7-8 days after ground application of glyphosate in the vine row at the rate of 3.37-3.94 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application with an application rate of 3.37-3.94 kg a.s./ha. This application rate is 17 % to 37 % higher than the critical GAP maximum seasonal application rate, which is acceptable since in all samples the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in grape plantations in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/012
Report author	[redacted]
Report year	2015
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 3 sites in Germany and 2 sites in Spain 2014
Report No	S14-04158
Document No	MSL0027070
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in grapes (bunch of grapes) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 5 field trials (3 trials in the northern zone and 2 trials in the southern zone). The grape plantations were treated once. The application was directed to the soil under the vine grapes and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of bunches of grapes were taken for analysis at normal harvest, which was 7 days after application. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S14-04158-01	Grape	*Vitis vinifera*	Portugieser (red)	Bunches	≥ 1 kg / 12 units
S14-04158-02	Grape	*Vitis vinifera*	Riesling (white)	Bunches	≥ 1 kg / 12 units
S14-04158-03	Grape	*Vitis vinifera*	Trollinger (red)	Bunches	≥ 2.5 kg / 12 units
S14-04158-05	Grape	*Vitis vinifera*	Cabernet Sauvignon (red)	Bunches	≥ 1.6 kg / 12 units
S14-04158-06	Grapes	*Vitis vinifera*	Garnacha (red)	Bunches	≥ 1 kg / 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S14-04158-01 and S14-04158-02):	Eurofins Agroscience Services GmbH, 69168 Wiesloch, Germany
Field phase (S14-04158-03):	Eurofins Agroscience Services GmbH, 71706 Markgröningen, Germany
Field phase (S14-04158-05 and S14-04158-06):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Five residue trials were conducted on grapes (outdoor) during the 2014 season in Germany (S14-04158-01, S14 04158-02, and S14-04158-03) and Spain (S14-04158-05 and S14-04158-06). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the vine grapes (6-12 plants per plot) at 7.5 L product/ha 7 days before harvest. In the trial S14-04158-03, however, the application was inadvertently overdosed at 22.6 L/ha (10.8 kg a.s./ha). The volume of water used to prepare the spray solution was in the range of 282-331 L/ha, except in the trial S14-04158-03, in which the water volume was 903 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-43: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S14-04158-01	2	87 BBCH	3.971	331
S14-04158-02	2	87 BBCH	3.940	328
S14-04158-03	2	85 BBCH	10.829	903
S14-04158-05	2	83-85 BBCH	3.772	314
S14-04158-06	2	87 BBCH	3.385	282

Regions, varieties and cultivation were typical for the cultivation of grapes.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of bunches were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 89), which was 7 days after application. Each field sample was taken from at least 4 plants from both sides of the rows and consists of at least 12 bunches. The first and last 1 m at the edges of the plots was not harvested. Separate samples were taken from the upper and lower bunch level. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 4.5 hours of sampling in the field).

Table 6.3.1-44: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S14-04158-01	Grape	Bunches	7	89	≥ 1 kg / 12 units	29.08.2014
S14-04158-02	Grape	Bunches	7	89	≥ 1 kg / 12 units	09.09.2014
S14-04158-03	Grape	Bunches	7	89	≥ 2.5 kg / 12 units	06.10.2014
S14-04158-05	Grape	Bunches	7	89	≥ 1.6 kg / 12 units	23.09.2014
S14-04158-06	Grape	Bunches	7	89	≥ 1 kg / 12 units	27.10.2014

Table 6.3.1-44: Crop sampling information

1 Separate samples were taken from the upper and lower bunch levels, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1% formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in bunches of grapes (EAS Chem study S14-05172). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. In the trials S14-04158-01 and S14-04158-02 the temperature during sample shipment exceeded -18°C for 15.5 hours (max temperature of -13°C). Since the temperature deviation was limited in time and the samples remained frozen, this does not impact the validity of the trial results. The maximum sample storage interval from harvest to extraction was 238 days, and the maximum interval from extraction to analysis was 2 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of grape (bunches) specimens, concurrent recoveries for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) were determined. The results were satisfactory, as shown in the table below.

Table 6.3.1-45: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Grape, bunches	Glyphosate	0.05	88	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-88	88	-	-	2
	AMPA	0.05	93	-	-	-	1
		0.5	96	-	-	-	1
		Overall	93-96	95	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of grape (bunches).

Detailed residue levels are shown in the table below.

Table 6.3.1-46: Residue levels of glyphosate and AMPA in grape after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S14-04158-01 / 75057 Kürnbach, Baden-Württemberg, Germany / NEU / 2014	Grape / Portugieser (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	25.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04158-02 / 67150 Niederkirchen, Rheinland-Pfalz, Germany / NEU / 2014	Grape / Riesling (white)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	25.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04158-03 / 71717 Beilstein, Baden-Württemberg, Germany / NEU / 2014	Grape / Trollinger (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	25.04.2015.- 26.04.2015
			Bunches lower plant level	< 0.05 < 0.05	n.d. n.d.		
S14-04158-05 / 50100 La Almunia de Dona Godina, Aragon, Spain / SEU / 2014	Grape / Cabernet Sauvignon (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	25.04.2015.- 26.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04158-06 / 50548 El Buste, Aragon, Spain / SEU / 2014	Grape / Garnacha (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	25.04.2015.- 26.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of grape (bunches) sampled at BBCH 89 (commercial maturity), 7 days after ground application of glyphosate in the vine row at the rate of 3.39-10.8 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application with an application rate of 3.39-10.8 kg a.s./ha. This application rate is 18 % to 276 % higher than the critical GAP maximum seasonal application rate, which is acceptable since in all samples the residues of both glyphosate and AMPA were below the limit of quantitation of 0.05 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in grape plantations in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/013
Report author	[redacted]
Report year	2015
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in vine grapes (outdoor) at 4 sites in Southern Europe 2014
Report No	S14-04226
Document No	MSL0027069
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in grapes (bunch of grapes) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials in the southern zone. The grape plantations were treated once. The application was directed to the soil under the vine grapes and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of bunches of grapes were taken for analysis at normal harvest, which was 7 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S14-04226-01	Grape	*Vitis vinifera*	Merlot (red)	Bunches	≥ 1 kg / 12 units
S14-04226-02	Grape	*Vitis vinifera*	Sauvignon blanc (white)	Bunches	≥ 1 kg / 12 units
S14-04226-03	Grape	*Vitis vinifera*	Garnacha (red)	Bunches	≥ 2 kg / 12 units
S14-04226-04	Grape	*Vitis vinifera*	Garnacha (red)	Bunches	≥ 2.3 kg / 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S14-04226-01 and S14-04226-01):	GAB Hellas, 57018 Melissohori, Greece
Field phase (S14-04226-03 and S14-04226-04):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on grapes (outdoor) during the 2014 season in Greece (S14-04226-01 and S14-04226-02) and Spain (S14-04226-03 and S14-04226-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the vine grapes (6-9 plants per plot) at 7.5 L product/ha 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 300-327 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-47: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S14-04226-01	2	85 BBCH	3.600	300
S14-04226-02	2	85 BBCH	3.643	304
S14-04226-03	2	87-89 BBCH	3.923	327
S14-04226-04	2	83-85 BBCH	3.857	321

Regions, varieties and cultivation were typical for the cultivation of grapes.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of bunches were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 89), which was 7 days after application. Each field sample was taken from at least 4 plants from both sides of the rows and consists of at least 12 bunches. The first and last 1 m at the edges of the plots was not harvested. Separate samples were taken from the upper and lower bunch level. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.1-48: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S14-04226-01	Grape	Bunches	7	89	≥ 1 kg / 12 units	28.08.2014
S14-04226-02	Grape	Bunches	7	89	≥ 1 kg / 12 units	28.08.2014
S14-04226-03	Grape	Bunches	7	89	≥ 2 kg / 12 units	10.09.2014
S14-04226-04	Grape	Bunches	7	87-89	≥ 2.3 kg / 12 units	02.10.2014

1 Separate samples were taken from the upper and lower bunch levels, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in bunches of grapes (EAS Chem study S14-05172). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. In the trial S14-04226-03 the temperature during sample shipment exceeded 18°C for twice 32 hours with max temperatures of -13°C and 15°C, respectively. Since the temperature deviation was limited to shipment and the samples remained frozen, this does not impact the validity of the trial results. The maximum sample storage interval from harvest to extraction was 229 days, and the maximum interval from extraction to analysis was 6 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of grape (bunches) specimens, concurrent recoveries for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) were determined. The results were satisfactory, as shown in the table below.

Table 6.3.1-49: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Grape, bunches	Glyphosate	0.05	93	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90-93	92	-	-	2
	AMPA	0.0496	93	-	-	-	1
		0.496	91	-	-	-	1
		Overall	91-93	92	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of grape (bunches).

Detailed residue levels are shown in the table below.

Table 6.3.1-50: Residue levels of glyphosate and AMPA in grape after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S14-04226-01 / 64008 Kariani, Kavala, Greece / SEU / 2014	Grape / Merlot (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	19.04.2015.-20.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04226-02 / 64008 Melissokomio, Kavala, Greece / SEU / 2014	Grape / Sauvignon blanc (white)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	19.04.2015.-20.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04226-03 / 50152 Mezalocha, Aragon, Spain / SEU / 2014	Grape / Garnacha (red)	89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	20.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		
S14-04226-04 / 50408 Aguaron, Aragon, Spain / SEU / 2014	Grape / Garnacha (red)	87-89	Bunches / upper plant level	n.d. n.d.	n.d. n.d.	7	20.04.2015
			Bunches / lower plant level	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of grape (bunches) sampled at BBCH 87-89 (commercial maturity), 7 days after ground application of glyphosate at the rate of 3.60-3.92 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application with an application rate of 3.60-3.92 kg a.s./ha. This application rate is 25 % to 36 % higher than the critical GAP maximum seasonal application rate, which is acceptable since in all samples the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in grape plantations in Southern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

Data point:	CA 6.3.1/014
Report author	Losseau, F.
Report year	1989
Report title	Glyphosate and AMPA residues in grapes following MON 8755 (Arcade) herbicide applications in vineyards. German field trials 1988
Report No	MLL 30227
Document No	Not available
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	Yes
Previous submission	RAR (2015)
Short description of study design and observations:	Six trials were conducted on grapes during the 1988 season in Germany. In all of the trials there were two applications of MON 8755 (180 g/L glyphosate) at the rate of 0.72 kg a.s./ha. Samples of grape bunches from the middle and lower parts of the vine as well as groundlying bunches were collected 0, 3 4, 8 10, 14 16, 20 21, and 65 days after the last treatment and analysed for glyphosate and AMPA. Residues of glyphosate and AMPA in grape samples were analysed by partition-extraction, ion exchange chromatography and determination by HPLC post column O-phthalaldehyde reaction system and quantification using internal standards. Percent recovery in grapes averaged at 75 % for glyphosate and 72 % for AMPA.
Short description of results:	Residues of glyphosate in grapes from the middle of the vine ranged from below the limit of detection (LOD) of 0.05 mg/kg to 0.2 mg/kg. Residues of glyphosate in grapes from low hanging bunches ranged from below the limit of detection (LOD) of 0.05 mg/kg to 0.3 mg/kg. Residues of glyphosate in grapes from groundlying bunches ranged from below the limit of detection (LOD) of 0.05 mg/kg to 0.4 mg/kg. No residues of AMPA above the limit of detection (LOD) of 0.05 mg/kg were found in the treated samples.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	• GLP assay and descriptive details of test material were not provided • First application date and crop variety for trial GGE 8904 cannot be determined from study report due to poor report legibility • Developmental scale / details of crop developmental stage was only provided for last sampling • Sample size / quantity, including number of plants sampled was not provided • The concurrent recoveries for glyphosate and AMPA were < 70 % (9 out of 28: 58 %-69 % and 12 out of 28; 50 %-69 %, respectively)
Category study in AIR 5 dossier (L docs)	Category 2b

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.1/015
Report author	[redacted]
Report year	1996
Report title	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with Roundup® herbicide. Spanish field trials, 1995
Report No	MLL 30469
Document No	95-GLY-20 Sp
Guidelines followed in study	OECD GLP FAO Guidelines
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 509: • Sample quantity and number of trees sampled were not provided
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in olive (fruit) and processed fraction olive oil after one application of Roundup, an SL formulation containing 360 g/L of glyphosate.

The study included 4 field trials in the southern zone. There was one treatment to the ground under the olive trees at a target rate of 2.16 kg glyphosate per hectare either 28, 14, or 7 days before commercial harvest. Olive samples were collected immediately after the application and at commercial harvest both from the tree and from the soil (ground fallen). No residues of glyphosate or AMPA above the limit of

detection (LOD) of 0.02 mg/kg were found in any olives from the tree harvested at or approaching commercial maturity. On the day of application, residues of glyphosate in ground fallen olives ranged from 4.2 to 12 mg/kg. Residues of glyphosate in ground fallen olives harvested 7 or 14 days after application ranged from 0.1 mg/kg to 0.9 mg/kg. Residues of glyphosate in ground fallen olives harvested 28 days after application were below the limit of quantification (LOQ) of 0.05 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 0, 7, 14, or 28 days after application.

I. Materials and Methods

A. Materials

1. Test material	
Description:	Roundup
Batch number:	010395
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	31.2 %
Formulation type:	SL
Appearance/colour:	Not provided
Certificate of analysis:	30.05.1995
Expiry date:	29.05.1996

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
AP/3065/ME/1	Olive	*Olea europaea*	Hojiblanca	Fruit	≥ 1 kg
AP/3065/ME/2	Olive	*Olea europaea*	Picual	Fruit	≥ 1 kg
AP/3065/ME/3	Olive	*Olea europaea*	Picual	Fruit	≥ 1 kg
AP/3065/ME/4	Olive	*Olea europaea*	Picual	Fruit	≥ 1 kg

Test facilities	
Study directory:	Monsanto Europe SA, 1348 Louvain-la-Neuve, Belgium
Field phase (AP/3065/ME/1, AP/3065/ME/2, AP/3065/ME/3, and AP/3065/ME/4):	Agrisearch UK, DE73 1AG Wilson, Derbyshire, England
Processing phase:	Viti R & D, 34400 Villetelle, France
Analytical phase:	Monsanto Technical Centre, 1348 Louvain-la-Neuve, Belgium

B. Methods

1. Field phase

Four residue trials were conducted on olives (outdoor) during the 1995 season in Spain (AP/3065/ME/1, AP/3065/ME/2, AP/3065/ME/3, and AP/3065/ME/4). One application of Roundup (360 g/L glyphosate) was performed onto the soil under the olive trees (6-10 plants per plot) at 6.0 L product/ha (2.16 kg a.s./ha) either 28, 14, or 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 381-440 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-51: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
AP/3065/ME/1	T3	7 days before harvest	2.141	396
	T2	14 days before harvest	2.188	405
	T1	28 days before harvest	2.147	398
AP/3065/ME/2	T3	7 days before harvest	2.341	433
	T2	14 days before harvest	2.374	440
	T1	28 days before harvest	2.160	400
AP/3065/ME/3	T3	7 days before harvest	2.281	422
	T2	14 days before harvest	2.143	397
	T1	28 days before harvest	2.056	381
AP/3065/ME/4	T3	7 days before harvest	2.279	422
	T2	14 days before harvest	2.132	395
	T1	28 days before harvest	2.151	398

Regions, varieties and cultivation were typical for the cultivation of olives.

Care was taken that the spray solution was properly homogenised by mixing before application. Ground spray applications were made via plot sprayer according to the label directions. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of olive were taken by hand from treated and untreated plots on the day of application (growth stage approaching maturity) and at 7, 14, and 28 days after treatment (commercial harvest). Specimens were taken from random points within each plot. No specimens were taken from plot edges or from the area of spray overlap. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use.

For residue analysis, separate specimens of olives were collected from the ground underneath the and directly from the tree, and duplicate specimens were taken as retain samples. Stones were removed from the olive samples used for residue analysis (replicate 1) within 24 hours of sampling and prior to freezing using a manual de-stoning tool.

Table 6.3.1-52: Crop sampling information

Trial	Crop	Commodity	Days before harvest[1]	Quantity	Date of sampling
AP/3065/ME/1	Olive	Fruit, from trees Fruit, from ground	28	≥ 1.0 kg ≥ 1.0 kg	07.11.1995
	Olive	Fruit, from trees Fruit, from ground	14	≥ 1.0 kg ≥ 1.0 kg	21.11.1995
	Olive	Fruit, from trees Fruit, from ground	7	Not recorded Not recorded	28.11.1995
	Olive	Fruit, from trees Fruit, from ground	NCH[1]	≥ 1.0 kg ≥ 1.0 kg	05.12.1995
AP/3065/ME/2	Olive	Fruit, from trees Fruit, from ground	28	≥ 1.0 kg ≥ 1.0 kg	09.11.1995

Table 6.3.1-52: Crop sampling information

Trial	Crop	Commodity	Days before harvest[1]	Quantity	Date of sampling
	Olive	Fruit, from trees Fruit, from ground	14	≥ 1.0 kg ≥ 1.0 kg	23.11.1995
	Olive	Fruit, from trees Fruit, from ground	7	≥ 1.0 kg ≥ 1.0 kg	01.12.1995
	Olive	Fruit, from trees Fruit, from ground	NCH	≥ 1.0 kg ≥ 1.0 kg	07.12.1995 (08.12.1995 – treatment 2)
AP/3065/ME/3	Olive	Fruit, from trees Fruit, from ground	28	≥ 1.0 kg ≥ 1.0 kg	06.11.1995
	Olive	Fruit, from trees Fruit, from ground	14	≥ 1.0 kg ≥ 1.0 kg	20.11.1995
	Olive	Fruit, from trees Fruit, from ground	7	≥ 1.0 kg ≥ 1.0 kg	27.11.1995
	Olive	Fruit, from trees Fruit, from ground	NCH	≥ 1.0 kg ≥ 1.0 kg	04.12.1995
AP/3065/ME/4	Olive	Fruit, from trees Fruit, from ground	28	≥ 1.0 kg ≥ 1.0 kg	08.11.1995
	Olive	Fruit, from trees Fruit, from ground	14	≥ 1.0 kg ≥ 1.0 kg	22.11.1995
	Olive	Fruit, from trees Fruit, from ground	7	≥ 1.0 kg ≥ 1.0 kg	29.11.1995
	Olive	Fruit, from trees Fruit, from ground	NCH	≥ 1.0 kg ≥ 1.0 kg	06.12.1995

1 Normal commercial harvest

3. Analytical phase

Residue analysis was conducted according to Monsanto method XA001. The residues of glyphosate and AMPA were extracted from the samples by water/dichloromethane partitioning/extraction followed by Chelex 100 resin isolation and anion exchange chromatographic clean-up. Quantification was based on a HPLC post column O-phthalaldehyde reaction system and comparison of peak area/height with known standards. The limit of quantitation (LOQ) for glyphosate and AMPA in olives (fruit) was 0.05 mg/kg with a limit of detection (LOD) of 0.02 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 207 days. Samples were stored frozen prior to analysis.

During analysis of olive (fruit) specimens, fortification experiments were performed with glyphosate and AMPA at fortification levels of 0.05, 0.1, 0.5, and 1.0 mg/kg, with additional fortifications at 10 and 20 mg/kg for glyphosate alone. The results are summarised in the table below.

Table 6.3.1-53: Recovery results

Matrix	Analyte	Fortification	Recovery[1]

		level (mg/kg)	Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Olives, fruit	Glyphosate	0.05	107, 110, 78, 63, 74	86	21	24	5
		0.1	105, 104, 100, 108, 109	105	3.6	3.4	5
		0.5	100, 98, 98, 99, 94	98	2.3	2.3	5
		1.0	105, 108, 98, 103, 104, 97	103	4.2	4.1	6
		10	79	-	-	-	1
		20	85	-	-	-	1
		Overall	63–110	97	12	13	23
	AMPA	0.05	80, 69, 90, 67, 68, 73, 74	74	8.2	11	7
		0.1	96, 74, 76, 78, 78, 64, 61, 67	74	11	15	8
		0.5	77, 80, 77, 80	79	1.7	2.2	4
		1.0	82	-	-	-	1
		Overall	61–96	76	8.4	11	20

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.02 mg/kg).
2 Mean and standard deviation values at each individual fortification level, as well as all relative standard deviation values, were calculated for this summary.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of Roundup when applied as per the study.

No residues of glyphosate or AMPA above the LOD of 0.02 mg/kg were found in any olives from the tree harvested at or approaching commercial maturity. On the day of application, residues of glyphosate in ground fallen olives ranged from 4.2 to 12 mg/kg. Residues of glyphosate in ground fallen olives harvested 7 or 14 days after application ranged from 0.1 mg/kg to 0.9 mg/kg. Residues of glyphosate in ground fallen olives harvested 28 days after application were below the LOQ of 0.05 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 0, 7, 14, or 28 days after application.
Detailed residue levels are shown in the table below.

Table 6.3.1-54: Residue levels of glyphosate and AMPA in olives after one application of Roundup (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Treat-ment	Commodity	Residue found[1,2] (mg/kg)		DALA[3] (days)	Date of analysis
				Glypho-sate	AMPA		
AP/3065/ME/1 / 29250 Cartaojal, Malaga, Spain / SEU / 1995	Olive / Hojiblanca	2	Fruit, from tree	n.d.	n.d.	0	April – May 1996
				n.d.	n.d.	7	
			Fruit from ground	9.15	n.d.	0	
				0.14	n.d.	7	
		3	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	14	
			Fruit from ground	12.5	<0.05	0	
				0.12	n.d.	14	
		4	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	28	
			Fruit from ground	9.81	<0.05	0	
				n.d.	n.d.	28	
AP/3065/ME/2 / 14100 La Carlota, Cordoba, Spain / SEU / 1995	Olive / Picual	2	Fruit, from tree	n.d.	n.d.	0	April – May 1996
				n.d.	n.d.	7	
			Fruit from ground	4.26	n.d.	0	
				0.11	n.d.	7	
		3	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	14	
			Fruit from ground	5.81	n.d.	0	
				0.11	n.d.	14	
		4	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	28	
			Fruit from ground	12.60	n.d.	0	
				n.d.	n.d.	28	
AP/3065/ME/3 / 14640 Villa del Rio, Cordoba, Spain / SEU / 1995	Olive / Picual	2	Fruit, from tree	n.d.	n.d.	0	April – May 1996
				n.d.	n.d.	7	
			Fruit from ground	5.89	n.d.	0	
				0.53	n.d.	7	
		3	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	14	
			Fruit from ground	11.8	0.05	0	
				0.13	n.d.	14	
		4	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	28	
			Fruit from ground	9.31	n.d.	0	
				<0.05	n.d.	28	

Table 6.3.1-54: Residue levels of glyphosate and AMPA in olives after one application of Roundup (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Treat-ment	Commodity	Residue found[1,2] (mg/kg)		DALA[3] (days)	Date of analysis
				Glypho-sate	AMPA		
AP/3065/ME/4 / 23400 Ubeda, Jaen, Spain / SEU / 1995	Olive / Picual	2	Fruit, from tree	n.d.	n.d.	0	April – May 1996
				n.d.	n.d.	7	
			Fruit from ground	6.05	<0.05	0	
				0.93	n.d.	7	
		3	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	14	
			Fruit from ground	6.75	n.d.	0	
				0.93	n.d.	14	
		4	Fruit, from tree	n.d.	n.d.	0	
				n.d.	n.d.	28	
			Fruit from ground	6.41	n.d.	0	
				<0.05	n.d.	28	

1 LOQ (limit of quantification): 0.05 mg/kg
2 n.d. (not detected): < 0.2mg/kg
3 Days after last application

III. Conclusion

No residues of glyphosate or AMPA above the LOD of 0.02 mg/kg were found in any olives from the tree harvested at or approaching commercial maturity. On the day of application, residues of glyphosate in ground fallen olives ranged from 4.2 to 12 mg/kg. Residues of glyphosate in ground fallen olives harvested 7 or 14 days after application ranged from 0.1 mg/kg to 0.9 mg/kg. Residues of glyphosate in ground fallen olives harvested 28 days after application were below the LOQ. No residues of AMPA above the LOQ were found in ground fallen olives harvested 0, 7, 14, or 28 days after application.
No residues of glyphosate or AMPA above the LOQ of 0.05 mg/kg were found in any of the untreated specimens.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. Even though the sample sizes and the number of sampled trees were not reported. In general, the sample sizes were above 1 kg per sample. Therefore, the study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative soil treatment between trees for glyphosate in orchards (and especially olives) in Southern Europe. However, with respect to the supported representative use in table olives, only the residue results measured in olives picked from the trees are considered relevant.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.1/016
Report author	[redacted]
Report year	1996
Report title	Glyphosate-trimesium: Residue levels in olives from trials carried out in Greece during 1995
Report No	RJ2217B
Document No	VV-381107
Guidelines followed in study	EEC Registration Directive 91/414/EEC Annex III
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, Crop Field Trial, 509: • Fewer than the guideline minimum number of olive trees were used per plot (1 large tree per treated plot vs. guideline indication of 4 trees) • Report is unclear if residue results are reported for whole fruit, including weight of stones, or if results are for flesh only • Report does not provide uncorrected residue values and does not clearly specify which recovery data were used for correction of each residue value (correction was used when mean recovery < 100 %)
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate-trimesium in olives (fruit) after one application of YF7712A, an SL formulation containing 480 g/L of glyphosate trimesium.

The study included 2 field trials in the southern zone. There was one treatment to the ground under the olive trees at a target rate of either 1.44 kg glyphosate-trimesium per hectare (0.99 kg/ha as glyphosate acid equivalents) or 4.8 kg glyphosate-trimesium per hectare (3.3 kg/ha as glyphosate acid equivalents). Actual application rates were within ± 5 % of the target rates; therefore, the study report listed the target / nominal rates as the application rates used in the study.

Samples of olives were collected separately from the ground under the olive trees or from the tree canopy during crop maturity, which was considered to span the sampling interval of 1 to 13 days after application. Residues of glyphosate-trimesium were determined as glyphosate (N-(phosphonomethyl)glycine or PMG). The metabolite AMPA was not measured. The study report indicates that stones were removed from the olive fruit during sample preparation, but weights of stones were not reported and there was no confirmation that reported residue values were calculated and

expressed based on the whole fruit, which would include the stones. Therefore, it is assumed that reported residue values are for the olive flesh only (not including the stones) rather than for the whole fruit.

No residues of glyphosate above the limit of quantitation (LOQ) of 0.05 mg/kg were found in the olive samples collected from the tree canopy, with the exception of a 1-day after application 4.8 kg/ha treated sample, in which residues were 0.17 and 0.32 mg/kg, respectively.

Low residues of glyphosate were determined in the olives collected from the ground. Across the two trials and two application rates at each trial site residues of glyphosate ranged from <0.05 to 0.39 mg/kg.

No residues of glyphosate above the LOQ (0.05 mg/kg) were found in untreated olive samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	YF7712A (SL formulation containing glyphosate-trimesium)
Batch number:	BDA2003
EAS test item code:	N/A
Active ingredient(s):	Glyphosate (in form of glyphosate-trimesium salt)
CAS number:	1071-83-6 (glyphosate); 81591-81-3 (glyphosate-trimesium)
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	37.99 % w/w (indicated as within ± 5 % of nominal)
Formulation type:	SL
Appearance/colour:	Not provided
Certificate of analysis:	Not provided
Expiry date:	Not provided. As indicated below, the study report stated the test material was used within 23 months of manufacture.

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
GR-95-H201	Olive	*Olea europaea*	Megaron	Fruit	≥ 1.0 kg
GR-95-H202	Olive	*Olea europaea*	Manaki	Fruit	≥ 1.0 kg

Test facilities	
Study directory:	Zeneca Agrochemicals, RG42 6ET Bracknell, Berkshire, UK
Field phase (GR-95-H201 and GR-95-H202):	Zeneca Hellas S.A., 171 21 N. Smyrni, Athens, Greece
Analytical phase:	Zeneca Agrochemicals, RG42 6ET Bracknell, Berkshire, UK

B. Methods

1. Field phase

Two residue trials were conducted on olives (outdoor) during the 1995 season in Greece (GR-95-H201 and GR-95-H202). One application of YF7712A (480 g/L glyphosate-trimesium) was performed to the soil under the olive trees (1 tree per plot) at either 3.0 L product/ha or 10.0 L product/ha at fruit maturity, and 1 to 13 days before samples were collected. The volume of water used to prepare the spray solution was in the range of 473-486 L/ha. Actual application rates were within ± 5 % of the target rates; therefore,

the study report listed the target / nominal rates as the application rates used in the study. The main application parameters are outlined in the table below.

Table 6.3.1-55: Application information

Trial no.	Plot number	Timing [1]	Application rate [2] kg a.s./ha	Water volume [3] L/ha
GR-95-H201	2	Mature fruit	1.44	473 - 486
GR-95-H201	3	Mature fruit	4.8	473 - 486
GR-95-H202	2	Mature fruit	1.44	473 - 486
GR-95-H202	3	Mature fruit	4.8	473 - 486

1 Study report indicated the growth stage was mature fruit at the time of application, although the growth stage was not expressed on BBCH or other growth scale. Samples were collected at 1 to 13 days after application. Therefore, application could be considered as being targeted at 1 to 13 days prior to harvest maturity.
2 Application rates were reported for glyphosate-trimesium. The application rates for glyphosate-trimesium at 1.44 and 4.8 kg as/ha expressed as glyphosate equivalents were 0.99 and 3.3 kg as/ha, respectively. The rates expressed as glyphosate equivalents were obtained by adjusting the indicated rates of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively.
3 The overall range of water volume used in the two treated plots in the two trials included in the study was reported, but the water volume was not reported by individual trial. Therefore, the overall range is listed for each plot / trial since these values bracket the actual volume used on an individual plot.

Regions, varieties and cultivation were typical for the cultivation of olives.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with a CO_2-pressurised knapsack sprayer equipped with a spray boom and flat fan nozzles, which were calibrated before use. Prior to application, the picking areas were cleared of olives.

2. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at olive fruit maturity, which spanned the 1 to13-day sampling interval following application. Each field sample was taken from 1 large tree, which was considered sufficiently large to provide a representative sample. Samples were collected randomly and included samples collected separately from the ground and from the tree canopy. Approximately 1 kg of olive fruit was collected for each field sample. The untreated plots were sampled first followed by the treated plots. All samples were bagged and labelled in the field immediately after sampling. All samples were placed in coolboxes with dry ice immediately after sampling and were transported to the freezer at the field test facility where they were maintained frozen at < -18°C. Samples were shipped to the analytical laboratory by air and were packed in dry ice to maintain frozen condition during shipment.

Table 6.3.1-56: Crop sampling information

Trial	Crop	Commodity	DALA[2]	Growth stage (BBCH)[3]	Quantity	Date of sampling
GR-95-H201	Olive	Fruit[1]	1	Mature fruit	≥ 1.0 kg	06.12.1995
			7	Mature fruit	≥ 1.0 kg	12.12.1995
			13	Mature fruit	≥ 1.0 kg	18.12.1995
GR-95-H202	Olive	Fruit[1]	1	Mature fruit	≥ 1.0 kg	06.12.1995
			7	Mature fruit	≥ 1.0 kg	12.12.1995
			13	Mature fruit	≥ 1.0 kg	18.12.1995

Table 6.3.1-56: Crop sampling information

Trial	Crop	Commodity	DALA[2]	Growth stage (BBCH)[3]	Quantity	Date of sampling

1 Separate samples were taken from the ground and from the tree.
2 Days after last application
3 The study report indicated that the olive fruit was mature at the time of sampling in each of the three sampling intervals in both trials. However, a growth stage based on BBCH or other scale was not provided.

3. Analytical phase

Each sample was prepared by removing stones and then grinding in a tecator homogeniser until a completely homogeneous sample was obtained.

Olives samples were analysed for residues of glyphosate (derived from glyphosate-trimesium) using method RR92-042B RES with modified clean up column elution conditions. The reference material used was N-(phosphonomethyl)glycine (purity 99.6 % w/w). Glyphosate was extracted from olives by maceration with water. The extracts were then cleaned up using a cation exchange resin column. The glyphosate-containing fraction was then derivatised with heptafluorobutanol and trifluoroacetic anhydride. The glyphosate derivative was analysed by gas chromatography with mass selective detection (GC-MSD). The LOQ of this method for olives was 0.05 mg/kg. The residues of the metabolite AMPA were not measured.

Treated and untreated specimens were maintained deep frozen during storage and shipment. The maximum sample storage interval from harvest to analysis was 9 months. Samples were stored frozen at ≤ -18 °C at the analytical facility prior to analysis.

During analysis of olive (fruit) specimens, concurrent recoveries were determined for glyphosate at fortification levels of 0.05 mg/kg (LOQ), and at 0.10, 0.20, 0.25, and 0.40 mg/kg. The recovery results are summarised in the table below.

Table 6.3.1-57: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Olive, fruit	Glyphosate	0.05	75, 68, 79	74.0	5.6	7.5	3
		0.10	87, 91	89.0	-	-	2
		0.20	71	-	-	-	1
		0.25	80	-	-	-	1
		0.40	65	-	-	-	1
		Overall	65-91	77.0	9.1	11.8	8

1 Means, standard deviations, and relative standard deviations were not included in the study report. These values were calculated during dossier assembly using the recovery data reported in the study.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow evaluation of the residue behaviour of glyphosate (derived from glyphosate-trimesium) after usage of YF7712A when applied as per the study.

No residues of glyphosate above the LOQ of 0.05 mg/kg were found in the olive samples collected from the tree canopy, with the exception of a 1-day after application of glyphosate-trimesium at 4.8 kg as/ha treated sample, in which residues were 0.17 and 0.32 mg/kg, respectively.

Low levels of glyphosate residues from <0.05 to 0.39 mg/kg were determined in the olives taken from the ground.

No residues of glyphosate above the LOQ (0.05 mg/kg) were found in the untreated olive samples.

The study report indicated that stones were removed from the olive fruit during sample preparation, but weights of stones were not reported and there was no confirmation that reported residue values were calculated and expressed based on the whole fruit, which would include the stones. Therefore, it is assumed that reported residue values are for the olive flesh only (not including the stones) rather than for the whole fruit.

The study report indicated that reported residue values had been corrected for recovery where mean recovery was <100 %. The report did not include the uncorrected values and did not specify which recovery results were used for correction of specific samples. Therefore, the residue results included in this summary are as provided in the study report and were corrected for recovery.

Detailed residue levels are shown in the table below.

Table 6.3.1-58: Residue levels of glyphosate in olives after one application of YF7712A (480 g/L glyphosate-trimesium)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Application rate[2] (kg as/ha)	Commodity	Residue found[3] (mg/kg)	DALA[4] (days)	Date of analysis
					Glypho-sate		
GR-95-H201 / 32011 Enofyta, Biotia, Greece / SEU / 1995	Olive / Megaron	Mature fruit; BBCH or other growth stage scale was not provided in study report	1.44	Fruit collected from ground	0.21	1	June – September, 1996
					<0.05	7	
					<0.05	13	
				Fruit taken from tree	<0.05	1	
					<0.05	7	
					<0.05	13	
			4.8	Fruit collected from ground	0.23	1	
					0.39	7	
					<0.05	13	
				Fruit taken from tree	0.17	1	
					<0.05	7	
					<0.05	13	
GR-95-H202 / 32011 Enofyta, Biotia, Greece / SEU / 1995	Olive / Manaki	Mature fruit; BBCH or other growth stage scale was not provided in study report	1.44	Fruit collected from ground	<0.05	1	June – September, 1996
					<0.05	7	
					<0.05	13	
				Fruit taken from tree	<0.05	1	
					<0.05	7	
					<0.05	13	
			4.8	Fruit collected from ground	0.17	1	
					0.14	7	

Table 6.3.1-58: Residue levels of glyphosate in olives after one application of YF7712A (480 g/L glyphosate-trimesium)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Application rate[2] (kg as/ha)	Commodity	Residue found[3] (mg/kg)	DALA[4] (days)	Date of analysis
					Glypho-sate		
					<0.05	13	
				Fruit taken from tree	<0.05	1	
					<0.05	7	
					<0.05	13	

1 Growth stage at harvest. Study report indicated "Maturity" as the growth stage for fruit at each sampling interval in both trials.
2 Application rates were reported for glyphosate-trimesium. The application rates for glyphosate-trimesium at 1.44 and 4.8 kg as/ha expressed as glyphosate equivalents were 0.99 and 3.3 kg as/ha, respectively. The rates expressed as glyphosate equivalents were obtained by adjusting the indicated rates of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively.
3 LOQ (limit of quantification): 0.05 mg/kg
4 Days after last application

III. Conclusion

Two residue trials were carried out on olives during 1995 in Greece. One application of glyphosate-trimesium was made at a rate of either 1.44 or 4.8 kg/ha, to the ground. The application rates for glyphosate-trimesium at 1.44 and 4.8 kg as/ha expressed as glyphosate equivalents were 0.99 and 3.3 kg as/ha, respectively.

Samples of olives were taken for analysis from the tree canopy and from the ground, at intervals of 1, 7 and 13 days after application. Glyphosate-trimesium residues are determined as N-(phosphonomethyl) glycine anion (PMG).
No residues of glyphosate above the LOQ of 0.05 mg/kg were found in the olive samples collected from the tree canopy, with the exception of a 1-day after application of glyphosate-trimesium at 4.8 kg as/ha treated sample, in which residues were 0.17 mg/kg.
Low levels of glyphosate residues from <0.05 to 0.39 mg/kg were determined in the olives taken from the ground.
No residues of glyphosate above the LOQ (0.05 mg/kg) were found in the untreated olive samples.
The residues of the metabolite AMPA were not measured.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was performed under GLP. The trials were conducted with one application with an application rate of either 1.44 kg glyphosate-trimesium/ ha or 4.8 kg glyphosate-trimesium/ ha (rates expressed in glyphosate equivalents are 0.99 or 3.3 kg a.s./ha, respectively). The lower of the two application rates is compliant with the maximum single application rate in the GAP, although the GAP allows for up to a total of 3 applications in 28-day intervals and a seasonal total of 2.88 kg a.s./ha. However, the higher of the two application rates, 4.8 kg glyphosate-trimesium/ ha (expressed as glyphosate equivalents: 3.3 kg a.s./ha) showed that glyphosate residues in fruit collected from the tree (not from the ground), remained below the LOQ (<0.05 mg/kg) even when ~117 % of the seasonal maximum rate is applied in a single application. Therefore, the study may provide results useful for supporting representative inter row use for glyphosate in orchards (and especially olives) in Southern Europe.

There were deviations from the current guideline, OECD Guideline for the Testing of Chemicals, 509, identified in the study (number of trees per plot was below the required number; unclear if residue results are reported for whole fruit, including stones, or if results are for flesh only (guideline indicates results should be expressed for the whole fruit); rather than residue results uncorrected for recovery, the report lists only values that were corrected for recovery if mean recovery <100 %). These deviations are deviations with the study relative to current guideline requirements. However, in the case of required minimum number of trees being used, the single tree used per plot was described as being large and capable of providing fully adequate representative sampling. The last two of the three deviations listed, if impacting results, would cause reported residue values to be larger. Therefore, for residue results reported as being <LOQ (<0.05 mg/kg), these deviations may result in a more conservative evaluation, but do not cause potential for the values reported as <0.05 mg/kg actually being higher than reported. Therefore, the treatments with residues <0.05 mg/kg may provide useful results despite the guideline deviations. However, since the metabolite AMPA was not measured, the study is considered at best supportive.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.1/017
Report author	[redacted]
Report year	1996
Report title	Glyphosate-trimesium: Residue levels in olives from trials carried out in Italy during 1995
Report No	RJ2218B
Document No	VV-381105
Guidelines followed in study	EEC Registration Directive 91/414/EEC Annex III
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, Crop Field Trial, 509: • Report is unclear if residue results are reported for whole fruit, including weight of stones, or if results are for flesh only • Report does not provide uncorrected residue values and does not clearly specify which recovery data were used for correction of each residue value (correction was used only when mean recovery <100 %)

Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate-trimesium in olives (fruit) after one application of YF7712A, an SL formulation containing 480 g/L of glyphosate trimesium.

The study included 2 field trials in the southern zone. There was one application to the soil under the olive trees at a target rate of either 1.44 kg glyphosate-trimesium per hectare (0.99 kg/ha as glyphosate acid equivalents) or 4.8 kg glyphosate-trimesium per hectare (3.3 kg/ha as glyphosate acid equivalents). Actual application rates were within ± 5 % of the target rates; therefore, the study report listed only the target / nominal rates as the application rates used in the study.

Samples of olives were collected separately from the ground under the olive trees or from the tree canopy during fruit ripening suitable for harvest, which was considered to span the sampling interval of 1 to 13 14 days after application. Residues of glyphosate-trimesium were determined as glyphosate (N (phosphonomethyl)glycine or PMG). The metabolite AMPA was not measured. The study report indicates that stones were removed from the olive fruit during sample preparation, but weights of stones or flesh were not included in the report and there was no confirmation that reported residue values were calculated and expressed based on the whole fruit, which would include the stones. Therefore, it is assumed that reported residue values are for the olive flesh only (not including the stones) rather than for the whole fruit.

No residues of glyphosate above the limit of quantitation (LOQ) of 0.05 mg/kg were found in the olive samples collected from the tree canopy, with the exception of a 13-day after application 1.44 kg/ha treated sample, in which residues of glyphosate were found at 0.06 mg/kg.

Low levels of glyphosate residues were determined in the olives collected from the ground. Across the two trials and two application rates at each trial site with sample collection at 1 to 13-14 days after application, residues of glyphosate ranged from <0.05 to 0.66 mg/kg.

No residues of glyphosate above the LOQ (0.05 mg/kg) were found in untreated olive samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	YF7712A (SL formulation containing glyphosate-trimesium)
Batch number:	BDA2003
EAS test item code:	N/A
Active ingredient(s):	Glyphosate (in form of glyphosate-trimesium salt)
CAS number:	1071-83-6 (glyphosate); 81591-81-3 (glyphosate-trimesium)
Content of a.s. nominal:	480 g/L

Content of a.s. analysed:	40.59 % w/w (indicated as within ± 5 % of nominal)
Formulation type:	SL
Appearance/colour:	Not provided
Certificate of analysis:	Not provided
Expiry date:	Not provided. As indicated below, the study report stated the test material was used within 22 months of manufacture.

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
IT10-95-H343	Olive	*Olea europaea*	Frantoio	Fruit	≥ 1.0 kg
IT10-95-H344	Olive	*Olea europaea*	Coratina	Fruit	≥ 1.0 kg

Test facilities	
Study directory:	Zeneca Agrochemicals, RG42 6ET Bracknell, Berkshire, UK
Field phase (IT10-95-H343 and IT10-95-H344):	Solplant S.p.A., 20122, Milan, Italy
Analytical phase:	Zeneca Agrochemicals, RG42 6ET Bracknell, Berkshire, UK

B. Methods

1. Field phase

Two residue trials were conducted on olives (outdoor) during the 1995 season in Italy (IT10-95-H343 and IT10-95-H344). One application of YF7712A (480 g/L glyphosate-trimesium) was performed to the soil under the olive trees (4 – 5 trees per plot) at either 3.0 L product/ha or 10.0 L product/ha at fruit ripening, and 1 to 13–14 days before samples were collected. The volume of water used to prepare the spray solution was in the range of 200-300 L/ha. Actual application rates were within ± 5 % of the target rates; therefore, the study report listed the target / nominal rates as the application rates used in the study. The main application parameters are outlined in the table below.

Table 6.3.1-59: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
IT10-95-H343	2	Beginning of ripening - ripening	1.44	200 - 300
IT10-95-H343	3	Beginning of ripening - ripening	4.8	200 - 300
IT10-95-H344	2	Commercial ripening	1.44	200 - 300
IT10-95-H344	3	Commercial ripening	4.8	200 - 300

1 Study report indicated the growth stage was beginning of ripening – ripening, or commercial ripening of fruit at the time of application, although the growth stage was not expressed on BBCH or other growth scale. Samples were collected at 1 to 13-14 days after application. Therefore, application could be considered as being targeted at 1 to 13-14 days prior to harvest maturity.

2 Application rates were reported for glyphosate-trimesium. The application rates for glyphosate-trimesium at 1.44 and 4.8 kg as/ha expressed as glyphosate equivalents were 0.99 and 3.3 kg as/ha, respectively. The rates expressed as glyphosate equivalents were obtained by adjusting the indicated rates of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively

3 The overall range of water volume used in the two treated plots in the two trials included in the study was reported, but the water volume was not reported by individual trial. Therefore, the overall range is listed for each plot / trial since these values bracket the actual volume used on an individual plot.

Regions, varieties and cultivation were typical for the cultivation of olives.

Care was taken that the spray solution was properly homogenised by mixing before application, and all applications were made within one hour of preparation. Applications were performed with a motorised knapsack sprayer equipped with a spray boom and flat fan nozzles, which were calibrated before use. Prior to application, the picking areas were cleared of olives.

2. Sampling

Specimens of crop from the untreated and treated plots were collected at olive fruit maturity (ripening fruit), which spanned the 1 to13-14 day sampling interval following application. Field samples were taken from 4-5 trees per plot. Samples were collected from the trees with use of a plastic comb with the fruit being caught in an upside down umbrella or on a plastic sheet. Olives from the ground were collected by hand. Approximately 1 kg of olive fruit was collected for each field sample. The untreated plots were sampled first followed by the treated plots. All samples were bagged and labelled in the field immediately after sampling.

All samples were frozen within 4 hours of collection and were store frozen at the field trial facility at ≤ -18°C until shipment to the analytical laboratory. Samples were transported frozen to the analytical laboratory where they were received in frozen condition.

Table 6.3.1-60: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)[3]	Quantity	Date of sampling
IT10-95-H343	Olive	Fruit	1	Ripening fruit, suitable for harvest	≥ 1.0 kg	10.11.1995
IT10-95-H343	Olive	Fruit	7	Ripening fruit, suitable for harvest	≥ 1.0 kg	16.11.1995
IT10-95-H343	Olive	Fruit	14	Ripening fruit, suitable for harvest	≥ 1.0 kg	23.11.1995
IT10-95-H344	Olive	Fruit	1	Ripening fruit, suitable for harvest	≥ 1.0 kg	08.11.1995
IT10-95-H344	Olive	Fruit	6	Ripening fruit, suitable for harvest	≥ 1.0 kg	13.11.1995
IT10-95-H344	Olive	Fruit	13	Ripening fruit, suitable for harvest	≥ 1.0 kg	20.11.1995

1 Separate samples were taken from the ground and from the tree.
2 Days after last application
3 The study report indicated that the olive fruit was mature at the time of sampling in each of the three sampling intervals in both trials. However, a growth stage based on BBCH or other scale was not provided.

3. Analytical phase

Each sample was prepared by removing stones and then grinding in a tecator homogeniser until a completely homogeneous sample was obtained.

Olives samples were analysed for residues of glyphosate (derived from glyphosate-trimesium) using method RR92-042B RES with modified clean up column elution conditions. The reference material used was N-(phosphonomethyl)glycine (purity 99.6 % w/w). Glyphosate was extracted from olives by maceration with water. The extracts were partitioned with chloroform and then cleaned up using a cation exchange resin column. The glyphosate -containing fraction was then derivatised with heptafluorobutanol and trifluoroacetic anhydride. The glyphosate derivative was analysed by gas chromatography with mass selective detection (GC-MSD). The LOQ of this method for olives was 0.05 mg/kg. Additionally, further experiments were conducted to ascertain suitable solvent alternatives to the chloroform used for

partitioning in the method. Results obtained indicated that dichloromethane and toluene are acceptable alternative solvents. The residues of the metabolite AMPA were not measured.

Treated and untreated specimens were maintained deep frozen during storage and shipment. The maximum sample storage interval from harvest to analysis was 11 months . Samples were stored frozen at ≤ -18 °C at the analytical facility prior to analysis.

During analysis of olive (fruit) specimens, concurrent recoveries were determined for glyphosate at fortification levels of 0.05 mg/kg (LOQ), and at 0.5 mg/kg. The recovery results are summarised in the table below.

Table 6.3.1-61: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Olive, fruit	Glyphosate	0.05	99, 79, 109, 101, 93, 76	92.8	13.0	14.0	6
		0.5	77, 77	77	-	-	2
		Overall	76 - 109	88.9	13.2	14.8	8

1 Means, standard deviations, and relative standard deviations were not included in the study report. These values were calculated during dossier assembly using the recovery data reported in the study.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow evaluation of the residue behaviour of glyphosate (derived from glyphosate-trimesium) after usage of YF7712A when applied as per the study.

No residues of glyphosate above the LOQ of 0.05 mg/kg were found in the olive samples collected from the tree canopy, with the exception of a 13-day after application of glyphosate-trimesium at 1.44 kg as/ha treated sample, in which glyphosate was found at 0.06 mg/kg.

Low levels of glyphosate residues were found in the olives collected from the ground. Across the two trials and two application rates at each trial site with sample collection at 1 to 13-14 days after application, residues of glyphosate ranged from <0.05 to 0.66 mg/kg.

No residues of glyphosate above the LOQ (0.05 mg/kg) were found in the untreated olive samples.

The study report indicated that stones were removed from the olive fruit during sample preparation, but weights of stones were not reported and there was no confirmation that reported residue values were calculated and expressed based on the whole fruit, which would include the stones. Therefore, it is assumed that reported residue values are for the olive flesh only (not including the stones) rather than for the whole fruit.

The study report indicated that reported residue values had been corrected for recovery where mean recovery was <100 %. The report did not include the uncorrected values and did not specify which recovery results were used for correction of specific samples. Therefore, the residue results included in this summary are as provided in the study report and were corrected for recovery.

Detailed residue levels are shown in the table below.

Table 6.3.1-62: Residue levels of glyphosate in olives after one application of YF7712A (480 g/L glyphosate-trimesium)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Application rate[2] (kg as/ha)	Commodity	Residue found[3] (mg/kg) Glypho-sate	DALA[4] (days)	Date of analysis
IT10-95-H343 / Cori, Latina, Italy / SEU / 1995	Olive / Frantoio	Ripening fruit, suitable for harvest; BBCH or other growth stage scale was not provided in study report	1.44	Fruit collected from ground	<0.05	1	July - October, 1996
					0.07	7	
					0.05	14	
				Fruit taken from tree	<0.05	1	
					<0.05	7	
					<0.05	14	
			4.8	Fruit collected from ground	0.66	1	
					0.27	7	
					0.19	14	
				Fruit taken from tree	<0.05	1	
					<0.05	7	
					<0.05	14	
IT10-95-H344 / 71037 Borgo Mezzanone, Foggia, Italy / SEU / 1995	Olive / Coratina	Ripening fruit, suitable for harvest; BBCH or other growth stage scale was not provided in study report	1.44	Fruit collected from ground	0.05	1	July - October, 1996
					0.16	6	
					<0.05	13	
				Fruit taken from tree	<0.05	1	
					<0.05	6	
					0.06	13	
			4.8	Fruit collected from ground	0.10	1	
					0.12	6	
					<0.05	13	
				Fruit taken from tree	<0.05	1	
					<0.05	6	
					<0.05	13	

1 Growth stage at harvest. Study report indicated "Ripening fruit" as the growth stage for fruit at each sampling interval.
2 Application rates were reported for glyphosate-trimesium. The application rates for glyphosate-trimesium at 1.44 and 4.8 kg as/ha expressed as glyphosate equivalents were 0.99 and 3.3 kg as/ha, respectively. The rates expressed as glyphosate equivalents were obtained by adjusting the indicated rates of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively.
3 LOQ (limit of quantification): 0.05 mg/kg
4 Days after last application

III. Conclusion

Two residue trials were carried out on olives during 1995 in Italy. One application of glyphosate-trimesium was made at a rate of either 1.44 or 4.8 kg/ha, to the ground. The application rates for glyphosate-trimesium at 1.44 and 4.8 kg as/ha expressed as glyphosate equivalents were 0.99 and 3.3 kg a.s./ha, respectively.

Samples of olives were taken for analysis from the tree canopy and from the ground, at intervals of 1, 6-7 and 13-14 days after application. Glyphosate-trimesium residues were determined as glyphosate.

No residues of glyphosate above the LOQ of 0.05 mg/kg were found in the olive samples collected from the tree canopy, with the exception of a 13-day after application of glyphosate-trimesium at 1.44 kg as/ha treated sample, in which glyphosate was found at 0.06 mg/kg.

Low levels of glyphosate residues were found in the olives collected from the ground. Across the two trials and two application rates at each trial site with sample collection at 1 to 13-14 days after application, residues of glyphosate ranged from <0.05 to 0.66 mg/kg.

No residues of glyphosate above the LOQ (0.05 mg/kg) were found in the untreated olive samples. The residues of the metabolite AMPA were not measured.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was performed under GLP. The trials were conducted with one application with an application rate of either 1.44 kg glyphosate-trimesium/ ha or 4.8 kg glyphosate-trimesium/ ha (rates expressed in glyphosate equivalents are 0.99 or 3.3 kg a.s./ha, respectively). The lower of the two application rates is compliant with the maximum single application rate in the GAP, although the GAP allows for up to a total of 3 applications in 28-day intervals and a seasonal total of 2.88 kg a.s./ha. However, the higher of the two application rates, 4.8 kg glyphosate-trimesium/ ha (expressed as glyphosate equivalents: 3.3 kg a.s./ha), showed that glyphosate residues in fruit collected from the tree (not from the ground), remained below the LOQ (<0.05 mg/kg) even when ~117 % of the seasonal maximum rate is applied in a single application. Therefore, the study may provide results useful for supporting representative inter row use for glyphosate in orchards (and especially olives) in Southern Europe.

There were deviations from the current guideline, OECD Guideline for the Testing of Chemicals, 509, identified in the study (unclear if residue results are reported for whole fruit, including stones, or if results are for flesh only (guideline indicates results should be expressed for the whole fruit); rather than residue results uncorrected for recovery, the report lists only values that were corrected for recovery if mean recovery <100 %). These deviations are deviations with the study relative to current guideline requirements. However, the deviations listed, if impacting results, would cause reported residue values to be larger. Therefore, for residue results reported as being <LOQ (<0.05 mg/kg), these deviations may result in a more conservative evaluation, but do not cause potential for the values reported as <0.05 mg/kg actually being higher than reported. Therefore, the treatments with residues <0.05 mg/kg may provide useful results despite the guideline deviations. However, since the metabolite AMPA was not measured, the study is considered at best supportive.

Assessment and conclusion by RMS:

Study previouslysubmitted to the EU

1. Information on the study

Data point:	CA 6.3.1/018
Report author	Sanderson, D.J., Austin, D.J.
Report year	1991
Report title	Phosphonomethylglycine: Residues in olive from ICIA0224 trials carried out in Italy during 1988
Report No	M5353B
Document No	VV-323340
Guidelines followed in study	None specified

Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, Crop Field Trial, 509: • GLP assay of the test material was not provided • Information on sampling method for fruit, husks, or oil was not provided. • Sample quantity (fruit, husks, and oil) was not provided • Report is unclear if residue results are reported for whole fruit, including weight of stones, or if results are for flesh only Report does not provide uncorrected residue values, although the recovery values used for correction were provided.
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 3a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate (PMG) in olives fruit, derived from glyphosate-trimesium, after one application of YF7712, an SL formulation containing 480 g/L of glyphosate-trimesium (referred to as ICIA0224 in the report text).

The study included one field trial in the southern zone (Italy). There was one application to the soil under the trees at either 0.73, 2.9, or 5.9 kg glyphosate-trimesium/ha (0.51, 2.0, or 4.1 kg a.s./ha, expressed as glyphosate equivalents, respectively). Samples of olive fruit were collected at 1 and 7 days after application. Samples of olive fruit were analysed for residues of glyphosate using an analytical method with a limit of quantitation (LOQ) of 0.05 mg/kg. The metabolite AMPA was not measured.

No residues of glyphosate above the LOQ of 0.05 mg/kg were found in olive fruit collected from plots treated with YF7712 at 0.73 or 2.5 kg a.s./ha at either 1 or 7 days after application. Olive fruit collected from the plot treated with YF7712 at 5.9 kg a.s./ha was found to have glyphosate residues <0.05 mg/kg at 1 day after application, but at 7 days after application glyphosate residues were found at 0.06 mg/kg.

No residues of glyphosate above the LOQ (0.05 mg/kg) were found in untreated olive matrices.

I. Materials and Methods

A. Materials

1. Test material	
Description:	YF7712 (SL formulation containing glyphosate-trimesium)
Batch number:	Not provided
EAS test item code:	ICIA0224
Active ingredient(s):	Glyphosate (in form of glyphosate-trimesium salt)
CAS number:	1071-83-6 (glyphosate); 81591-81-3 (glyphosate-trimesium)
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	Not provided
Formulation type:	SL

Appearance/colour:	Not provided
Certificate of analysis:	Not provided
Expiry date:	Not provided

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
IT1089E001	Olive	*Olea europaea*	Perananzana	Fruit	Not specified

Test facilities	
Study directory:	ICI Agrochemicals, RG12 6EY Bracknell, Berks, UK
Field phase (IT1089E001):	ICI Solplant S.p.A., Italy
Analytical phase:	ICI Agrochemicals, RG12 6EY Bracknell, Berks, UK

B. Methods

1. Field phase

One residue trial was conducted on olives (outdoor) during the 1988 season in Italy (IT1089E001). One application of YF7712A (480 g/L glyphosate-trimesium) was performed to the soil under the olive trees (6 trees per plot) at either 1.53, 6.13, or 12.25 L product/ha (0.73, 2.9, or 5.9 kg glyphosate-trimesium/ha) at 1 to 7 days before samples were collected. The main application parameters are outlined in the table below.

Table 6.3.1-63: Application information

Trial no.	Plot	Timing [1]	Application rate [2] kg a.s./ha	Water volume L/ha
IT1089E001	1	Fruit ripening	0.734	200
	2	Fruit ripening	2.941	200
	3	Fruit ripening	5.882	200

1 Study report indicated the growth stage was fruit ripening, although the growth stage was not expressed on BBCH or other growth scale. Samples were collected at 1 or 7 days after application.

2 Application rates were reported for glyphosate-trimesium. The application rates for glyphosate-trimesium at 0.734, 2.941, and 5.882 a.s./ha expressed as glyphosate equivalents were 0.51, 2.03, and 4.06 kg a.s./ha, respectively. The rates expressed as glyphosate equivalents were obtained by adjusting the indicated rates of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively

Regions, varieties and cultivation were typical for the cultivation of olives. The spray solution was prepared and applied in a volume of 200 L/ha using a motorised sprayer with a spray boom equipped with 3 flat-fan spray nozzles.

2. Sampling

Specimens of olive fruit from the untreated and treated plots were collected at olive fruit maturity (ripening fruit) at either 1 or 7 days after treatment application. The field trial included 6 trees per plot, but details on sample collection method or quantity of olive fruit sampled as well as production of husks and oil from some of the collected fruit were not included in the study report.

Samples were stored frozen at the field test facility, and upon shipment were received in frozen condition at the analytical laboratory in January, 1989. As indicated below, samples were stored frozen at ≤ -18 °C at the analytical facility.

A summary of the sampling information is shown in the table below.

Table 6.3.1-64: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage	Quantity	Date of sampling
IT1089E001	Olive	Fruit	1	Ripening fruit	Not provided	16.11.1988
IT1089E001	Olive	Fruit	7	Ripening fruit	Not provided	22.11.1988

1 Days after last application.

3. Analytical phase

The olive fruit samples were prepared using a Tecator Homogeniser.

Samples were analysed for residues of glyphosate derived from ICIA0224 using ICI Tentative Residue Analytical Method 172/1. The glyphosate was extracted from crop samples with water. The extracts were cleaned up first using a BOND ELUT™ SCX cartridge followed by use of a S5SAX HPLC column. The glyphosate containing fraction was derivatised with 9-fluorenylmethyl chloroformate. The glyphosate derivative was determined by HPLC using a second S5SAX column and a fluorescence detector. The LOQ for quantification of glyphosate in olive matrices was 0.05 mg/kg. The residues of the metabolite AMPA were not measured.
Treated and untreated specimens were maintained frozen during storage and shipment. The maximum sample storage interval was approximately 22 months (653 days). Samples were stored frozen at ≤ -18 °C at the analytical facility prior to analysis.
Detailed information on concurrent recovery analysis was not provided in the study report. Although details on recovery analysis or fortification levels were not included in the report, footnotes with tables providing analytical results for study samples indicated that recovery data was collected from two samples for olive fruit. The mean recovery of glyphosate in olive fruit was 46 %. According to the example chromatograms included in the study report, at least one of the samples of each matrix was fortified with glyphosate at 0.1 mg/kg.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow evaluation of the residue behaviour of glyphosate (derived from glyphosate-trimesium) in olive fruit after usage of YF7712 when applied as per the study.

No residues of glyphosate above the LOQ of 0.05 mg/kg were found in olive fruit collected from plots treated with YF7712 at 0.73 or 2.5 kg a.s./ha at either 1 or 7 days after application. Olive fruit collected from the plot treated with YF7712 at 5.9 kg a.s./ha was found to have glyphosate residues <0.05 mg/kg at 1 day after application, but at 7 days after application glyphosate residues were found at 0.06 mg/kg.

The study report did not address the point concerning removal of stones during sample preparation and if the residue values reported for olive fruit were adjusted to account for the weight of the whole fruit, including stones, rather than just the residue level in the flesh of the olive fruit. However, the reported residue value for olive fruit can be taken as potentially a worst case value since the residue level in olive flesh, if not adjusted for stone weight, would be higher than if the stone weight were added.

The study report indicated that reported residue values for olive matrices were corrected for recovery. The uncorrected residue values were not included in the report, but the recovery values used for correction were listed along with the corrected residue values. Residues of glyphosate were <0.05 mg/kg in olive fruit, except for the 7-day after application sample treated with YF7712 at 5.9 kg a.s./ha in which glyphosate residues were found at 0.06 mg/kg. Recovery values listed in the report as being used for

correction were <100 %. Therefore, there would be no impact of correction on any values for olive fruit that were reported as <0.05 mg/kg. The only potential impact would be that the value of 0.06 mg/kg for olive fruit found in samples collected at 7 days after treatment from the plot treated with YF7712 at 5.9 a.s./ha would be reduced if reported as an uncorrected residue value rather than after correction for recovery.

Detailed residue levels are shown in the table below.

Table 6.3.1-65: Residue levels of glyphosate in olives after one application of YF7712A (480 g/L glyphosate-trimesium)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Application rate[2] (kg as/ha)	Commodity	Residue found[3] (mg/kg) Glypho-sate	DALA[4] (days)	Date of analysis
IT1089E001 / San Severo, Torremaggiore, Italy / SEU / 1988	Olive / Perananzana	Ripening fruit	0.734	Fruit	<0.05	1	July - August, 1990
					<0.05	7	
			2.941	Fruit	<0.05	1	
					<0.05	7	
			5.882	Fruit	<0.05	1	
					0.06	7	

1 Growth stage at harvest. Study report indicated "Ripening fruit" as the growth stage for fruit at each sampling interval.
2 Application rates were reported for glyphosate-trimesium. The application rates for glyphosate-trimesium at 0.734, 2.941, or 5.882 kg as/ha expressed as glyphosate equivalents were 0.51, 2.03, and 4,06 kg as/ha, respectively. The rates expressed as glyphosate equivalents were obtained by adjusting the indicated rates of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively.
3 LOQ (limit of quantification): 0.05 mg/kg
4 Days after last application

III. Conclusion

One residue trial was carried out on olives during 1988 in Italy. In this trial YF7712 was applied at 0.73, 2.5 or 5.9 kg a.s./ha. Samples of olive fruit were collected at either 1 or 7 days after application. All samples were analysed for residues of glyphosate using an analytical method with a LOQ of 0.05 mg/kg. No residues of glyphosate above the LOQ of 0.05 mg/kg were found in olive fruit collected from plots treated with YF7712 at 0.73 or 2.5 kg a.s./ha at either 1 or 7 days after application. Olive fruit collected from the plot treated with YF7712 at 5.9 kg a.s./ha was found to have glyphosate residues <0.05 mg/kg at 1 day after application, but at 7 days after application glyphosate residues were found at 0.06 mg/kg. The residues of the metabolite AMPA were not measured.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was performed under GLP. The trial was conducted with one application with an application rate of glyphosate-trimesium at 0.73, 2.5, or 5.9 kg a.s./ha (0.51, 2.0, or 4.1 kg a.s./ha, expressed as glyphosate equivalents, respectively) applied to the ground under olive trees at 1 to 7 days before harvest maturity.

As indicated above, there were several deviations from the current guideline, OECD Guideline for the Testing of Chemicals, 509, identified in the study (i.e. GLP assay of the test material was not provided; information on sampling method and sample quantity not provided for olive fruit; report is unclear regarding whether or not residue values for fruit were corrected concerning weight of stones (i.e. on basis of whole fruit, including stones or just on basis of flesh without correction for weight of stones); report does not provide residue results uncorrected for recovery, although the mean recovery value used for correction of fruit was provided). These deviations are deviations with the study relative to current guideline specifications. The treatments with residues <0.05 mg/kg may provide useful results despite the guideline deviations. However, since the metabolite AMPA was not measured, the study is considered at best supportive.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/019
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in kiwi fruit (outdoor) at 2 sites in Southern Europe 2015
Report No	S15-00469
Document No	MSL0027501
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in kiwi fruit processed commodities peel and pulp after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 2 field trials in the southern zone. The kiwi plantations were treated once. The application was directed to the soil between the kiwi plants and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of kiwi fruit were taken at normal harvest, which was 7 days after application. The fruits were separated into peel and pulp for analysis. No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00469-01	Kiwi fruit	*Actinidia chinensis*	Hayward	Fruit (peel) Fruit (pulp)	≥ 0.4 kg / > 20 units ≥ 1.4 kg / > 20 units
S15-00469-02	Kiwi fruit	*Actinidia chinensis*	Hayward	Fruit (peel) Fruit (pulp)	≥ 0.3 kg / > 14 units ≥ 1.0 kg / > 14 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00469-01):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00469-02):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Two residue trials were conducted on kiwi fruit during the 2015 season, one trial in Italy (S15-00469-01) and one trial in Southern France (S15-00469-02). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the plants (6 plants per plot) at 7.5 L product/ha 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 293-323 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-66: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S15-00469-01	2	85-87 BBCH	3.878	323
S15-00469-02	2	87 BBCH	3.510	293

Regions, varieties and cultivation were typical for the cultivation of kiwi.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of fruits were taken by hand from the untreated and treated plots 7 days after application at BBCH 87. Each field sample was taken from at least 4 trees from all segments of the tree or plant, high and low, exposed and protected by foliage, avoiding the ends of the row. At least 12 sampling locations per plot were chosen. Whole fruit samples were manually separated into peel and pulp at the test site facilities using knives (S15 00469-01) or a manual peeling machine (S15-00469-02). During peeling, the contact to the pulp was reduced to a minimum to avoid cross contamination between peel and pulp. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.1-67: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00469-01	Kiwi fruit	Peel	7	87	≥ 0.4 kg / > 20 units	19.10.2015
S15-00469-01	Kiwi fruit	Pulp	7	87	≥ 1.4 kg / > 20 units	19.10.2015
S15-00469-02	Kiwi fruit	Peel	7	87	≥ 0.3 kg / > 14 units	23.10.2015
S15-00469-02	Kiwi fruit	Pulp	7	87	≥ 1.0 kg / > 14 units	23.10.2015

1 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in bunches of grapes, which is a plant commodity with a high acid content (EAS Chem study S14-05172). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 79 days, and the

maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in kiwi peel and kiwi pulp (3 replicates per matrix and analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.1-68: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Kiwi, peel	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	91, 91, 87	90	-	2.6	3
		0.5	89, 89, 87	88		1.3	3
		Overall	87-91	89	-	2.0	6
		Confirmation transition 168 > 79 m/z					
		0.05	89, 90, 92	90	-	1.7	3
		0.5	86, 86, 84	85	-	1.4	3
		Overall	84-92	88	-	3.4	6
Kiwi, pulp	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	92, 91, 91	91	-	0.6	3
		0.5	87, 88, 89	88	-	1.1	3
		Overall	87-92	90	-	2.2	6
		Confirmation transition 168 > 79 m/z					
		0.05	90, 93, 96	93	-	3.2	3
		0.5	87, 86, 89	87	-	1.7	3
		Overall	86-96	90	-	4.2	6
Kiwi, peel	AMPA	Quantification transition 110 > 63 m/z					
		0.05	90, 85, 90	88	-	3.3	3
		0.5	87, 88, 87	87	-	0.7	3
		Overall	85-90	88	-	2.2	6
		Confirmation transition 110 > 79 m/z					
		0.05	89, 86, 87	87	-	1.7	3
		0.5	87, 83, 85	85	-	2.4	3
		Overall	83-89	86	-	2.4	6
Kiwi, pulp	AMPA	Quantification transition 110 > 63 m/z					
		0.05	92. 85, 91	89	-	4.2	3
		0.5	89, 91, 92	91	-	1.7	3
		Overall	85-92	90	-	3.0	6
		Confirmation transition 110 > 79 m/z					
		0.05	92, 89, 92	91	-	1.9	3
		0.5	85, 86, 94	88	-	5.6	3
		Overall	85-94	90	-	4.0	6

Table 6.3.1-68: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of kiwi fruit (peel and pulp).

Detailed residue levels are shown in the table below.

Table 6.3.1-69: Residue levels of glyphosate and AMPA in kiwi after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00469-01 / 48018 Faenza, Emilia Romagna, Italy / SEU / 2015	Kiwi fruit / Hayward	87	Peel	<0.05 <0.05	n.d. n.d.	7	06.01.2016 – 07.01.2016
			Pulp	n.d. n.d.	n.d. n.d.		
S15-00469-02 / 66200 Elne, Pyreneés-Orientales, France / SEU / 2015	Kiwi fruit / Hayward	87	Peel	n.d. n.d.	n.d. n.d.	7	06.01.2016 – 07.01.2016
			Pulp	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of kiwi fruit (peel and pulp) sampled at BBCH 87 (commercial maturity), 7 days after ground application of glyphosate in the plant row at the rate of 3.51-3.88 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application with an application rate of 3.51-3.88 kg a.s./ha. This application rate is 22 % to 35 % higher than the critical GAP maximum seasonal application rate, which is acceptable since in all samples the residues of both glyphosate and AMPA were below the limit of quantitation of 0.05 mg/kg. Therefore, the study adequately supports the representative use for glyphosate in fruit plantations (and especially kiwi plantations) in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.1/020
Report author	[redacted]
Report year	2015
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in bananas (outdoor) at 4 sites in Spain (Canary Islands) 2014
Report No	S14-04159
Document No	MSL0027222
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in raw agricultural commodity specimens of banana (RAC whole fruit) as well as processed commodities peel and pulp after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials in the southern zone. The banana plantations were treated once. The application was directed to the soil between the banana plants and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of banana whole fruit, peel, and pulp were taken for analysis at

normal harvest, which was 1 day after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S14-04159-01	Banana	*Musa x paradisiaca*	Gruesa Palmera	Fruit	≥ 3 kg / 24 units
S14-04159-02	Banana	*Musa x paradisiaca*	Del Pais	Fruit	≥ 3 kg / 24 units
S14-04159-03	Banana	*Musa x paradisiaca*	Del Pais	Fruit	≥ 3.5 kg / 24 units
S14-04159-04	Banana	*Musa x paradisiaca*	Del Pais	Fruit	≥ 3 kg / 24 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S14-04159-01, S14-04159-02, S14-04159-03, and S14-04159-04):	Eurofins Agroscience Services SL, 41900 Camas, Seville, Spain
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on banana (outdoor) during the 2014 season in Spain, Canary Islands (S14-04159-01, S14-04159-02, S14-04159-03, and S14-04159-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the banana trees (4 trees per plot) at 7.5 L product/ha 1 day before harvest. The volume of water used to prepare the spray solution was in the range of 287-325 L/ha. The main application parameters are outlined in the table below.

Table 6.3.1-70: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S14-04159-01	2	78 BBCH	3.900	325
S14-04159-02	2	78 BBCH	3.645	304
S14-04159-03	2	78 BBCH	3.450	287
S14-04159-04	2	78 BBCH	3.900	325

Regions, varieties and cultivation were typical for the cultivation of banana.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of fruits were taken by hand from the untreated and treated plots 1 day after application, at BBCH 78. Each field sample was taken from at least 4 plants. Two fingers from top, middle, and lowest hand of four harvestable bunches were taken. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.1-71: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S14-04159-01	Banana	Whole Fruit	1	78	≥ 3 kg / 24 units	22.08.2014
S14-04159-02	Banana	Whole Fruit	1	78	≥ 3 kg / 24 units	22.08.2014
S14-04159-03	Banana	Whole Fruit	1	78	≥ 3.5 kg / 24 units	02.09.2014
S14-04159-04	Banana	Whole Fruit	1	78	≥ 3 kg / 24 units	23.09.2014

1 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in bunches of grapes (EAS Chem study S14-05172). The limit of quantitation (LOQ) for glyphosate and AMPA in grapes (bunches) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

From each sample, prior to homogenisation some of the fruit were selected and the pulp separated from the peel. These samples were analysed to determine distribution of any residues observed in the whole fruit to the peel and pulp fractions.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. During sample shipment the temperature exceeded 18°C first for 13 hours and then for 18 hours with max temperatures of -17°C and 13°C, respectively. Since the temperature deviations were limited to shipment and the samples remained frozen, this does not impact the validity of the trial results. The maximum sample storage interval from harvest to extraction was 348 days, and the maximum interval from extraction to analysis was 6 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in banana fruit (3 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. Furthermore, concurrent recoveries were also determined for glyphosate and AMPA in banana pulp and banana peel at fortification levels of 0.05 mg/kg and 0.50 mg/kg. The results were satisfactory, as shown in the table below.

Table 6.3.1-72: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Banana (whole fruit)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	106, 100, 96	101	-	5.0	3
		0.5	86, 86, 84	85	-	1.4	3
		Overall	84-106	93	-	9.7	6
		Confirmation transition 168 > 79 m/z					
		0.05	103, 105, 96	101	-	4.7	3
		0.5	92, 88, 87	89	-	3.0	3
		Overall	87-105	95	-	8.0	6
Banana (pulp)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	91	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90-91	-	-	-	2
Banana (peel)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	84	-	-	-	1
		0.5	86	-	-	-	1
		Overall	84-86	-	-	-	2
Banana (whole fruit)	AMPA	Quantification transition 110 > 63 m/z					
		0.05	92, 86, 83	87	-	5.3	3
		0.5	82, 80, 86	83	-	3.7	3
		Overall	80-92	85	-	5.0	6
		Confirmation transition 110 > 79 m/z					
		0.05	94, 90. 86	90	-	4.4	3
		0.5	83, 82, 83	83	-	0.7	3
		Overall	82-94	86	-	5.5	6
Banana (pulp)	AMPA	Quantification transition 110 > 63 m/z					
		0.05	92	-	-	-	1

Table 6.3.1-72: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		0.5	85	-	-	-	1
		Overall	85-92	-	-	-	2
Banana (peel)	AMPA	Quantification transition 110 > 63 m/z					
		0.05	83	-	-	-	1
		0.5	84	-	-	-	1
		Overall	83-84	-	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of banana (whole fruit, peel and pulp).

Detailed residue levels are shown in the table below.

Table 6.3.1-73: Residue levels of glyphosate and AMPA in banana after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S14-04159-01 / 38730 Villa de Mazo, La Palma, Spain / SEU / 2014	Banana / Gruesa Palmera	78	Whole fruit	n.d. n.d.	n.d. n.d.	1	19.04.2015.-07.08.2015
			Pulp	n.d.	n.d.		
			Peel	n.d.	n.d.		
S14-04159-02 / 38730 Villa de Mazo, La Palma, Spain / SEU / 2014	Banana / Del Pais	78	Whole fruit	n.d. n.d.	n.d. n.d.	1	19.04.2015.-07.08.2015
			Pulp	n.d.	n.d.		
			Peel	n.d.	n.d.		
S14-04159-03 / 38760 Los Llanos, La Palma, Spain / SEU / 2014	Banana / Del Pais	78	Whole fruit	n.d. n.d.	n.d. n.d.	1	19.04.2015.-07.08.2015
			Pulp	n.d.	n.d.		
			Peel	n.d.	n.d.		
S14-04159-04 / 38715 Puntallana,	Banana / Del Pais	78	Whole fruit	n.d. n.d.	n.d. n.d.	1	19.04.2015.-07.08.2015

Table 6.3.1-73: Residue levels of glyphosate and AMPA in banana after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
La Palma, Spain / SEU / 2014			Pulp	n.d.	n.d.		
			Peel	n.d.	n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of banana (whole fruit, peel, and pulp) sampled at BBCH 78 (commercial maturity), 1 days after ground application of glyphosate in the plant row at the rate of 3.45-3.90 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. Hence, the study can be regarded as reliable and acceptable. The trials were conducted with one application with an application rate of 3.45-3.90 kg a.s./ha. This application rate is 20 % to 35 % higher than the critical GAP maximum seasonal application rate. Moreover, the samples were taken 1 day instead of 7 days after the application according to the critical GAP. Since the higher application rate and the earlier harvest represent a worst case compared to the critical GAP and since all samples showed residues of both glyphosate and AMPA below the limit of detection of 0.015 mg/kg, the study adequately supports the representative use for glyphosate in fruit plantations (and especially banana plantations) in Southern Europe.

Assessment and conclusion by RMS:

CA 6.3.2 Use before emergence or before transplanting

For the use of glyphosate before emergence, before sowing or before transplanting sufficient data to support the representative GAP are available. The critical GAP is presented in the table below.

Table 6.3.2-1: Critical GAP of glyphosate for the post-harvest, pre-sowing, pre-planting use

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Application			Application rate			PHI (days)
		Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product/ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	

Table 6.3.2-1:Critical GAP of glyphosate for the post-harvest, pre-sowing, pre-planting use

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Application			Application rate			PHI (days)
		Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product/ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min./ max	
Root & tuber vegetables, Bulb vegetables, Fruiting vegetables, Brassica, Leafy vegetables, Stem vegetables, Sugar beet	F	Tractor mounted broadcast spray	Post-harvest, pre-sowing, pre-planting	a) 1 - 2 (28 days) b) 1 - 2 (28 days)	a) 3 – 4 L/ha b) 6 L/ha	a) 1.08 – 1.44 kg as/ha b) 2.16 kg as/ha	100 – 400	N/A

The magnitude of residues of glyphosate and its metabolite AMPA was investigated in 10 studies with glyphosate which are considered valid to address the data point.

For the use before emergence or before transplanting, residues of glyphosate and AMPA were always below the LOQ of 0.05 mg/kg in all trials (16 trials in Northern Europe and 19 trials in Southern Europe), in the most cases even below the LOD of 0.015 mg/kg. Therefore, the data set is sufficient to derive an MRL for vegetables grown after pre-emergence, pre-sowing or pre-transplanting treatment of the soil.

Table 6.3.2-2: Overview on residue studies in vegetables treated before emergence or before transplanting

Data Point	Crop, commodities	Analyte(s)	Number of trials	Reference	Status
CA 6.3.2/001	Potato, tuber	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2012 Report No. S11-00258	Valid
CA 6.3.2/002	Carrot, roots	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted]2012 Report No. S11-00259	Valid
CA 6.3.2/003	Onion, bulbs	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted]2012 Report No. S11-00260	Valid
CA 6.3.2/004	Tomato, fruit	Glyphosate AMPA	Northern Europe: 2	[redacted] 2012 Report No. S11-00267	Valid
CA 6.3.2/005	Cucumber, fruit Zucchini, fruit	Glyphosate AMPA	Northern Europe: 1 Southern Europe: 2	[redacted] 2012 Report No. S11-00261	Valid
CA 6.3.2/006	Cauliflower, heads	Glyphosate AMPA	Northern Europe: 1 Southern Europe: 3	[redacted] 2012 Report No. S11-00263	Valid
CA 6.3.2/007	Cabbage, heads	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2012 Report No. S11-00262	Valid
CA 6.3.2/008	Lettuce, leaves	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2012 Report No. S11-00264	Valid
CA 6.3.2/009	Leek, plants	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2012 Report No. S11-00265	Valid
CA 6.3.2/010	Sugar beet, leaves Sugar beet, roots	Glyphosate AMPA	Southern Europe: 2	[redacted], 2012 Report No. S11-00266	Valid

Table 6.3.2-2: Overview on residue studies in vegetables treated before emergence or before transplanting

Data Point	Crop, commodities	Analyte(s)	Number of trials	Reference	Status
CA 6.3.2/011	Strawberry	Glyphosate AMPA	Northern Europe: 4	[redacted] 1978 Report No. A25	Invalid
CA 6.3.2/012	Salads Onions Carots Peas Beans	Glyphosate AMPA	Europe: 19	[redacted] 1977 Report No. A16	Invalid
CA 6.3.2/013	Kale Swedes	Glyphosate AMPA	Northern Europe: 6	[redacted] 1977 Report No. A13	Invalid
CA 6.3.2/014	Kale Serradella Turnips	Glyphosate AMPA	Northern Europe: 3	[redacted] 1976 Report No. A10	Invalid
CA 6.3.2/015	Sugar beet tops Sugar beet roots	Glyphosate AMPA	Northern Europe: 2	[redacted] 1975 Report No. A3	Invalid

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/001
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in potatoes (outdoor) at 4 sites in France, Germany and Italy 2011
Report No	S11-00258
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in potato (tubers) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 4 field trials (2 trials in the northern zone and 2 trials in the southern zone). The potato fields were treated once, at least 3 days after planting and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Samples of potato tuber were taken for analysis at normal harvest, which was 98-138 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00258-01	Potato	*Solanum tuberosum*	Charlotte	Tubers	≥ 2 kg / ≥ 24 units
S11-00258-02	Potato	*Solanum tuberosum*	Milva	Tubers	≥ 2 kg / ≥ 24 units
S11-00258-03	Potato	*Solanum tuberosum*	Noisette	Tubers	≥ 2 kg / ≥ 24 units
S11-00258-04	Potato	*Solanum tuberosum*	Primura	Tubers	≥ 2 kg / ≥ 24 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00258-01):	Eurofins Agroscience Services / GAB France SAS, 67140 Saint Pierre, France
Field phase (S11-00258-02):	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00258-03):	Eurofins Agroscience Services SAS, 82290 Meauzac, France
Field phase (S11-00258-04):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on potatoes (outdoor) during 2011 in Northern France (S11-00258-01), Germany (S11-00258-02), Southern France (S11-00258-03) and Italy (S11-00258-04). One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha at least 3 days after planting and before crop emergence. The volume of water used to prepare the spray solution was in the range of 175-200 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-3: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00258-01	2	00 BBCH	2.173	175
S11-00258-02	2	00 BBCH	2.276	184
S11-00258-03	2	00 BBCH	2.218	187
S11-00258-04	2	00 BBCH	2.374	200

Regions, varieties and cultivation were typical for the cultivation of potatoes.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49), which was 98-138 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Leaves and soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

Table 6.3.2-4: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00258-01	Potato	Tuber	119	49	≥ 2 kg / ≥ 24 units	26.07.2011
S11-00258-02	Potato	Tuber	138	49	≥ 2 kg / ≥ 24 units	05.09.2011
S11-00258-03	Potato	Tuber	114	49	≥ 2 kg / ≥ 24 units	03.11.2011
S11-00258-04	Potato	Tuber	98	49	≥ 2 kg / ≥ 24 units	24.06.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in potato (tubers) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 227 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of potato (tuber) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-5: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Potato, tubers	Glyphosate	0.05	88	-	-	-	1
		0.5	88	-	-	-	1
		Overall	88	88	-	-	2
	AMPA	0.05	85	-	-	-	1
		0.5	87	-	-	-	1
		Overall	85-87	86	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of potato (tubers).

Detailed residue levels are shown in the table below.

Table 6.3.2-6: Residue levels of glyphosate and AMPA in potato after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location /	Crop/ Variety	Growth stage[1]	Commodity	Residue found[2,3] (mg/kg)	DALA[4] (days)	Date of analysis

EU zone / Year		(BBCH)		Glypho-sate	AMPA		
S11-00258-01 / 67870 Griesheim, Bas-Rhin, France / NEU / 2011	Potato / Charlotte	49	Tuber	n.d.	n.d.	119	06.-07.02.2012
S11-00258-02 / 21739 Dollern, Lower Saxony, Germany / NEU / 2011	Potato / Milva	49	Tuber	n.d.	n.d.	138	06.-07.02.2012
S11-00258-03 / 82290 Meauzac, Midi Pyrenees, France / SEU / 2011	Potato / Noisette	49	Tuber	n.d.	n.d.	114	06.-07.02.2012
S11-00258-04 / 40024, Gaiana, Bologna, Italy / SEU / 2011	Potato / Primura	49	Tuber	n.d.	n.d.	98	07.02.2012

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of potato (tubers) sampled at BBCH 49 (commercial maturity), 98-138 days after pre-emergence application of glyphosate at the rate of 2.17-2.37 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially potatoes) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/002
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in carrots (outdoor) at 4 sites in France, Spain and Poland 2011
Report No	S11-00259
Document No	Not available
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in carrot (roots without leaves) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 4 field trials (2 trials in the northern zone and 2 trials in the southern zone). The carrot fields were treated once, at least 3 days after seeding and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Samples of carrot root without leaves were taken for analysis at normal harvest, which was 93-176 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L

Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00259-01	Carrot	*Daucus carota* subsp. *sativus*	Montdibell	Roots without leaves	≥ 2 kg / ≥ 12 units
S11-00259-02	Carrot	*Daucus carota* subsp. *sativus*	Laguna	Roots without leaves	≥ 2 kg / ≥ 12 units
S11-00259-04	Carrot	*Daucus carota* subsp. *sativus*	Maestro	Roots without leaves	≥ 2 kg / ≥ 12 units
S11-00259-05	Carrot	*Daucus carota* subsp. *sativus*	Maestro	Roots without leaves	≥ 2 kg / ≥ 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00259-01):	Eurofins Agroscience Services SAS, 49350 Gennes, France
Field phase (S11-00259-02):	Eurofins Agroscience Services sp. Z o. o., 64500 Szamotuly, Poland
Field phase (S11-00259-04):	Eurofins Agroscience Services SAS, 82290 Meauzac, France
Field phase (S11-00259-05):	Eurofins Agroscience Services SL, 46650 Canals, Valencia, Spain
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on carrots (outdoor) during 2011 in Northern France (S11-00259-01), Poland (S11-00259-02), Southern France (S11-00259-04) and Spain (S11-00259-05). Due to problems in seed emergence, trial S11-00259-03 was stopped and replaced by S11-00259-05. One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil the nominal rate of at 6.0 L product/ha at least 3 days after seeding and before crop emergence The volume of water used to prepare the spray solution was in the range of 169-210 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-7: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00259-01	2	00 BBCH	2.311	187
S11-00259-02	2	00 BBCH	2.295	193
S11-00259-04	2	00-03 BBCH	2.492	210
S11-00259-05	2	05 BBCH	2.080	169

The actual application rate across the four trials ranged from 2.08 to 2.49 kg a.s./ha.

Regions, varieties and cultivation were typical for the cultivation of carrots.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49, which was 93-176 days after application.. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Leaves and soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

Table 6.3.2-8: Crop sampling information

Trial	Crop	Commodity	DALA [1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00259-01	Carrot	Roots without leaves	93	49	≥ 2 kg / ≥ 12 units	15.09.2011
S11-00259-02	Carrot	Roots without leaves	176	49	≥ 2 kg / ≥ 12 units	12.10.2011
S11-00259-04	Carrot	Roots without leaves	137	49	≥ 2 kg / ≥ 12 units	14.10.2011
S11-00259-05	Carrot	Roots without leaves	154	49	≥ 2 kg / ≥ 12 units	21.09.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in potato (tubers) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 116 days, and the maximum interval from extraction to analysis was 0 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of potato (tuber) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-9: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Carrot, roots without leaves	Glyphosate	0.05	97	97	-	-	1
		0.5	91	91	-	-	1
		Overall	91-97	94	-	-	2
	AMPA	0.05	93	93	-	-	1
		0.5	90	90	-	-	1
		Overall	90-93	92	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of carrot (roots without leaves).

Detailed residue levels are shown in the table below.

Table 6.3.2-10: Residue levels of glyphosate and AMPA in carrot after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00259-01 / 49650 Allonnes, Maine et Loire, France / NEU / 2011	Carrot / Montdibell	49	Roots without leaves	n.d.	n.d.	93	09.01.2012
S11-00259-02 / 64-600 Uscikowo, Wielkopolska, Poland / NEU / 2011	Carrot / Laguna	49	Roots without leaves	n.d.	n.d.	176	09.01.2012
S11-00259-04 / 31700 Balgnac, Haute-Garonne, France / SEU / 2011	Carrot / Maestro	49	Roots without leaves	n.d.	n.d.	137	09.01.2012
S11-00259-05 / 03400 Villena, Alicante, Spain / SEU / 2011	Carrot / Maestro	49	Roots without leaves	n.d.	n.d.	154	09.01.2012

1 Growth stage at last harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of carrot (roots without leaves) sampled at BBCH 49 (commercial maturity), 93-176 days after pre-emergence application of glyphosate at the rate of 2.08-2.49 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially carrots) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/003
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in bulb onions (outdoor) at 4 sites in France, Spain and Bulgaria 2011
Report No	S11-00260
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in bulb onion (bulb) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 4 field trials (2 trials in the northern zone and 2 trials in the southern zone). The onion fields were treated once, at least 3 days after seeding and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Samples of onion bulb were taken for analysis at normal harvest, which was 143-154 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL

Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00260-01	Bulb onion	*Allium cepa*	Takmark F1	Bulbs	≥ 2 kg / ≥ 12 units
S11-00260-02	Bulb onion	*Allium cepa*	Kristine	Bulbs	≥ 2 kg / ≥ 12 units
S11-00260-03	Bulb onion	*Allium cepa*	Eso	Bulbs	≥ 2 kg / ≥ 12 units
S11-00260-04	Bulb onion	*Allium cepa*	Stutgarten rijsen	Bulbs	≥ 2 kg / ≥ 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00260-01):	Eurofins Agroscience Services GAB France SAS, 67140 Saint Pierre, France
Field phase (S11-00260-02):	Eurofins Agroscience Services sp. Z o. o., 64500 Szamotuly, Poland
Field phase (S11-00260-03):	Eurofins Agroscience Services SL, 46650 Canals, Valencia, Spain
Field phase (S11-00260-04):	Eurofins Agroscience Services EOOD, 5570 Letniza, Bulgaria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on bulb onions (outdoor) during 2011 in Northern France (S11-00260-01), Poland (S11-00260-02), Spain (S11-00260-03) and Bulgaria (S11-00260-04). One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha at least 3 days after seeding and before crop emergence. The volume of water used to prepare the spray solution was in the range of 187-205 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-11: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00260-01	2	01 BBCH	2.311	187
S11-00260-02	2	03 BBCH	2.413	203
S11-00260-03	2	03 BBCH	2.433	205
S11-00260-04	2	00 BBCH	2.386	193

Regions, varieties and cultivation were typical for the cultivation of bulb onions.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49), which was 129-154 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Roots and adhering soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep frozen immediately after arrival at the test sites (i.e. within less than 4 hours of sampling in the field).

Table 6.3.2-12: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00260-01	Bulb onion	Bulb	129	49	≥ 2 kg / ≥ 12 units	11.08.2011
S11-00260-02	Bulb onion	Bulb	143	49	≥ 2 kg / ≥ 12 units	01.09.2011
S11-00260-03	Bulb onion	Bulb	154	49	≥ 2 kg / ≥ 12 units	26.08.2011
S11-00260-04	Bulb onion	Bulb	149	49	≥ 2 kg / ≥ 12 units	27.08.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in potato (tubers) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 151 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of onions (bulb) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-13: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Bulb onion, bulb	Glyphosate	0.05	92	92	-	-	1
		0.5	91	91	-	-	1

Table 6.3.2-13: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	91-92	92	-	-	2
	AMPA	0.05	89	89	-	-	1
		0.5	88	88	-	-	1
		Overall	88-89	89	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of onion (bulb).

Detailed residue levels are shown in the table below.

Table 6.3.2-14: Residue levels of glyphosate and AMPA in bulb onion after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00260-01 / 67370 Woellenheim, Bas-Rhin, France / NEU / 2011	Bulb onion / Takmark Fl	49	Bulb	n.d.	n.d.	129	09.-10.01.2012
S11-00260-02 / 64-600 Uscikowo, Wielkopolska, Poland / NEU / 2011	Bulb onion / Kristine	49	Bulb	n.d.	n.d.	143	09.-10.01.2012
S11-00260-03 / 02140 El Salobral, Albacete, Spain / SEU / 2011	Bulb onion / Eso	49	Bulb	n.d.	n.d.	154	09.-10.01.2012
S11-00260-04 / 5570 Letnitsa, Letnitsa, Bulgaria / SEU / 2011	Bulb onion / Stutgarten rijsen	49	Bulb	n.d.	n.d.	149	09.-10.01.2012

Table 6.3.2-14: Residue levels of glyphosate and AMPA in bulb onion after one application of MON 52276 (360 g/L glyphosate)

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of bulb onions (bulbs) sampled at BBCH 49 (commercial maturity), 129-154 days after pre-emergence application of glyphosate at the rate of 2.31-2.43 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially bulb onions) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/004
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in tomato (outdoor) at 2 sites in Hungary and Germany 2011
Report No	S11-00267
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA tomato (fruits) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 2 field trials in the northern zone. The tomato fields were treated once, 3 days before planting of seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Samples of tomato fruit were taken for analysis at normal harvest, which was 93-94 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00267-01	Tomato	*Solanum lycopersicum*	Vanessa	Fruits	≥ 2 kg / ≥ 12 units
S11-00267-02	Tomato	*Solanum lycopersicum*	Claudius F1	Fruits	≥ 2 kg / ≥ 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00267-01):	Eurofins Agroscience Services GmbH, 69168 Wiesloch, Germany
Field phase (S11-00267-02):	Eurofins Agroscience Services Kft., 8000 Székesfehérvár, Hungary
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Two residue trials were conducted on tomato (outdoor) during 2011 in Germany (S11-00267-01) and Hungary (S11-00267-02). One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha at 3 days before planting the tomato seedlings. The

volume of water used to prepare the spray solution was in the range of 185-187 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-15: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00267-01	2	3 days before transplanting crop seedlings	2.304	187
S11-00267-02	2	3 days before transplanting crop seedlings	2.283	185

n/a not applicable; application was conducted 3 days before planting the tomato seedlings

Regions, varieties and cultivation were typical for the cultivation of tomato.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at BBCH 89, 93-94 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.2-16: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00267-01	Tomato	Fruit	93	89	≥ 2 kg / ≥ 12 units	04.08.2011
S11-00267-02	Tomato	Fruit	94	89	≥ 2 kg / ≥ 12 units	14.10.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in tomato (fruit) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 168 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of potato (tuber) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-17: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Tomato, fruit	Glyphosate	0.05	90	90	-	-	1
		0.5	87	87	-	-	1
		Overall	87-90	89	-	-	2
	AMPA	0.05	90	90	-	-	1
		0.5	88	88	-	-	1
		Overall	88-90	89	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of tomato (fruits) sampled at BBCH 89 (commercial maturity).

Detailed residue levels are shown in the table below.

Table 6.3.2-18: Residue levels of glyphosate and AMPA in tomato after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00267-01 / 69124, Kirchheim, Baden-Württemberg, Germany / NEU / 2011	Tomato / Vanessa	89	Fruit	n.d.	n.d.	93	20.01.2012

Table 6.3.2-18: Residue levels of glyphosate and AMPA in tomato after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00267-02 / 2454, Iváncsa, Fejér, Hungary / NEU / 2011	Tomato / Claudius F1	89	Fruit	n.d.	n.d.	94	20.01.2012

1 Growth stage at last harvest. Application is pre-plant to bare soil 3 days before tomato seedlings are planted
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of tomato (fruits) sampled at BBCH 89 (commercial maturity), 93-94 days after pre-emergence application of glyphosate at the rate of 2.30-2.28 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially tomatoes) in Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/005
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in cucumber and zucchini (outdoor) at 3 sites in Italy, France and Germany 2011
Report No	S11-00261
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test	None

guideline	
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in zucchini and cucumber (fruit) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 3 field trials (two trials on zucchini and one trial on cucumber). The fields were treated once 3 days before transplanting the crop seedlings, at a target rate of 2.16 kg glyphosate acid per hectare (6.0 L product/ha). Samples of zucchini and cucumber fruits were taken for analysis at normal harvest, which was 42-55 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00261-01	Zucchini	*Cucurbita pepo* var. giromontiina	Monitor	Fruit	≥ 2 kg / ≥ 12 units
S11-00261-03	Zucchini	*Cucurbita pepo* var. giromontiina	Cigal F1	Fruit	≥ 2 kg / ≥ 12 units
S11-00261-04	Cucumber	*Cucumis sativus*	Ekron	Fruit	≥ 2 kg / ≥ 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00261-01):	Eurofins Agroscience Services GmbH, 69124 Heidelberg, Germany
Field phase (S11-00261-03):	Eurofins Agroscience Services / GAB France Sarl, 66200 Elne, France
Field phase (S11-00261-04):	Eurofins Agroscience Services SRL, 04022 Fondi (Latina), Italy
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Three residue trials were completed on cucumber or zucchini (outdoor) during 2011 in Germany (S11-00261-01), Southern France (S11-00261-03) and Italy (S11-00261-04). A fourth trial was initiated in Hungary (S11-00261-02), but this trial could not be completed due to crop failure. One application of MON 52276 (nominal 360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha (2.16 kg a.s./ha) at 3 days before transplanting the crop seedlings. The volume of water used to prepare the spray solution was in the range of 180-207 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-19: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00261-01	2	3 days before transplanting crop seedlings	2.551	207
S11-00261-03	2	3 days before transplanting crop seedlings	2.222	180
S11-00261-04	2	3 days before transplanting crop seedlings	2.239	181

Regions, varieties and cultivation were typical for the cultivation of cucumber or zucchini.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at at normal commercial harvest (BBCH 89), which was 42-55 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Stems were removed and control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 1.5 hours of sampling in the field).

Table 6.3.2-20: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00261-01	Zucchini	Fruit	55	89	≥ 2 kg / ≥ 12 units	15.08.2011
S11-00261-03	Zucchini	Fruit	52	89	≥ 2 kg / ≥ 12 units	30.06.2011
S11-00261-04	Cucumber	Fruit	42	89	≥ 2 kg / ≥ 12 units	23.06.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in cucumber and zucchini (fruit) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Table 6.3.2-21: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Zucchini, fruit	Glyphosate	0.05	92	92	-	-	1
		0.5	88	88	-	-	1
		Overall	88-92	90	-	-	2
	AMPA	0.05	90	90	-	-	1
		0.5	90	90	-	-	1
		Overall	90	90	-	-	2
Cucumber, fruit	Glyphosate	0.05	90	90	-	-	1
		0.5	87	87	-	-	1
		Overall	87-90	89	-	-	2
	AMPA	0.05	87	87	-	-	1
		0.5	90	90	-	-	1
		Overall	87-90	89	-	-	2

1 Residues of glyphosate and AMPA in blank / untreated matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of cucumber and zucchini (fruits).

Detailed residue results are shown in the table below.

Table 6.3.2-22: Residue levels of glyphosate and AMPA in zucchini and cucumber fruit after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00261-01 / 69124, Neurott, Baden-Württemberg, Germany / NEU / 2011	Zucchini / Monitor	89	Fruit	n.d.	n.d.	55	10.01.2012
S11-00261-03 / 66540, Baho, Pyrénées-Orientales, France / SEU / 2011	Zucchini / Cigal F1	89	Fruit	n.d.	n.d.	52	10.01.2012
S11-00261-04 / 04022, Fondi, Latina, Italy / SEU / 2011	Cucumber / Ekron	89	Fruit	n.d.	n.d.	42	10.01.2012

1 Growth stage at last harvest; treatment applied prior to transplanting zucchini or cucumber seedlings
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of cucumber and zucchini (fruits) sampled at BBCH 89 (commercial maturity), 42-55 days after pre-emergence application of glyphosate at the rate of 2.22-2.55 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially cucumber and zucchini) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/006
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in cauliflower (outdoor) at 4 sites in France, Hungary, Bulgaria and Italy 2011
Report No	S11-00263
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA cauliflower (inflorescence) after one application of MON 52276, an SL formulation containing 360 g/l of glyphosate acid equivalents.

The study included 4 field trials (1 trial in the northern zone and 3 trials in the southern zone). The cauliflower fields were treated once, at a target rate of 2.16 kg glyphosate acid per hectare (6.0 L product/ha) at 3 days before transplanting the crop seedlings. Samples of cauliflower (inflorescence) were taken for analysis at normal harvest, which was 75-125 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L

Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00263-01	Cauliflower	*Brassica oleracea* var. botrytis	Aviso	Inflorescence	≥ 2 kg / ≥ 12 units
S11-00263-02	Cauliflower	*Brassica oleracea* var. botrytis	Cortes	Inflorescence	≥ 3.1 kg / ≥ 12 units[1]
S11-00263-03	Cauliflower	*Brassica oleracea* var. botrytis	Castellum	Inflorescence	≥ 2 kg / ≥ 12 units
S11-00263-04	Cauliflower	*Brassica oleracea* var. botrytis	Snowball	Inflorescence	≥ 2.5 kg / ≥ 12 units[1]

1 Sample weight was reduced by sectioning at minimum the required number of heads and collecting representative portions

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00263-01):	Eurofins Agroscience Services SAS, 45300 Rouvres St Jean, France
Field phase (S11-00263-02):	Eurofins Agroscience Services Kft., 8000 Székesfehérvár, Hungary
Field phase (S11-00263-03):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S11-00263-04):	Eurofins Agroscience Services EOOD, 5570 Letniza, Bulgaria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on cauliflower (outdoor) during 2011 in Northern France (S11-00263-01), Hungary (S11-00263-02), Italy (S11-00263-03) and Bulgaria (S11-00263-04). One application of MON 52276 (nominal 360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha (2.16 kg a.s./ha) at 3 days before transplanting the crop seedlings. The volume of water used to prepare the spray solution was in the range of 175-200 L/ha.. The main application parameters are outlined in the table below.

Table 6.3.2-23: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00263-01	2	3 days before transplanting crop seedlings	2.256	182
S11-00263-02	2	3 days before transplanting crop seedlings	2.172	176
S11-00263-03	2	3 days before transplanting crop seedlings	2.413	203
S11-00263-04	2	3 days before transplanting crop seedlings	2.332	189

n/a not applicable; application was conducted 3 days before planting the cauliflower seedlings

Regions, varieties and cultivation were typical for the cultivation of cauliflower.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49), which was 98-138 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Stalks and leaves were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

In two of the four trials, sample weight was reduced by cutting heads into four to eight parts and collecting at least the two opposite parts from each plant for use in the sample. The required minimum number of plants were sampled.

Table 6.3.2-24: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00263-01	Cauliflower	Inflorescence	75	49	≥ 2 kg / ≥ 12 units	07.09.2011
S11-00263-02	Cauliflower	Inflorescence	125	49	≥ 3.1 kg / ≥ 12 units[2]	14.11.2011
S11-00263-03	Cauliflower	Inflorescence	80	49	≥ 2 kg / ≥ 12 units	13.06.2011
S11-00263-04	Cauliflower	Inflorescence	120	49	≥ 2.5 kg / ≥ 12 units[2]	10.11.2011

1 Days after last application.
2 Sample weight was reduced by sectioning at minimum the required number of heads and collecting representative portions.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in potato (tubers) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 210 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of potato (tuber) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-25: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Cauliflower, inflorescence	Glyphosate	0.05	95	95	-	-	1
		0.5	90	90	-	-	1
		Overall	90-95	93	-	-	2
	AMPA	0.05	84	84	-	-	1
		0.5	89	89	-	-	1
		Overall	84-89	87	-	-	2

1 Residues of glyphosate and AMPA in blank / untreated matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of cauliflower (inflorescence).

Detailed residue results are shown in the table below.

Table 6.3.2-26: Residue levels of glyphosate and AMPA in cauliflower inflorescence after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop / Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00263-01 / 91140, La Poitevine, Essonne, France / NEU / 2011	Cauliflower / Aviso	49	Inflorescence	n.d.	n.d.	75	10.01.2012
S11-00263-02 / 2454 Ivánsca, Fejër, Hungary / NEU / 2011	Cauliflower / Cortes	49	Inflorescence	n.d.	n.d.	125	10.01.2012

Table 6.3.2-26: Residue levels of glyphosate and AMPA in cauliflower inflorescence after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00263-03 / 40057, Granarolo, Emilia, Romagna, Italy / SEU / 2011	Cauliflower / Castellum	49	Inflorescence	n.d.	n.d.	80	10.01.2012
S11-00263-04 / 40024, Letnitsa, Letnitsa, Bulgaria / SEU / 2011	Cauliflower / Snowball	49	Inflorescence	n.d.	n.d.	120	10.01.2012

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of cauliflower (inflorescence) sampled at BBCH 49 (commercial maturity), 75-125 days after pre-emergence application of glyphosate at the rate of 2.17-2.41 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially cauliflower) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/007
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in head cabbage (outdoor) at 4 sites in Hungary, France (North), Spain and Bulgaria 2011
Report No	S11-00262
Document No	---

Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in head cabbage (heads) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 4 field trials (2 trials in the northern zone and 2 trials in the southern zone). The head cabbage fields were treated once, at a target rate of 2.16 kg glyphosate acid per hectare (6.0 L product/ha) at least 3 days before transplanting the crop seedlings. Samples of head cabbage (heads) were taken for analysis at normal harvest, which was 98-138 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00262-01	Head cabbage	*Brassica oleracea* var. capitata	Padoc	Heads	≥ 1 kg / ≥ 12 units

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00262-02	Head cabbage	*Brassica oleracea* var. capitata	Pandion	Heads	≥ 1 kg / ≥ 12 units
S11-00262-03	Head cabbage	*Brassica oleracea* var. capitata	Melissa	Heads	≥ 1 kg / ≥ 12 units
S11-00262-04	Head cabbage	*Brassica oleracea* var. capitata	Kyose	Heads	≥ 1 kg / ≥ 12 units[1]

[1] Sample weight was reduced by sectioning at minimum the required number of heads and collecting representative portions.

Test facilities	
Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00262-01):	Eurofins Agroscience Services SAS, 71700 Uchizy, France
Field phase (S11-00262-02):	Eurofins Agroscience Services Kft., 8000 Székesfehérvár, Hungary
Field phase (S11-00262-03):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S11-00262-04):	Eurofins Agroscience Services EOOD, 5570 Letniza, Bulgaria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on head cabbage (outdoor) during 2011 in Northern France (S11-00262-01), Hungary (S11-00262-02), Spain (S11-00262-03), and Bulgaria (S11-00262-04). One application of MON 52276 (nominal 360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha (2.16 kg a.s./ha) 3 days before transplanting the crop seedlings. The volume of water used to prepare the spray solution was in the range of 172-207 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-27: Application information

Application code	Treatment number	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00262-01	2	3 days before transplanting crop seedlings	2.558	207
S11-00262-02	2	3 days before transplanting crop seedlings	2.127	172
S11-00262-03	2	3 days before transplanting crop seedlings	2.140	173
S11-00262-04	2	3 days before transplanting crop seedlings	2.345	190

Regions, varieties and cultivation were typical for the cultivation of head cabbage.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49), which was 67-99 days after application.. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Leaves and soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample).. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

In one trial, sample size was reduced by cutting each fruit into eight equal segments and collecting two opposite segments of each head.

Table 6.3.2-28: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00262-01	Head cabbage	Heads	67	49	≥ 1 kg / ≥ 12 units	13.09.2011
S11-00262-02	Head cabbage	Heads	97	49	≥ 1 kg / ≥ 12 units	13.12.2011
S11-00262-03	Head cabbage	Heads	98	49	≥ 1 kg / ≥ 12 units	14.11.2011
S11-00262-04	Head cabbage	Heads	99	49	≥ 1 kg / ≥ 12 units[2]	24.11.2011

1 Days after last application.
2 Sample weight was reduced by sectioning at minimum the required number of heads and collecting representative portions.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in potato (tubers) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained in a deep frozen condition and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 146 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of cabbage (heads) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-29: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Cabbage, heads	Glyphosate	0.05	87	87	-	-	1
		0.5	87	87	-	-	1
		Overall	87	87	-	-	2
	AMPA	0.05	91	91	-	-	1
		0.5	90	90	-	-	1
		Overall	90-91	91	-	-	2

1 Residues of glyphosate and AMPA in blank / untreated matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of head cabbage (heads).

Detailed residue results are shown in the table below.

Table 6.3.2-30: Residue levels of glyphosate and AMPA in cabbage heads after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00262-01 / 21130, Auxonne, Côte d'Or, France / NEU / 2011	Head cabbage / Padoc	49	Heads	n.d.	n.d.	67	07.02.2012
S11-00262-02 / 6635 Szegvár, Csongrád, Hungary / NEU / 2011	Head cabbage / Pandion	49	Heads	n.d.	n.d.	97	07.02.2012
S11-00262-03 / 50180, Utebo, Zaragoza, Spain / SEU / 2011	Head cabbage / Melissa	49	Heads	n.d.	n.d.	98	07.02.2012

Table 6.3.2-30: Residue levels of glyphosate and AMPA in cabbage heads after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00262-04 / 5570 Letnitsa, Letnitsa, Bulgaria / SEU / 2011	Head cabbage / Kyose	49	Heads	n.d.	n.d.	99	07.02.2012

1 Growth stage at harvest;
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of head cabbage (head) sampled at BBCH 49 (commercial maturity), 67-99 days after pre-emergence application of glyphosate at the rate of 2.13-2.56 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially head cabbage) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/008
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in leaf and head lettuce (outdoor) at 4 sites in France, Spain, UK and Germany 2011
Report No	S11-00264
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4

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

2. Full summary of the study according to OECD format

Executive Summary
The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in leaf and head lettuce (leaves and heads, respectively) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 4 field trials (2 trials on leaf lettuce in the northern zone and 2 trials on head lettuce in the southern zone). The lettuce fields were treated once, at a target rate of 2.16 kg glyphosate acid per hectare applied to bare soil at 3 days before transplanting the crop seedlings. Samples of leaf lettuce (leaves) or head lettuce (heads) were taken for analysis at normal harvest, which was 38-56 days after application. No residues of glyphosate and AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any treated or untreated specimens of leaf lettuce (leaves) and head lettuce (heads).

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00264-01	Leaf lettuce	*Lactuca sativa* var. crispa	Kirinia	Leaves	≥ 1 kg / ≥ 12 units
S11-00264-02	Leaf lettuce	*Lactuca sativa* var. crispa	Oak Leaf - Red	Leaves	≥ 1 kg / ≥ 12 units
S11-00264-03	Head lettuce	*Lactuca sativa* var. capitata	Sucrine	Heads	≥ 1 kg / ≥ 12 units

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00264-04	Head lettuce	*Lactuca sativa* var. capitata	Cervantes	Heads	≥ 1 kg / ≥ 12 units

Test facilities	
Study directory:	Eurofins Agroscience Services, 21684 Stade, Germany
Field phase (S11-00264-01):	Eurofins Agroscience Services GmbH, 69168 Wiesloch, Germany
Field phase (S11-00264-02):	Eurofins Agroscience Services Ltd / EAS UK South East, C07 8SD Little Bentley, Colchester, Essex, UK
Field phase (S11-00264-03):	Eurofins Agroscience Services / GAB France Sarl, 66200 Elne, France
Field phase (S11-00264-04):	Eurofins Agroscience Services SL, 46650 Canals, Valencia, Spain
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on leaf or head lettuce (outdoor) during 2011 in Germany (S11-00264-01), UK (S11-00264-02), Southern France (S11-00264-03), and Spain (S11-00264-04). One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha (2.16 kg a.s./ha) at 3 days before transplanting the crop seedlings. The volume of water used to prepare the spray solution was in the range of 190-203 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-31: Application information

Application code	Treatment number	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00264-01	2	3 days before transplanting crop seedlings	2.469	200
S11-00264-02	2	3 days before transplanting crop seedlings	2.258	190
S11-00264-03	2	3 days before transplanting crop seedlings	2.334	197
S11-00264-04	2	3 days before transplanting crop seedlings	2.413	203

Regions, varieties and cultivation were typical for the cultivation of leaf or head lettuce.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49), which was 38-56 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Decayed outer leaves, roots and soil were removed. Control specimens were taken before treated specimens. Sampling

equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites.

Table 6.3.2-32: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00264-01	Leaf lettuce	Leaves	42	49	≥ 1 kg / ≥ 12 units	30.05.2011
S11-00264-02	Leaf lettuce	Leaves	56	49	≥ 1 kg / ≥ 12 units	15.07.2011
S11-00264-03	Head lettuce	Heads	38	49	≥ 1 kg / ≥ 12 units	30.06.2011
S11-00264-04	Head lettuce	Heads	48	49	≥ 1 kg / ≥ 12 units[2]	25.07.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in leaf lettuce (leaves) and head lettuce (heads) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 252 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of potato (tuber) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-33: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Leaf lettuce, leaves	Glyphosate	0.05	91	-	-	-	1
		0.5	86	-	-	-	1
		Overall	86-91	89	-	-	2
	AMPA	0.05	86	-	-	-	1
		0.5	85	-	-	-	1
		Overall	85-86	86	-	-	2
Head lettuce, heads	Glyphosate	0.05	93	-	-	-	1
		0.5	88	-	-	-	1

Table 6.3.2-33: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	88-93	91	-	-	2
	AMPA	0.05	85	-	-	-	1
		0.5	84	-	-	-	1
		Overall	84-85	85	-	-	2

1 Residues of glyphosate and AMPA in blank / untreated matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of leaf lettuce (leaves) or head lettuce (heads).

Detailed residue results are shown in the table below.

Table 6.3.2-34: Residue levels of glyphosate and AMPA in leaf and head lettuce after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00264-01 / 69124, Neurott, Baden-Württemberg, Germany / NEU / 2011	Leaf lettuce / Kirinia	49	Leaves	<0.05	n.d.	42	07.02.2012
S11-00264-02 / C07 7KU Ardleigh, Essex, UK / NEU / 2011	Leaf lettuce / Oak Leaf - Red	49	Leaves	<0.05	n.d.	56	07.02.2012
S11-00264-03 / 66600, Rivesaltes, Pyrénés-Orientales, France / SEU / 2011	Head lettuce / Sucrine	49	Heads	n.d.	n.d.	38	07.02.2012

Table 6.3.2-34: Residue levels of glyphosate and AMPA in leaf and head lettuce after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00264-04 / 46820, Anna, Valencia, Spain / SEU / 2011	Head lettuce / Cervantes	49	Heads	<0.05	n.d.	48	07.02.2012

1 Growth stage at last harvest
2 <0.05 mg/kg (< limit of quantitation (LOQ) of 0.05 mg/kg)
3 n.d. (not detected): < 0.015 mg/kg (<LOD of 0.015 mg/kg)
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of leaf lettuce (leaves) or head lettuce (heads) sampled at BBCH 49 (commercial maturity), 38-56 days after soil application of glyphosate at the rate of 2.26-2.47 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially lettuce) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.2.3/009
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in leek (outdoor) at 4 sites in France, United Kingdom, Bulgaria and Italy 2011
Report No	S11-00265
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None

Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in raw agricultural commodity specimens of leek (RAC whole plant w/o roots) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 4 field trials (2 trials in the northern zone and 2 trials in the southern zone). The leek fields were treated once, at a target rate of 2.16 kg glyphosate acid per hectare at 2 to 3 days before transplanting the crop seedlings. Samples of leek (whole plant w/o roots) were taken for analysis at normal harvest, which was 65-183 days after application. No residues of glyphosate and AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material	
Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00265-01	Leek	*Allium porrum*	Kenton	Whole plant w/o roots	≥ 2 kg / ≥ 12 units
S11-00265-02	Leek	*Allium porrum*	Parvella	Whole plant w/o roots	≥ 2 kg / ≥ 12 units
S11-00265-03	Leek	*Allium porrum*	Maxim	Whole plant w/o roots	≥ 2 kg / ≥ 12 units
S11-00265-04	Leek	*Allium porrum*	Starozagorski 72	Whole plant w/o roots	≥ 2 kg / ≥ 12 units

Test facilities

Study directory:	Eurofins Agroscience Services, 21684 Stade, Germany
Field phase (S11-00265-01):	Eurofins Agroscience Services SAS, 71700 Uchizy, France
Field phase (S11-00265-02):	Eurofins Agroscience Services Ltd / EAS UK North West, L40 2QU, Towngate Works, Mawdesley, Lancashire, UK
Field phase (S11-00265-03):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S11-00265-04):	Eurofins Agroscience Services EOOD, 5570 Letniza, Bulgaria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on leek (outdoor) during 2011 in Northern France (S11-00265-01), UK (S11-00265-02), Italy (S11-00265-03), and Bulgaria (S11-00265-04). One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha (2.16 kg a.s./ha) at 2 to 3 days before transplanting the crop seedlings. The volume of water used to prepare the spray solution was in the range of 173-213 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-35: Application information

Application code	Treatment number	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00265-01	2	3 days before transplanting crop seedlings	2.539	213
S11-00265-02	2	2 days before transplanting crop seedlings	2.413	203
S11-00265-03	2	3 days before transplanting crop seedlings	2.255	190
S11-00265-04	2	3 days before transplanting crop seedlings	2.140	173

Regions, varieties and cultivation were typical for the cultivation of leek.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49, except for trial S11-00265-02, in which the growth stage at sampling was BBCH 47-49), which was 65-183 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Samples were collected by hand. Roots and adhering soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. . Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling, the control specimens

and treated specimens were kept separated by an adequate space at all times. Specimens were deep frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

Table 6.3.2-36: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00265-01	Leek	Whole plant w/o roots	77	49	≥ 2 kg / ≥ 12 units	13.09.2011
S11-00265-02	Leek	Whole plant w/o roots	183	47-49	≥ 2 kg / ≥ 12 units	22.09.2011
S11-00265-03	Leek	Whole plant w/o roots	65	49	≥ 2 kg / ≥ 12 units	05.08.2011
S11-00265-04	Leek	Whole plant w/o roots	125	49	≥ 2 kg / ≥ 12 units[2]	19.10.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in potato tubers (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in leek (plants) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 167 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of leek (plants) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.2-37: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Leek whole plant w/o roots	Glyphosate	0.05	90	-	-	-	1
		0.5	89	-	-	-	1
		Overall	89-90	90	-	-	2
	AMPA	0.05	89	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-89	88	-	-	2

Table 6.3.2-37: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
1 Residues of glyphosate and AMPA in blank / untreated matrix were below the limit of detection (< 0.015 mg/kg).							

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of leek (whole plant w/o roots).

Detailed residue results are shown in the table below.

Table 6.3.2-38: Residue levels of glyphosate and AMPA in leek after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00265-01 / 21130, Auxone, Cote-d'Or, France / NEU / 2011	Leek / Kenton	49	Whole plant w/o roots	n.d.	n.d.	77	19.01.2012
S11-00265-02 / L40 0RF Burscough, Lancashire, UK / NEU / 2011	Leek / Parvella	47-49	Whole plant w/o roots	<0.05	n.d.	183	19.01.2012
S11-00265-03 / 45020, Lusia, Rovigo, Italy / SEU / 2011	Leek / Maxim	49	Whole plant w/o roots	n.d.	n.d.	65	19.01.2012
S11-00265-04 / 5570 Letnitsa, Letnitsa, Bulgaria / SEU / 2011	Leek / Starozagorski 72	49	Whole plant w/o roots	n.d.	n.d.	125	19.01.2012

1 Growth stage at harvest
2 <0.05 mg/kg (< limit of quantitation (LOQ) of 0.05 mg/kg)
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any treated or untreated specimens of leek (whole plant w/o roots) sampled at BBCH 49 (commercial maturity), 65-183 days after pre-emergence application of glyphosate at the rate of 2.14-2.54 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially leek) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.3.2/010
Report author	[redacted]
Report year	2012
Report title	Determination of residues of glyphosate and AMPA after one application of MON 52276 in sugar beet (outdoor) at 2 sites in Spain and Italy 2011
Report No	S11-00266
Document No	---
Guidelines followed in study	EU Directive 91/414/EEC as amended by Commission Directive 96/68/EC EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

The objective of the study is to determine the magnitude of the residues of glyphosate and AMPA in sugar beet (leaves with top, roots) after one application of MON 52276, an SL formulation containing 360 g/L of glyphosate acid equivalents.

The study included 2 field trials in the southern zone. The sugar beet fields were treated once, at least 3 days after seeding and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Samples of sugar beet (leaves with tops and roots) were taken for analysis at normal harvest, which was 144-165 days after application. No residues of glyphosate and AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 52276
Batch number:	A9K0106104
EAS test item code:	2011-000115
Active ingredient(s):	Glyphosate (in form of isopropylamine salt)
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	358.8 g/L
Formulation type:	SL
Appearance/colour:	Liquid/yellowish-brown
Certificate of analysis:	12.07.2010
Expiry date:	01.10.2013

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00266-01	Sugar beet	*Beta vulgaris*	Sandrina	Leaves with top	≥ 1 kg
				Roots	≥ 2 kg / ≥ 12 units
S11-00266-02	Sugar beet	*Beta vulgaris*	Gea	Leaves with top	≥ 1 kg
				Roots	≥ 2 kg / ≥ 12 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S11-00266-01):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S11-00266-02):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Italy
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Two residue trials were conducted on sugar beets (outdoor) during 2011 in Spain (S11-00266-01) and Italy (S11-00266-02). One application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at the nominal rate of 6.0 L product/ha at least 3 days after seeding and before crop emergence. The volume of water used to prepare the spray solution was in the range of 180-207 L/ha. The main application parameters are outlined in the table below.

Table 6.3.2-39: Application information

Application code	Treatment number	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00266-01	2	BBCH 00	2.222	180
S11-00266-02	2	BBCH 00	2.453	207

Regions, varieties and cultivation were typical for the cultivation of sugar beets.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with boom sprayers equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

1. Sampling

Specimens of crop from the untreated and treated plots were taken by hand at normal commercial harvest (BBCH 49), which was 144-165 days after application. Each field specimen was taken from at least 12 different points distributed over the plot. The ends of the rows (1 m) were left unharvested. No specimens were taken from the outer plants of the plots or from the area of spray overlap. Leaves with top were separated from roots and soil was removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. Specimens were taken in duplicate (with one of the duplicates serving as retain sample). After sampling the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

Table 6.3.2-40: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00266-01	Sugar beet	Leaves with top	165	49	≥ 1 kg	26.09.2011
		Roots	165	49	≥ 2 kg / ≥ 12 units	26.09.2011
S11-00266-02	Sugar beet	Leaves with top	144	49	≥ 1 kg	09.08.2011
		Roots	144	49	≥ 2 kg / ≥ 12 units	09.08.2011

1 Days after last application.

2. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant materials (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in sugar beet (leaves with top, roots) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained in a deep frozen condition and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 181 days, and the maximum interval from extraction to analysis was 0 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of sugar beet (leaves with top, root) specimens, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) each were performed. The results are summarised in the table below.

Table 6.3.2-41: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Sugar beet, leaves with top	Glyphosate	0.05	96	-	-	-	1
		0.5	93	-	-	-	1
		Overall	93-96	95	-	-	2
	AMPA	0.05	94	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-94	91	-	-	2
Sugar beet, roots	Glyphosate	0.05	91	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90-91	91	-	-	2
	AMPA	0.05	93	-	-	-	1
		0.5	89	-	-	-	1
		Overall	89-93	91	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied, specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 52276 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of sugar beet (leaves with top, roots).

Detailed residue levels are shown in the table below.

Table 6.3.2-42: Residue levels of glyphosate and AMPA in sugar beet after one application of MON 52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2, 3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S11-00266-01 / 42210, Barca, Soria, Spain / SEU / 2011	Sugar beet / Sabdrina	49	Leaves with top	n.d.	n.d.	165	06.02.2012
			Root	n.d.	n.d.	165	06.02.2012
S11-00266-02 / 40054 Budrio, Bologna, ltaly / SEU / 2011	Sugar beet / Gea	49	Leaves with top	n.d.	n.d.	144	06.02.2012
			Root	n.d.	n.d.	144	06.02.2012

1 Growth stage at harvest
2 <0.05 mg/kg (< limit of quantitation (LOQ) of 0.05 mg/kg)
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of sugar beet (leaves with top, roots) sampled at BBCH 49 (commercial maturity), 144-165 days after pre-emergence application of glyphosate at the rate of 2.22-2.45 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative pre-emergence use for glyphosate in vegetables (and especially sugar beet) both in Southern and Northern Europe.

Assessment and conclusion by RMS:

Study previously submitted to the EU

Data point:	CA 6.3.2/011
Report author	[redacted]
Report year	1978
Report title	Glyphosate residues in strawberry samples following pre-plant Roundup application
Report No	A25
Document No	-
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Glyphosate Monograph (1998)

Short description of study design and observations:	Four trials were conducted on strawberry during the 1977 season in the United Kingdom. In all trials there was a single application of Roundup® at a rate of 1.8 or 3.6 kg a.s./ha. Samples of strawberry fruit were collected between 233 and 305 days after treatment and analysed for glyphosate and AMPA. The residues of glyphosate and AMPA in strawberry samples were analysed by partition-extraction, ion exchange chromatography derivatisation to the N-trifluoroacetyl methyl esters and determination by GLC using a phosphorus specific flame photometric detector. Percent recovery in strawberry fruit averaged at 68 % for glyphosate and 59 % for AMPA.
Short description of results:	No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated strawberry samples.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted to GLP. Furthermore the average recoveries for glyphosate and AMPA are very low with 68 % and 59 %, respectively.
Category study in AIR 5 dossier (L docs)	Category 3b

Study previously submitted to the EU

Data point:	CA 6.3.2/012
Report author	[redacted]
Report year	1977
Report title	CP 67573 : Determination of crop residues in salads, onions, carots, peas and beans
Report No	A16
Document No	
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Glyphosate Monograph (1998)
Short description of study design and observations:	Six trials were conducted on salads during the 1977 season in France. In all trials there was a single application of Roundup (MON 2139) at a rate ranging from 1.44 to 8.64 kg a.s./ha. Samples of salads were collected between 54 and 89 days after treatment and analysed for glyphosate and AMPA. Six trials were conducted on onions during the 1977 season in France and the United Kingdom. In all trials there was a single application of Roundup (MON 2139) at a rate ranging from 1.44 to 8.64 kg a.s./ha. Samples of onions were collected between 80 and 181 days after treatment and analysed for glyphosate and AMPA. Four trials were conducted on carrots during the 1977 season in France. In all trials there was a single application of Roundup

	(MON 2139) at a rate ranging from 1.44 to 8.64 kg a.s./ha. Samples of carrots were collected between 101 and 140 days after treatment and analysed for glyphosate and AMPA. Two trials were conducted on peas during the 1976 and 1977 seasons in the United Kingdom. In both trials there was a single application of Roundup (MON 2139) at a rate ranging from 2.16 to 4.32 kg a.s./ha. Samples of peas were collected 242 days after treatment and analysed for glyphosate and AMPA. One trial was conducted on beans during the 1977 season in the United Kingdom. There was a single application of Roundup (MON 2139) at a rate ranging from 2.16 to 4.32 kg a.s./ha. Samples of peas were collected 100 days after treatment and analysed for glyphosate and AMPA. The residues of glyphosate and AMPA in samples of the various crops (salad, onion, carrots, peas, and beans) were analysed by partition-extraction, ion exchange chromatography, derivatisation to the N-trifluoroacetyl methyl esters and determination by GLC using a phosphorus specific flame photometric detector. Percent recovery in salads averaged at 59 % for glyphosate and 42 % for AMPA. Percent recovery in onions averaged at 47 % for glyphosate and 37 % for AMPA. Percent recovery in carrots averaged at 62 % for glyphosate and 48 % for AMPA. Percent recovery in peas averaged at 49 % for glyphosate and 36 % for AMPA. Percent recovery in beans averaged at 53 % for glyphosate and 41 % for AMPA.
Short description of results:	Glyphosate residues ranged from below the limit of quantitation (LOQ) of 0.05 to 0.1 mg/kg in treated salad samples. Glyphosate residues ranged from below the LOQ to 0.050 mg/kg in treated onion samples. Glyphosate residues ranged from below the LOQ to 0.08 mg/kg in treated carrot samples. No residues of glyphosate above the limit of quantitation were found in any of the treated pea samples. Glyphosate residues ranged from below the LOQ to 0.1 mg/kg in treated bean samples. No residues of glyphosate above the limit of quantitation were found in any of the untreated salads, onions, carrots, peas, and beans samples. No residues of AMPA above the limit of quantitation were found in any of the treated or untreated salads, onions, carrots, peas, and beans samples. Contamination during sample collection is strongly suspected in the trial in which glyphosate residue at 0.1 mg/kg was found in salad, as residues in higher-rate treatments in the same trial remained below the LOQ.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted to GLP. Furthermore the average recoveries are very low with values between 49 % and 62 % for glyphosate and between 36 % and 48 % for AMPA.
Category study in AIR 5 dossier (L docs)	Category 3b

Study previously submitted to the EU

Data point:	CA 6.3.2/013
Report author	[redacted]
Report year	1977
Report title	CP 67573 : Determination of crop residues in kale and swedes
Report No	A13
Document No	RIP95-01215 MON
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Glyphosate Monograph (1998)
Short description of study design and observations:	Four trials were conducted on kale during the 1976 season in the United Kingdom. All of the trials were conducted with a single application of Roundup (MON 2139) directed to the ground prior to planting (pre-drilling of crop) at a rate ranging from 1.44 to 3.6 kg a.s./ha. Samples of kale roots and leaves were collected between 144 and 199 days after treatment and analysed for glyphosate and AMPA. Two trials were conducted on swede during the 1976 season in the United Kingdom. Both of the trials were conducted with a single application of Roundup (MON 2139) directed to the ground prior to planting (pre-drilling of crop) at a rate ranging from 1.44 to 3.6 kg a.s./ha. Samples of swede root were collected between 199 and 229 days after treatment and analysed for glyphosate and AMPA. The residues of glyphosate and AMPA in kale and swede samples were analysed by partition-extraction, ion exchange chromatography, derivatisation to the N-trifluoroacetyl methyl esters, and determination by GLC using a phosphorus specific flame photometric detector. Percent recovery in kale root averaged at 78 % for glyphosate and 67 % for AMPA. Percent recovery in kale leaves averaged at 71 % for glyphosate and 56 % for AMPA. Percent recovery in swede root averaged at 74 % for glyphosate and 72 % for AMPA.
Short description of results:	No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated kale or swede samples.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Although application date and sampling dates are listed in the report, no date is given for crop planting. The report does state that the crops were "sown" after glyphosate application, but since the date of sowing / planting is not given, it is not possible to determine the interval between application and planting. Furthermore the study was not conducted according to GLP.
Category study in AIR 5 dossier (IL docs)	Category 3b

Study previously submitted to the EU

Data point:	CA 6.3.2/014
Report author	[redacted]
Report year	1976
Report title	CP 67573 : Determination of crop residues in kale, serradella, turnips
Report No	A10
Document No	RIP95-01215 MON
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Glyphosate Monograph (1998)
Short description of study design and observations:	Three trials were conducted on kale during the 1975 season in Germany. All of the trials were treated with a single application of Roundup (MON 2139) applied pre-plant (stubble treatment) at a rate of 2.16 kg a.s./ha. Samples of kale leaves were collected between 89 and 106 days after treatment and analysed for glyphosate and AMPA. Three trials were conducted on serradella (pasture crop) during the 1975 season in Germany. All of the trials were conducted with a single application of Roundup (MON 2139) applied pre-plant (stubble treatment) at a rate of 2.16 kg a.s./ha. Samples of serradella were collected between 89 and 113 days after treatment and analysed for glyphosate and AMPA. Three trials were conducted on turnips during the 1974 and 1975 seasons in Germany. All of the trials were conducted with a single application of Roundup (MON 2139) applied pre-plant (stubble treatment) at a rate of 2.16 kg a.s./ha. Samples of turnip roots and leaves were collected between 89 and 106 days after treatment and analysed for glyphosate and AMPA. The residues of glyphosate and AMPA in kale, serradella, and turnip samples were analysed by partition-extraction, ion exchange chromatography derivatisation to the N-trifluoroacetyl methyl esters, and determination by GLC using a phosphorus specific flame photometric detector. Percent recovery in kale root averaged at 90 % for glyphosate and 75 % for AMPA. Percent recovery in kale leaves averaged at 84 % for glyphosate and 71 % for AMPA. Percent recovery in serradella averaged at 79 % for glyphosate and 70 % for AMPA. Percent recovery in turnip root averaged at 87 % for glyphosate and 80 % for AMPA.
Short description of results:	No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated kale, serradella, or turnip samples.

Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted according to GLP.
Category study in AIR 5 dossier (L docs)	Category 3b

Study previously submitted to the EU

Data point:	CA 6.3.2/015
Report author	[redacted]
Report year	1975
Report title	CP 67573: Determination of crop residues in sugar beets tops and roots
Report No	A3
Document No	
Guidelines followed in study	Not provided
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Previous submission	Glyphosate Monograph (1998)
Short description of study design and observations:	Four trials were conducted on sugar beet during the 1973 and 1974 seasons in Belgium and the United Kingdom. All of the trials were conducted with a single application of Roundup (MON 2139) applied pre-plant and/or postharvest at a rate ranging from 1.44 to 2.88 kg a.s./ha. Samples of root and tops were collected between 205 and 375 days after treatment and analysed for glyphosate and AMPA. The residues of glyphosate and AMPA in sugar beet samples were analysed by aqueous extraction, ion exchange chromatography, derivatisation to the N-trifluoroacetyl methyl esters and determination by GLC using a phosphorus specific flame photometric detector. Percent recovery in sugar beet tops averaged at 69 % for glyphosate and 69 % for AMPA. Percent recovery in sugar beet roots averaged at 62 % for glyphosate and 67 % for AMPA.
Short description of results:	No residues of glyphosate or AMPA above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated or untreated sugar beet root or top samples.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The study was not conducted according to GLP. Furthermore the average recoveries are low with 62 % and 69 % (sugar beet roots and tops, respectively) for glyphosate and 67 % and 69 % (sugar beet roots and tops, respectively) for AMPA
Category study in AIR 5 dossier (L docs)	Category 3b

CA 6.3.3 Inter-row use

For the use of glyphosate as an inter-row treatment of vegetables sufficient data to support the representative GAP are available. The critical GAP is presented in the table below.

Table 6.3.3-1:Critical GAP for the inter-row treatment of vegetables

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Application			Application rate			PHI (days)
		Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product/ha a) max. rate per appl. b) max. total rate per crop/season	g, kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max	
Vegetables (Root and tuber vegetables Bulb vegetables, Fruiting vegetables Legume vegetables Leafy vegetables)	F	Inter-row application: ground directed, shielded spray	Crop BBCH < 20	a) 1 b) 1	a) 3 L/ha b) 3 L/ha	a) 1.08 kg as/ha b) 1.08 kg as/ha	100 - 400	60

The magnitude of residues of glyphosate and its metabolite AMPA was investigated in 9 studies with glyphosate which are considered valid to address the data point.

For the inter-row use, residues of glyphosate and AMPA in the different vegetables were always below the LOD of 0.015 mg/kg in all trials (14 trials in Northern Europe and 30 trials in Southern Europe). Therefore, the data set is sufficient to derive an MRL for vegetables (inter-row treatment).

Table 6.3.3-2:Overview on residue studies in orchards and vines

Data Point	Crop, commodities	Analyte(s)	Number of trials	Reference	Status
CA 6.3.3/001	Carrot	Glyphosate AMPA	Southern Europe: 4	[redacted] 2016 Report No. S15-00482	Valid
CA 6.3.3/002	Radish	Glyphosate AMPA	Southern Europe: 2	[redacted] 2016 Report No. S15-00467	Valid
CA 6.3.3/003	Bulb onion	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 6	[redacted] 2016 Report No. S15-00466	Valid
CA 6.3.3/004	Tomato	Glyphosate AMPA	Southern Europe: 4	[redacted] 2016 Report No. S15-00465	Valid
CA 6.3.3/005	Cucumber	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2016 Report No. S15-00464	Valid
CA 6.3.3/006	Courgette	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2016 Report No. S15-00463	Valid
CA 6.3.3/007	Lettuce head	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 4	[redacted] 2016 Report No. S15-00460	Valid
CA 6.3.3/008	Parsley	Glyphosate AMPA	Northern Europe: 2 Southern Europe: 2	[redacted] 2016 Report No. S15-00459	Valid

Table 6.3.3-2:Overview on residue studies in orchards and vines

Data Point	Crop, commodities	Analyte(s)	Number of trials	Reference	Status
CA 6.3.3/009	Green beans	Glyphosate AMPA	Northern Europe: 4 Southern Europe: 4	[redacted] 2016 Report No. S15-00461	Valid

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/01
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in carrots (outdoor) at 4 sites in Southern Europe 2015
Report No	S15-00482
Document No	---
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in carrot (roots without leaves) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials in the southern zone. The carrot fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of carrot root without leaves were taken for analysis at normal harvest, which was 60-61 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00482-01	Carrot	*Daucus carota* subsp. sativus	Dordonia	Roots	≥ 2 kg / 48 units
S15-00482-02	Carrot	*Daucus carota* subsp. sativus	Samson	Roots	≥ 2 kg / ≥ 29 units
S15-00482-03	Carrot	*Daucus carota* subsp. sativus	Primo	Roots	≥ 2 kg / > 50 units
S15-00482-04	Carrot	*Daucus carota* subsp. sativus	Chambord	Roots	≥ 2 kg / ≥ 45 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00482-01):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00482-02 and S15-00482-03):	Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00482-04):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on carrots (outdoor) during the 2015 season in Italy (S15-00482-01), Bulgaria (S15-00482-02 and S15-00482-03), and France (S15-00482-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha 60 days before harvest. The volume of water used to prepare the spray solution was in the range of 299-313 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-3: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00482-01	2	14 BBCH	1.109	308
S15-00482-02	2	16 BBCH	1.127	313
S15-00482-03	2	16 BBCH	1.126	313
S15-00482-04	2	19 BBCH	1.077	299

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of carrots.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of carrots were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 49), which was 60-61 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 15 cm) and far-off the application band (approx. 30 to 250 cm). Leaves and soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.3-4: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00482-01	Carrot	Roots	60	49	≥ 2 kg / 48 units	26.10.2015
S15-00482-02	Carrot	Roots	60	49	≥ 2 kg / ≥ 29 units	25.10.2015
S15-00482-03	Carrot	Roots	61	49	≥ 2 kg / > 50 units	16.09.2015
S15-00482-04	Carrot	Roots	61	49	≥ 2 kg / ≥ 45 units	06.07.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in carrot roots (EAS Chem study S11-03331). The limit of quantitation (LOQ) in carrot (roots) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 240 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of carrot (roots) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.3-5: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Carrot, roots	Glyphosate	0.05	82	-	-	-	1
		0.5	72	-	-	-	1
		Overall	72-82	77	-	-	2
	AMPA	0.05	88	-	-	-	1
		0.5	84	-	-	-	1
		Overall	84-88	86	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of carrots (roots).

Detailed residue levels are shown in the table below.

Table 6.3.3-6: Residue levels of glyphosate and AMPA in carrot after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00482-01 / 44026 Bosco Mesola, Ferrara, Italy / SEU / 2015	Carrot / Dordonia	49	Roots / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	03.03.2016
			Roots / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

Table 6.3.3-6: Residue levels of glyphosate and AMPA in carrot after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00482-02 / 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Carrot / Samson	49	Roots / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	03.03.2016
			Roots / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00482-03 / 4455 Chernogorovo, Pazardjik, Bulgaria / SEU / 2015	Carrot / Primo	49	Roots / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	61	03.03.2016
			Roots / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00482-04 / 66700 Argelès-sur-Mer, Pyrénées-Orientales, France / SEU / 2015	Carrot / Chambord	49	Roots / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	61	03.03.2016
			Roots / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of carrot (roots) sampled at BBCH 49 (commercial maturity), 60-61 days after inter row band application of glyphosate at the rate of 1.08-1.13 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative inter row use for glyphosate in vegetables (and especially carrots) in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/002
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in radish (outdoor) at 2 sites in Southern Europe 2015
Report No	S15-00467
Document No	---
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in radish (tops and roots) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 2 field trials in the southern zone. The radish fields were treated once. The test item was applied, to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of radish were taken for analysis at normal harvest, which was 30-31 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L

Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00467-01	Radish	*Raphanus sativus var. niger*	Candela di Ghiaccio	Roots	≥ 2 kg / > 50 units
				Tops (leaves)	≥ 1 kg / > 50 units
S15-00467-02	Radish	*Raphanus sativus var. niger*	Celesta	Roots	≥ 2 kg / > 50 units
				Tops (leaves)	≥ 1 kg / > 50 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00467-01):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00467-02):	Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Two residue trials were conducted on radish (outdoor) during the 2015 season in Italy (S15-00467-01) and Bulgaria (S15-00467-02). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha about 30 days before harvest. The volume of water used to prepare the spray solution was in the range of 307-317 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-7: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00467-01	2	14 BBCH	1.104	307
S15-00467-02	2	11 BBCH	1.142	317

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of radish.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of radishes were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 49), which was 30-31 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 30 cm) and far-off the application band (approx. 30 to 60 cm). Adhering soil was removed. Roots and

tops (leaves) were separated in distinct analytical samples. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 0.5 hours of sampling in the field).

Table 6.3.3-8: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00467-01	Radish	Roots	31	49	≥ 2 kg / > 50 units	24.09.2015
S15-00467-01	Radish	Tops (leaves)	31	49	≥ 1 kg / > 50 units	24.09.2015
S15-00467-02	Radish	Roots	30	49	≥ 2 kg / > 50 units	04.06.2015
S15-00467-02	Radish	Tops (leaves)	30	49	≥ 1 kg / > 50 units	04.06.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant commodities with a high water content (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 176 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in radish roots and radish tops (3 replicates per analyte and matrix at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.3-9: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Radish, roots	Glyphosate	Quantification transition 168 >63 m/z					
		0.05	104, 107, 91	101	-	8.4	3
		0.5	86, 80, 79	82	-	4.6	3
		Overall	79-107	91	-	13	6
		Confirmation transition 168 >79 m/z					
		0.05	93, 100, 90	94	-	5.4	3
		0.5	86, 78, 77	80	-	6.1	3
		Overall	77-100	87	-	10	6
Radish, tops (leaves)	Glyphosate	Quantification transition 168 >63 m/z					
		0.05	78, 86, 87	84	-	5.9	3
		0.5	82, 79, 77	79	-	3.2	3
		Overall	77-87	82	-	5.2	6
		Confirmation transition 168 >79 m/z					
		0.05	74, 83, 85	81	-	7.3	3
		0.5	81, 77, 76	78	-	3.4	3
		Overall	74-85	79	-	5.4	6
Radish, roots	AMPA	Quantification transition 110 >63 m/z					
		0.05	87, 86, 83	85	-	2.4	3
		0.5	92, 84, 90	89	-	4.7	3
		Overall	83-92	87	-	4.0	6
		Confirmation transition 110 >79 m/z					
		0.05	81, 87, 76	81	-	6.8	3
		0.5	87, 95, 87	90	-	5.2	3
		Overall	76-95	86	-	7.5	6
Radish, tops (leaves)	AMPA	Quantification transition 110 >63 m/z					
		0.05	80, 82, 83	82	-	1.9	3
		0.5	88, 82, 92	87	-	5.8	3
		Overall	80-92	85	-	5.4	6
		Confirmation transition 110 >79 m/z					
		0.05	72, 88, 81	80	-	10	3
		0.5	100, 87, 90	92	-	7.4	3
		Overall	72-100	86	-	11	6

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of radish (tops and roots).

Detailed residue levels are shown in the table below.

Table 6.3.3-10: Residue levels of glyphosate and AMPA in radish after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00467-01 / 40054 Mezzolara, Bologna, Italy / SEU / 2015	Radish / Candela di Ghiaccio	49	Roots / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	31	27–28.11.2015
			Roots / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
			Tops (leaves) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.		27–28.11.2015
			Tops (leaves) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00467-02 / 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Radish / Celesta	49	Roots / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	30	27–28.11.2015
			Roots / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
			Tops (leaves) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.		27–28.11.2015
			Tops (leaves) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of radish (tops and roots) sampled at BBCH 49 (commercial maturity), 30-31 days after inter row band application of glyphosate at the rate of 1.10-1.14 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. The samples were taken 30 days after the application instead of 60 days as stated for the representative use for inter row application in vegetables. The shorter PHI can be considered as a worst case. Furthermore, the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative inter row use for glyphosate in vegetables (and especially radish) in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/003
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in bulb onions (outdoor) at 4 sites in Southern and 2 sites in Northern Europe 2015
Report No	S15-00466
Document No	-
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in bulb onions (bulb) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 6 field trials (2 trials in the northern zone and 4 trials in the southern zone). The onion fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of onion bulbs were taken for analysis at normal harvest, which was 59-61 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00466-01	Bulb onion	*Allium cepa*	Rose de Figueres	Bulb	≥ 2 kg / ≥ 12 units
S15-00466-02	Bulb onion	*Allium cepa*	Cassiopea	Bulb	≥ 2 kg / ≥ 31 units
S15-00466-03	Bulb onion	*Allium cepa*	Derek	Bulb	≥ 2 kg / 16 units
S15-00466-04	Bulb onion	*Allium cepa*	Dulce Fuentes	Bulb	≥ 2 kg / ≥ 12 units
S15-00466-05	Bulb onion	*Allium cepa*	Sturon	Bulb	≥ 2 kg / 30 units
S15-00466-06	Bulb onion	*Allium cepa*	Rawhide	Bulb	≥ 2 kg / > 12 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00466-01):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S15-00466-02):	Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00466-03):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00466-04):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S15-00466-05):	Eurofins Agroscience Services GmbH, 21684 Stade, Germany

Field phase (S15-00466-06): Eurofins Agroscience Services Austria GmbH, 8200 Gleisdorf, Austria
Analytical phase: Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Six residue trials were conducted on bulb onion (outdoor) during the 2015 season in Southern France (S15-00466-01), Bulgaria (S15-00466-02), Italy (S15-00466-03), Spain (S15-00466-04), Germany (S15-00466-05), and Austria (S15-00466-06). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha 60 days before harvest. The volume of water used to prepare the spray solution was in the range of 280-343 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-11: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00466-01	2	12-13 BBCH	1.065	296
S15-00466-02	2	19 BBCH	1.157	321
S15-00466-03	2	41 BBCH	1.236	343
S15-00466-04	2	41 BBCH	1.008	280
S15-00466-05	2	15 BBCH	1.124	312
S15-00466-06	2	17 BBCH	1.185	329

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of onions.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of onions were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 49), which was 59-61 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 15 cm) and far-off the application band (approx. 30 to 85 cm). Leaves and soil were removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

Table 6.3.3-12: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00466-01	Bulb onion	Bulb	60	49	≥ 2 kg / ≥ 12 units	23.06.2015
S15-00466-02	Bulb onion	Bulb	60	49	≥ 2 kg / ≥ 31 units	08.09.2015
S15-00466-03	Bulb onion	Bulb	59	49	≥ 2 kg / 16 units	23.07.2015
S15-00466-04	Bulb onion	Bulb	60	49	≥ 2 kg / ≥ 12 units	25.08.2015
S15-00466-05	Bulb onion	Bulb	61	49	≥ 2 kg / 30 units	10.08.2015
S15-00466-06	Bulb onion	Bulb	61	49	≥ 2 kg / ≥ 12 units	18.08.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in onion bulb (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 195 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of onion (bulb) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.3-13: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Onion, bulb	Glyphosate	0.05	90	-	-	-	1
		0.5	82	-	-	-	1
		Overall	82-90	86	-	-	2
	AMPA	0.05	93	-	-	-	1
		0.5	83	-	-	-	1
		Overall	83-93	88	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of onion (bulbs).

Detailed residue levels are shown in the table below.

Table 6.3.3-14: Residue levels of glyphosate and AMPA in onions after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00466-01 / 66250 Saint-Laurent de la Salanque, Pyrénées-Orientales, France / SEU / 2015	Bulb onion / Rose de Figueres	49	Bulbs from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	05.01.2016
			Bulbs from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00466-02 / 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Bulb onion / Cassiopea	49	Bulbs from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	05.01.2016
			Bulbs from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00466-03 / 40061 Minerbio, Emilia Romagna, Italy / SEU / 2015	Bulb onion / Derek	49	Bulbs from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	59	05.01.2016
			Bulbs from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00466-04 / 50740 Fuentes de Ebro, Aragon, Spain / SEU / 2015	Bulb onion / Dulce Fuentes	49	Bulbs from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	05.01.2016
			Bulbs from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

Table 6.3.3-14: Residue levels of glyphosate and AMPA in onions after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00466-05 / 27449 Kutenholz, Lower Saxony, Germany / NEU / 2015	Bulb onion / Sturon	49	Bulbs from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	61	05.01.2016
			Bulbs from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00466-06 / 8142 Wundschuh, Styria, Austria / NEU / 2015	Bulb onion / Rawhide	49	Bulbs from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	61	05.01.2016
			Bulbs from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of onion (bulbs) sampled at BBCH 49 (commercial maturity), 59-61 days after inter row band application of glyphosate at the rate of 1.01-1.24 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative inter row use for glyphosate in vegetables (and especially bulb onions) in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/004
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in tomato (outdoor) at 4 sites in Southern Europe 2015
Report No	S15-00465
Document No	MSL0027498
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in tomato (fruit) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials in the southern zone. The tomato fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of tomato fruit were taken for analysis at normal harvest, which was 57-59 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L

Formulation type: SL
Appearance/colour: Liquid/brown
Certificate of analysis: 16.07.2015
Expiry date: 18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00465-01	Tomato	*Solanum lycopersicum*	H9036	Fruit	≥ 2 kg / 35 units
S15-00465-02	Tomato	*Solanum lycopersicum*	Hector	Fruit	≥ 2 kg / ≥ 12 units
S15-00465-03	Tomato	*Solanum lycopersicum*	Gamlex	Fruit	≥ 2 kg / ≥ 38 units
S15-00465-04	Tomato	*Solanum lycopersicum*	Rugby F1	Fruit	≥ 2.2 kg / 24 units

Test facilities

Study directory: Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00465-01): Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S15-00465-02): Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S15-00465-03): Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00465-04): Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Analytical phase: Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on tomato (outdoor) during the 2015 season in Spain (S15-00465-01), France (S15 00465-02), Italy (S15-00465-03), and Bulgaria (S15-00465-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha 57 59 days before harvest. The volume of water used to prepare the spray solution was in the range of 297-338 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-15: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00465-01	2	51 BBCH	1.216	338
S15-00465-02	2	62 BBCH	1.097	305
S15-00465-03	2	29 BBCH	1.075	298
S15-00465-04	2	25 BBCH	1.071	297

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of tomato.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of tomatoes were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 87-89), which was 57-59 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 50 cm) and far-off the application band (approx. 15 to 350 cm). Stems/calyces were removed from the fruits. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3 hours of sampling in the field).

Table 6.3.3-16: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00465-01	Tomato	Fruit	57	87-89	≥ 2 kg / 35 units	10.09.2015
S15-00465-02	Tomato	Fruit	59	87-89	≥ 2 kg / ≥ 12 units	24.08.2015
S15-00465-03	Tomato	Fruit	59	89	≥ 2 kg / ≥ 38 units	28.08.2015
S15-00465-04	Tomato	Fruit	59	89	≥ 2.2 kg / 24 units	18.09.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in tomato (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 102 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of tomato specimens, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ) each were performed. The results are summarised in the table below.

Table 6.3.3-17: Recovery results

Matrix	Analyte	Fortification	Recovery[1]

		level (mg/kg)	Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Tomato, fruit	Glyphosate	0.05	86	-	-	-	1
		0.5	81	-	-	-	1
		Overall	81-86	84	-	-	2
	AMPA	0.05	84	-	-	-	1
		0.5	89	-	-	-	1
		Overall	84-89	87	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of tomato (fruit).

Detailed residue levels are shown in the table below.

Table 6.3.3-18: Residue levels of glyphosate and AMPA in tomato after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00465-01 / 50637 Remolinos, Aragon, Spain / SEU / 2015	Tomato / H9036	87-89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	57	04.12.2015
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00465-02 / 66200 Elne, Pyrénées-Orientales, France / SEU / 2015	Tomato / Hector	87-89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	59	04.12.2015-05.12.2015
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00465-03 / 44023 Lagosanto, Emilia Romagna, Italy /	Tomato / Gamlex	89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	59	05.12.2015

Table 6.3.3-18: Residue levels of glyphosate and AMPA in tomato after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
SEU / 2015			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00465-04 / 4455 Chernogorovo, Pazardjik, Bulgaria / SEU / 2015	Tomato / Rugby F1	89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	59	05.12.2015
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of tomato (fruit) sampled at BBCH 87-89 (commercial maturity), 57-59 days after inter row band application of glyphosate at the rate of 1.07-1.22 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative inter row use for glyphosate in vegetables (and especially tomato) in Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/005
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in cucumber (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015
Report No	S15-00464
Document No	MSL0027497

Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in cucumber (fruit) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials (2 in the northern zone and 2 in the southern zone). The cucumber fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of cucumber fruit were taken for analysis at normal harvest, which was 60 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00464-01	Cucumber	*Cucumis sativus*	Timor F1	Fruit	≥ 2.5 kg / 13 units
S15-00464-02	Cucumber	*Cucumis sativus*	Raider	Fruit	≥ 3.7 kg / 12 units
S15-00464-03	Cucumber	*Cucumis sativus*	Tanja	Fruit	≥ 2.1 kg / 12 units
S15-00464-04	Cucumber	*Cucumis sativus*	Raider	Fruit	≥ 5 kg / 12 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00464-01):	Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00464-02):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S15-00464-03):	Eurofins Agroscience Services GmbH, 71706 Markgröningen, Germany
Field phase (S15-00464-04):	Eurofins Agroscience Services SAS, 49350 Gennes, France
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on cucumber (outdoor) during the 2015 season in Bulgaria (S15-00464-01), Southern France (S15-00464-02), Germany (S15-00464-03), and Northern France (S15-00464-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha 60 days before harvest. The volume of water used to prepare the spray solution was in the range of 293-326 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-19: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00459-01	2	14 BBCH	1.091	303
S15-00459-02	2	24 BBCH	1.101	306
S15-00459-03	2	15 BBCH	1.056	293
S15-00459-04	2	61 BBCH	1.174	326

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of cucumber.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of cucumber were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 79-89), which was 60 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 40 cm) and far-off the application band (approx. 15 to 150 cm). Stems were removed from the fruits before freezing. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 4.5 hours of sampling in the field).

Table 6.3.3-20: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00464-01	Cucumber	Fruit	60	79	≥ 2.5 kg / 13 units	31.07.2015
S15-00464-02	Cucumber	Fruit	60	89	≥ 3.7 kg / 12 units	07.08.2015
S15-00464-03	Cucumber	Fruit	60	89	≥ 2.1 kg / 12 units	28.09.2015
S15-00464-04	Cucumber	Fruit	60	89	≥ 5 kg / 12 units	04.09.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in cucumber fruit (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 164 days, and the maximum interval from extraction to analysis was 2 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of cucumber specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.3-21: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Cucumber, fruit	Glyphosate	0.05	90	-	-	-	1
		0.5	84	-	-	-	1
		Overall	84-90	87	-	-	2
	AMPA	0.05	92	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90-92	91	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of cucumber (fruit).

Detailed residue levels are shown in the table below.

Table 6.3.3-22: Residue levels of glyphosate and AMPA in cucumber after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00464-01 / 4455 Chernogorovo, Pazardjik, Bulgaria / SEU / 2015	Cucumber / Timor F1	79	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	06.01.2016
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00464-02 / 66250 Saint-Laurent de la Salanque, Pyrénées-Orientales, France / SEU / 2015	Cucumber / Raider	89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	06.01.2016
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00464-03 / 71706 Markgröningen, Baden-Württemberg, Germany / NEU / 2015	Cucumber / Tanja	89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	06.01.2016
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00464-04 / 49730 Varennes sur Loire, Pays de la Loire, France / NEU / 2015	Cucumber / Raider	89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	06.01.2016
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of cucumber (fruit) sampled at BBCH 79-89 (commercial maturity), 60 days after inter row band application of glyphosate at the rate of 1.06-1.17 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative inter row use for glyphosate in vegetables (and especially cucumber) in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/006
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in courgette (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015
Report No	S15-00463
Document No	MSL0027496
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in courgette (fruit) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials (2 in the northern zone and 2 in the southern zone). The courgette fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of courgette were taken for analysis at normal harvest, which was 59-60 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00463-01	Courgette	*Cucurbita pepo* var. giromontiina	Beara	Fruit	≥ 2 kg / 12 units
S15-00463-02	Courgette	*Cucurbita pepo* var. giromontiina	Lipan	Fruit	≥ 8.7 kg / 12 units
S15-00463-03	Courgette	*Cucurbita pepo* var. giromontiina	Opera	Fruit	≥ 2 kg / ≥ 14 units
S15-00463-04	Courgette	*Cucurbita pepo* var. giromontiina	Super Jedida F1	Fruit	≥ 2.8 kg / 12 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00463-01):	Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00463-02):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S15-00463-03):	Eurofins Agroscience Services GmbH, 69168 Wiesloch, Germany
Field phase (S15-00463-04):	Eurofins Agroscience Services SAS, 45300 Rouvres St Jean, France
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on courgette (outdoor) during the 2015 season in Bulgaria (S15-00463-01), Southern France (S15-00463-02), Germany (S15-00463-03), and Northern France (S15-00463-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha 59 60 days before harvest. The volume of water used to prepare the spray solution was in the range of 299-328 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-23: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00463-01	2	10 BBCH	1.179	328
S15-00463-02	2	29 BBCH	1.077	299
S15-00463-03	2	10 BBCH	1.098	305
S15-00463-04	2	00 BBCH	1.129	314

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of courgette.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of courgette were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 75-89), which was 59-60 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Stems were removed from the fruits before freezing. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. According to the study protocol it was planned to take separate samples from plants close to the application band (approx. 0 to 15 cm) and far-off the application band (approx. 10 to 45 cm). Moreover, on the treated plot the field samples for analysis were to be taken in duplicate and a third field sample was to be taken as a retain sample. However, in practice, during the conduct of the field trials, some of the samples were not taken in duplicate and/or the retain samples were omitted since not enough fruits were available at harvest (trials S15-00463-02 and S15-00463-03). Furthermore, in the trial S15 00463 04 it was not deemed possible to differentiate between plants close to or far-off the application band. Therefore, only one type of samples was taken. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 1.5 hours of sampling in the field).

Table 6.3.3-24: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00463-01	Courgette	Fruit	60	85	≥ 2 kg / 12 units	30.08.2015
S15-00463-02	Courgette	Fruit	60	89	≥ 8.7 kg / 12 units	07.08.2015
S15-00463-03	Courgette	Fruit	59	75	≥ 2 kg / ≥ 14 units	10.09.2015
S15-00463-04	Courgette	Fruit	60	84	≥ 2.8 kg / 12 units	18.09.2015

1 Separate samples were taken, close to and far off the application band, respectively, except in the trial S15-00463-04 where only one type of samples was taken (about 10 cm away from the application band).

2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-

up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant commodities with a high water content (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 208 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ -18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in courgette fruit (3 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.3-25: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Courgette, fruit	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	79, 83, 83	82	-	2.8	3
		0.5	83, 85, 87	85	-	2.4	3
		Overall	79-87	83	-	3.2	6
		Confirmation transition 168 > 79 m/z					
		0.05	85, 83, 78	81	-	4.3	3
		0.5	88, 81, 86	85	-	4.2	3
		Overall	78-88	83	-	4.5	6
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	77, 81, 85	81	-	4.9	3
		0.5	88, 92, 85	88	-	4.0	3
		Overall	77-92	85	-	6.2	6
		Confirmation transition 110 > 79 m/z					
		0.05	76, 80, 80	79	-	2.9	3
		0.5	78, 77, 80	78	-	2.0	3
		Overall	76-80	79	-	2.2	6

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of courgette (fruit).

Detailed residue levels are shown in the table below.

Table 6.3.3-26: Residue levels of glyphosate and AMPA in courgette after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00463-01 / 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Courgette / Beara	85	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	02.03.2016
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00463-02 / 66250 Saint-Laurent de la Salanque, Pyrénées-Orientales, France / SEU / 2015	Courgette / Lipan	89	Fruit / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	02.03.2016
			Fruit / from plants far-off the application band(s)	n.d.	n.d.		
S15-00463-03 / 69124 Heidelberg-Neurott, Baden-Württemberg, Germany / NEU / 2015	Courgette / Opera	75	Fruit / from plants next to the application band(s)	n.d.	n.d.	59	02.03.2016
			Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00463-04 / 45480 Boisseaux, Loiret, France / NEU / 2015	Courgette / Super Jedida F1	84	Fruit / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.	60	02.03.2016-03.03.2016

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of courgette (fruit) sampled at BBCH 75-89 (commercial maturity), 59-60 days after inter row band application of glyphosate at the rate of 1.08-1.18 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It is noted that in the trial S15-00463-04 the application was performed before crop emergence, which may be challenged for a typical inter row application. However, the study plan only requested that the application be performed 60 days before harvest without specifying any crop growth stage and, therefore, this is not a deviation to the study plan. It is conclude that at least three of the study trials adequately support the representative inter row use for glyphosate in vegetables (and especially courgette) in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/007
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in head lettuce (outdoor) at 4 sites in Southern and 2 sites in Northern Europe 2015
Report No	S15-00460
Document No	MSL0027493
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in head lettuce (heads) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 6 field trials (2 in the northern zone and 4 in the southern zone). The lettuce fields were treated once at BBCH 15. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of lettuce heads were taken for analysis at

normal harvest, which was 19-45 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L
Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00460-01	Head lettuce	*Lactuca sativa* var. capitata	David	Heads	≥ 1.1 kg / 12 units
S15-00460-02	Head lettuce	*Lactuca sativa* var. capitata	Iceberg	Heads	≥ 3.7 kg / 12 units
S15-00460-03	Head lettuce	*Lactuca sativa* var. capitata	Iceberg	Heads	≥ 1.2 kg / 12 units
S15-00460-04	Head lettuce	*Lactuca sativa* var. capitata	Aitana	Heads	≥ 3.7 kg / 12 units
S15-00460-05	Head lettuce	*Lactuca sativa* var. capitata	Laruna NAS	Heads	≥ 2.7 kg / 12 units
S15-00460-06	Head lettuce	*Lactuca sativa* var. capitata	Vitalis	Heads	≥ 3 kg / 12 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00460-01):	Eurofins Agroscience Services EEOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00460-02):	Eurofins Agroscience Services France SAS, 66200 Elne, France
Field phase (S15-00460-03):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00460-04):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S15-00460-05):	Eurofins Agroscience Services GmbH, 69168 Wiesloch, Germany
Field phase (S15-00460-06):	Eurofins Agroscience Services Austria GmbH, 8200 Gleisdorf, Austria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Six residue trials were conducted on head lettuce (outdoor) during the 2015 season in Bulgaria (S15-00460-01), Southern France (S15-00460-02), Italy (S15-00460-03), Spain (S15-00460-04), Germany (S15-00460-05), and Austria (S15-00460-06). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha at BBCH 15 of the crop. The volume of water used to prepare the spray solution was in the range of 284-335 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-27: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00460-01	2	15 BBCH	1.138	316
S15-00460-02	2	15 BBCH	1.022	284
S15-00460-03	2	15 BBCH	1.206	335
S15-00460-04	2	15 BBCH	0.984	273
S15-00460-05	2	15 BBCH	1.059	294
S15-00460-06	2	15 BBCH	1.159	322

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of head lettuce.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of lettuces were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 49), which was 19-45 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 30 cm) and far-off the application band (approx. 25 to 100 cm). Lettuce heads were sampled by hand. Decayed outer leaves (if any), adhering soil and roots were removed from the heads and discarded. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 3.5 hours of sampling in the field).

Table 6.3.3-28: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00460-01	Head lettuce	Heads; leaves	22	49	≥ 1.1 kg / 12 units	17.06.2015[3]
S15-00460-02	Head lettuce	Heads; leaves	19	49	≥ 3.7 kg / 12 units	04.05.2015
S15-00460-03	Head lettuce	Heads; leaves	45	49	≥ 1.2 kg / 12 units	16.07.2015

Table 6.3.3-28: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00460-04	Head lettuce	Heads; leaves	28	49	≥ 3.7 kg / 12 units	24.07.2015
S15-00460-05	Head lettuce	Heads; leaves	31	49	≥ 2.7 kg / 12 units	15.06.2015
S15-00460-06	Head lettuce	Heads; leaves	34	49	≥ 3 kg / 12 units	30.09.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.
3 The sampling date of 27.06.2015 stated in Table 5 on page 20 of the report is probably erroneous. Based on the date of application and the DALA of 22 days, the sampling date of 17.06.2015 stated in the Tier 1 table on page 51 of the report is probably correct.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in lettuce (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 224 days, and the maximum interval from extraction to analysis was 1 day. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

During analysis of lettuce (heads) specimens, concurrent recoveries were determined for glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and 0.50 mg/kg (10x LOQ). The results are summarised in the table below.

Table 6.3.3-29: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Head lettuce, leaves	Glyphosate	0.05	91	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-91	89	-	-	2
	AMPA	0.05	89	-	-	-	1
		0.5	89	-	-	-	1
		Overall	89	89	-	-	2

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of lettuce (heads).

Detailed residue levels are shown in the table below.

Table 6.3.3-30: Residue levels of glyphosate and AMPA in head lettuce after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00460-01/ 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Head lettuce / David	49	Leaves (head) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	22	14.12.2015 to 15.12.2015
			Leaves (head) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00460-02 / 66200, Alenya, Pyrénées-Orientales, France / SEU / 2015	Head lettuce / Iceberg	49	Leaves (head) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	19	14.12.2015 to 15.12.2015
			Leaves (head) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00460-03 / 40016, San Marino di Bentivoglio, Bologna, Italy / SEU / 2015	Head lettuce / Iceberg	49	Leaves (head) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	45	14.12.2015 to 15.12.2015
			Leaves (head) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00460-04 / 50008, Zaragoza, Aragon, Spain / SEU / 2015	Head lettuce / Aitana	49	Leaves (head) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	28	14.12.2015 to 15.12.2015
			Leaves (head) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00460-05 / 69124, Heidelberg, Baden-Württemberg, Germany / NEU / 2015	Head lettuce / Laruna NAS	49	Leaves (head) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	31	14.12.2015 to 15.12.2015
			Leaves (head) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00460-06 / 8141, Zettling, Styria, Austria / NEU / 2015	Head lettuce / Vitalis	49	Leaves (head) / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	34	14.12.2015 to 15.12.2015
			Leaves (head) / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

Table 6.3.3-30: Residue levels of glyphosate and AMPA in head lettuce after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of lettuce (heads) sampled at BBCH 49 (commercial maturity), 19-45 days after inter row band application of glyphosate at the rate of 0.98-1.21 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. The samples were taken 19-45 days after the application instead of 60 days after the application, as stated for the representative use for inter row application in vegetables. The shorter PHI can be considered as a worst case. Furthermore, the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative inter row use for glyphosate in vegetables (and especially lettuce) in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/008
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in parsley (outdoor) at 2 sites in Southern and 2 sites in Northern Europe 2015
Report No	S15-00459
Document No	MSL0027492
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in parsley (leaves) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 4 field trials (2 in the northern zone and 2 in the southern zone). The parsley fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of parsley were taken for analysis at normal harvest, which was 59-61 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L

Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00459-01	Parsley	*Petroselinum crispum*	Gaia	Leaves	≥ 0.5 kg / > 50 units
S15-00459-02	Parsley	*Petroselinum crispum*	Italian Gigant	Leaves	≥ 0.5 kg / > 50 units
S15-00459-03	Parsley	*Petroselinum crispum*	Gigante d'Italia	Leaves	≥ 0.5 kg / > 50 units
S15-00459-04	Parsley	*Petroselinum crispum*	Gigante d'Italia	Leaves	≥ 0.5 kg / > 12 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00459-01):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00459-02):	Eurofins Agroscience Services EOOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00459-03):	Eurofins Agroscience Services GmbH, 69168 Wiesloch, Germany
Field phase (S15-00459-04):	Eurofins Agroscience Services Austria GmbH, 8200 Gleisdorf, Austria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Four residue trials were conducted on parsley (outdoor) during the 2015 season in Italy (S15-00459-01), Bulgaria (S15-00459-02), Germany (S15-00459-03), and Austria (S15-00459-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha 59-61 days before harvest. The volume of water used to prepare the spray solution was in the range of 302-353 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-31: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00459-01	2	14 BBCH	1.239	344
S15-00459-02	2	12 BBCH	1.151	320
S15-00459-03	2	10 BBCH	1.087	302
S15-00459-04	2	12 BBCH	1.271	353

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of parsley.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of parsley leaves were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 49), which was 59-61 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 30 cm) and far-off the application band (approx. 30 to 60 cm). Adhering soil was removed. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 0.5 hours of sampling in the field).

Table 6.3.3-32: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00459-01	Parsley	Leaves	61	49	≥ 0.5 kg / > 50 units	14.07.2015
S15-00459-02	Parsley	Leaves	60	49	≥ 0.5 kg / > 50 units	11.08.2015
S15-00459-03	Parsley	Leaves	61	49	≥ 0.5 kg / > 50 units	29.06.2015
S15-00459-04	Parsley	Leaves	59	49	≥ 0.5 kg / > 12 units	07.09.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant commodities with a high water content (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 164 days, and the maximum interval from extraction to analysis was 2 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in parsley leaves (3 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.3-33: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Parsley, leaves	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	95, 90, 94	93	-	2.8	3
		0.5	90, 82, 81	84	-	5.8	3
		Overall	81-95	89	-	6.7	6
		Confirmation transition 168 > 79 m/z					
		0.05	91, 92, 90	91	-	1.1	3
		0.5	87, 83, 86	85	-	2.4	3
		Overall	83-92	88	-	3.9	6
Parsley, leaves	AMPA	Quantification transition 110 > 63 m/z					
		0.05	88, 89, 85	87	-	2.4	3
		0.5	90, 91, 91	91	-	0.6	3
		Overall	85-91	89	-	2.6	6
		Confirmation transition 110 > 79 m/z					
		0.05	87, 99, 87	91	-	7.6	3
		0.5	83, 88, 86	86	-	2.9	3
		Overall	83-99	88	-	6.2	6

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of parsley (leaves).

Detailed residue levels are shown in the table below.

Table 6.3.3-34: Residue levels of glyphosate and AMPA in parsley after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00459-01 40057 Lovoleto, Bologna, Italy / SEU / 2015	Parsley / Gaia	49	Leaves / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	61	11.12.2015
			Leaves / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

Table 6.3.3-34: Residue levels of glyphosate and AMPA in parsley after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00459-02 / 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Parsley / Italian Gigant	49	Leaves / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	11.12.2015
			Leaves / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00459-03 / 69207 Bruchhausen, Baden-Württemberg, Germany / NEU / 2015	Parsley / Gigante d'Italia	49	Leaves / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	61	11.12.2015
			Leaves / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00459-04 / 8073 Feldkirchen b. Graz, Styria, Austria / NEU / 2015	Parsley / Gigante d'Italia	49	Leaves / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	59	11.12.2015- 12.12.2015
			Leaves / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of parsley (leaves) sampled at BBCH 49 (commercial maturity), 59-61 days after inter row band application of glyphosate at the rate of 1.09-1.27 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. It adequately supports the representative inter row use for glyphosate in herbs (and especially parsley) in Northern and Southern Europe.

Assessment and conclusion by RMS:

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.3.3/009
Report author	[redacted]
Report year	2016
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in green beans (outdoor) at 4 sites in Southern and 4 sites in Northern Europe 2015
Report No	S15-00461
Document No	MSL0027494
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 1607/VI/97, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in green beans (whole pods) after one application of MON 79351, an SL formulation containing 480 g/L of glyphosate acid equivalents (as the potassium salt).

The study included 8 field trials (4 in the northern zone and 4 in the southern zone). The bean fields were treated once. The test item was applied to the soil between crop rows at a target rate of 1.08 kg glyphosate acid equivalents per hectare. Samples of radish were taken for analysis at normal harvest, which was 29-60 days after application. No residues of glyphosate or AMPA above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated or untreated samples.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 79351
Batch number:	AGF172310C
EAS test item code:	2014-004603
Active ingredient(s):	Glyphosate (in form of potassium salt)
CAS number:	1071-83-6
Content of a.s. nominal:	480 g/L
Content of a.s. analysed:	469.6 g/L

Formulation type:	SL
Appearance/colour:	Liquid/brown
Certificate of analysis:	16.07.2015
Expiry date:	18.06.2019

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00461-01	Green beans	*Phaseolus vulgaris*	Lodi	Whole pod	≥ 0.8 kg / > 50 units
S15-00461-02	Green beans	*Phaseolus vulgaris*	Venice	Whole pod	≥ 0.5 kg / > 24 units
S15-00461-03	Green beans	*Phaseolus vulgaris*	Schubert	Whole pod	≥ 0.5 kg / > 50 units
S15-00461-04	Green beans	*Phaseolus vulgaris*	Cocobel	Whole pod	≥ 0.6 kg / > 50 units
S15-00461-05	Green beans	*Phaseolus vulgaris*	Flagrano	Whole pod	≥ 0.5 kg / ≥ 24 units
S15-00461-06	Green beans	*Phaseolus vulgaris*	Maxi	Whole pod	≥ 0.5 kg / > 50 units
S15-00461-07	Green beans	*Phaseolus vulgaris*	Maxi	Whole pod	≥ 0.5 kg / > 50 units
S15-00461-08	Green beans	*Phaseolus vulgaris*	Imayca	Whole pod	≥ 0.5 kg / > 24 units

Test facilities

Study directory:	Eurofins Agroscience Services GmbH, 16321 Bernau, Germany
Field phase (S15-00461-01):	Eurofins Agroscience Services EEOD, 5570 Letnitsa, Bulgaria
Field phase (S15-00461-02):	Eurofins Agroscience Services France SAS, 30390 Aramon, France
Field phase (S15-00461-03):	Eurofins Agroscience Services SRL, 40016 San Giorgio di Piano, Bologna, Italy
Field phase (S15-00461-04):	Eurofins Agroscience Services SL, 50016 Zaragoza, Spain
Field phase (S15-00461-05):	Eurofins Agroscience Services France SAS, 56860 Sene, France
Field phase (S15-00461-06):	Eurofins Agroscience Services GmbH, 21684 Stade, Germany
Field phase (S15-00461-07):	Eurofins Agroscience Services GmbH, 71706 Markgröningen, Germany
Field phase (S15-00461-08):	Eurofins Agroscience Services Austria GmbH, 8200 Gleisdorf, Austria
Analytical phase:	Eurofins Agroscience Services Chem GmbH, 21079 Hamburg, Germany

B. Methods

1. Field phase

Eight residue trials were conducted on green beans (outdoor) during the 2015 season, one trial each in Bulgaria (S15 00461-01), Southern France (S15-00461-02), Italy (S15-00461-03), Spain (S15-00461-04), Northern France (S15-00461-05), Austria (S15-00461-08), and two trials in Germany (S15-00461-06 and S15-00461-07). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil between crop rows at the nominal rate of 2.25 L product/ha at BBCH 15 of the crop. The volume of water used to prepare the spray solution was in the range of 291-343 L/ha. The main application parameters are outlined in the table below.

Table 6.3.3-35: Application information

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00461-01	2	15 BBCH	1.171	325
S15-00461-02	2	15 BBCH	1.185	329
S15-00461-03	2	15 BBCH	1.234	343
S15-00461-04	2	15 BBCH	1.080	300
S15-00461-05	2	15 BBCH	1.199	333
S15-00461-06	2	15 BBCH	1.117	310
S15-00461-07	2	15 BBCH	1.049	291
S15-00461-08	2	15 BBCH	1.236	343

1 Expressed as acid equivalents

Regions, varieties and cultivation were typical for the cultivation of green bean.

Care was taken that the spray solution was properly homogenised by mixing before application. Application was performed with motorised knapsack sprayer equipped with flat fan nozzles, which were duly calibrated before use. Spray shields were used to minimise crop contamination. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of green beans (pods with seeds) were taken by hand from the untreated and treated plots at normal commercial harvest (BBCH 79), which was 29-60 days after application. Each field sample was taken from at least 12 areas distributed over of the whole plot. A 0.5 m wide strip round the edge of the plot or the end of the rows was not harvested. Separate samples were taken from plants close to the application band (approx. 0 to 50 cm) and far-off the application band (approx. 5 to 90 cm). Whole pods were separated manually from the plants. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use. No diseased or damaged crop was collected. On the treated plot the field samples for analysis were taken in duplicate and a further field sample was taken as a retain sample. After sampling, the control specimens and treated specimens were kept separated by an adequate space at all times. Specimens were deep-frozen immediately after arrival at the test sites (i.e. within less than 10 hours of sampling in the field).

Table 6.3.3-36: Crop sampling information

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00461-01	Green beans	Whole pods	30	79	≥ 0.8 kg / > 50 units	31.07.2015
S15-00461-02	Green beans	Whole pods	35	79	≥ 0.5 kg / > 24 units	09.06.2015
S15-00461-03	Green beans	Whole pods	47	79	≥ 0.5 kg / > 50 units	06.07.2015
S15-00461-04	Green beans	Whole pods	45	79	≥ 0.6 kg / > 50 units	21.09.2015
S15-00461-05	Green beans	Whole pods	60	79	≥ 0.5 kg / ≥ 24 units	15.09.2015
S15-00461-06	Green beans	Whole pods	44	79	≥ 0.5 kg / > 50 units	20.08.2015
S15-00461-07	Green beans	Whole pods	29	79	≥ 0.5 kg / > 50 units	03.09.2015
S15-00461-08	Green beans	Whole pods	45	79	≥ 0.5 kg / > 24 units	06.08.2015

1 Separate samples were taken, close to and far off the application band, respectively.
2 Days after last application.

3. Analytical phase

Residue analysis was conducted according to Monsanto method AG-ME-1294-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water and dichloromethane. Following centrifugation, an aliquot of aqueous phase extract was cleaned-up by solid phase extraction. The analytes were determined by LC-MS/MS using internal standards. The method was previously validated by the same laboratory for the determination of glyphosate and AMPA in various plant commodities with a high water content (EAS Chem study S11-03331). The limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.015 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 189 days, and the maximum interval from extraction to analysis was 2 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

A reduced method validation for the determination of glyphosate and AMPA in green bean pods (3 replicates per analyte at 0.05 mg/kg and 0.5 mg/kg, respectively) was conducted concurrently with the analysis of the study specimens. The results were satisfactory, as shown in the table below.

Table 6.3.3-37: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Green beans (pods)	Glyphosate	Quantification transition 168 > 63 m/z					
		0.05	90, 96, 91	92	-	3.5	3
		0.5	97, 87, 87	90	-	6.4	3
		Overall	87-97	91	-	4.7	6
		Confirmation transition 168 > 79 m/z					
		0.05	94, 95, 84	91	-	6.7	3
		0.5	95, 88, 91	91	-	3.8	3
		Overall	84-95	91	-	4.9	6
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	93, 100, 93	95	-	4.2	3
		0.5	94, 88, 92	91	-	3.3	3
		Overall	88-100	93	-	4.2	6
		Confirmation transition 110 > 79 m/z					
		0.05	89, 95, 93	92	-	3.3	3
		0.5	100, 91, 88	93	-	6.7	3
		Overall	88-100	93	-	4.8	6

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.015 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON 79351 when applied as per the study.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of green beans (whole pods).

Detailed residue levels are shown in the table below.

Table 6.3.3-38: Residue levels of glyphosate and AMPA in parsley after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00461-01/ 5570 Letnitsa, Lovech, Bulgaria / SEU / 2015	Green beans / Lodi	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	30	16.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00461-02 / 30300 Beaucaire, Gard, France / SEU / 2015	Green beans, / Venice	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	35	16.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00461-03 / 48012 Boncellino, Emilia, Romagna, Italy / SEU / 2015	Green beans, / Schubert	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	47	16.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00461-04 / 31360, Funes, Navarra, Spain / SEU / 2015	Green beans, / Cocobel	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	45	16.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00461-05 / 56220, Malansac, Morbihan, France / NEU / 2015	Green beans, / Flagrano	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	60	16.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00461-06 / 27478, Altenbruch, Lower Saxony, Germany / NEU / 2015	Green beans, / Maxi	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	44	16.12.201- 17.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

Table 6.3.3-38: Residue levels of glyphosate and AMPA in parsley after one application of MON 79351 (480 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S15-00461-07 / 71706, Markgröningen, Baden-Württemberg, Germany / NEU / 2015	Green beans, / Maxi	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	29	16.12.201-17.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		
S15-00461-08 / 8200, Hofstätten, Styria, Austria / NEU / 2015	Green beans, / Imayca	79	Whole pods / from plants next to the application band(s)	n.d. n.d.	n.d. n.d.	45	16.12.201-17.12.2015
			Whole pods / from plants far-off the application band(s)	n.d. n.d.	n.d. n.d.		

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): < 0.015 mg/kg
4 Days after last application

III. Conclusion

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any treated or untreated specimens of green beans (whole pods) sampled at BBCH 79 (commercial maturity), 28-60 days after inter row band application of glyphosate at the rate of 1.05-1.24 kg a.s./ha.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was not previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 509. In 7 trials the samples were taken 29-47 days after the application instead of 60 days as stated for the representative use for inter row application in vegetables. The shorter PHI can be considered as a worst case. Furthermore, the residues of both glyphosate and AMPA were below the limit of detection of 0.015 mg/kg. Therefore, the study adequately supports the representative inter row use for glyphosate in vegetables (and especially green beans) in Northern and Southern Europe.

Assessment and conclusion by RMS:

CA 6.4 Feeding Studies

Glyphosate is authorised for use on crops that might be fed to livestock and consideration of the occurrence of residues in commodities of animal origin is required.

The residue studies conducted to the in orchards, vines and in vegetables (pre-emergence, pre-planting and inter-row treatment) show, that residues of glyphosate and AMPA above the LOQ of 0.05 mg/kg are not to be expected for these crops.

Nevertheless, the possible transfer of residues in animal commodities from the proposed uses has been considered. Livestock intake calculations and feeding studies undertaken are provided below. All uses considered in the critical GAP will be included as those cover worst case residues.

To take AMPA into account for the feed burden calculation the total residues for risk assessment were calculated as:

mg/kg glyphosate + mg/kg AMPA x 1.5 (equivalency factor)

Table 6.4-1: Input values for the dietary burden calculation

Feed Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
1 - Forages				
Beet, mangel fodder	0.125	pre-emergence application	0.125	pre-emergence application
Beet, sugar tops	0.125	pre-emergence application	0.125	pre-emergence application
Cabbage, heads leaves	0.125	pre-emergence application	0.125	pre-emergence application
Kale leaves (forage)	0.125	pre-emergence application	0.125	pre-emergence application
Turnip tops (leaves)	0.125	pre-emergence application	0.125	pre-emergence application
2 - Roots & Tubers				
Carrot culls	0.125	pre-emergence application	0.125	pre-emergence application
Cassava/tapioca roots	0.125	pre-emergence application	0.125	pre-emergence application
Potato culls	0.125	pre-emergence application	0.125	pre-emergence application
Swede roots	0.125	pre-emergence application	0.125	pre-emergence application
Turnip roots	0.125	pre-emergence application	0.125	pre-emergence application
4 - By-products				
Apple pomace, wet	0.125	ground application in orchards	0.125	ground application in orchards
Beet, sugar dried pulp	0.125	pre-emergence application	0.125	pre-emergence application
Beet, sugar ensiled pulp	0.125	pre-emergence application	0.125	pre-emergence application
Beet, sugar molasses	0.125	pre-emergence application	0.125	pre-emergence application
Citrus dried pulp	0.125	ground application in orchards	0.125	ground application in orchards
Potato process waste	0.125	pre-emergence application	0.125	pre-emergence application

The results of the calculations are reported in Table 6.4-2.

Table 6.4-2: Results of the dietary burden calculation

Animal species	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)	Trigger exceeded (Yes/No) 0.004 mg/kg bw
	mg/kg bw/d		mg/kg DM				
	Median	Maximum	Median	Maximum			
Cattle (all diets)	0.030	0.030	1.08	1.08	Dairy cattle	Swede roots	Yes
Sheep (all diets)	0.030	0.030	0.79	0.79	Dairy cattle	Swede roots	Yes
Sheep (ewe only)	0.031	0.031	0.92	0.92	Ram/Ewe	Swede roots	Yes

Table 6.4-2: Results of the dietary burden calculation

Animal species	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)	Trigger exceeded (Yes/No) 0.004 mg/kg bw
	mg/kg bw/d		mg/kg DM				
	Median	Maximum	Median	Maximum			
Swine (all diets)	0.031	0.031	0.92	0.92	Swine (breeding)	Swede roots	Yes
Poultry (all diets)	0.019	0.019	0.83	0.83	Poultry layer	Swede roots	Yes
Poultry (layer only)	0.013	0.013	0.19	0.19	Poultry layer	Swede roots	Yes

The calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.004 mg/kg bw.

Livestock feeding studies are available investigating the behaviour of glyphosate, AMPA, *N*-acetyl-glyphosate in ruminants (all analytes), poultry (all analytes) and swine (glyphosate and AMPA).

Table 6.4-3:Overview on feeding studies

Data Point	Animal, matrices Analyte(s)	Reference	Status
CA 6.4.1/001	Magnitude of residues of N-Acetylglyphosate and degradates in laying hen tissues and eggs	[redacted] 2007 Report no. 28212	Valid
CA 6.4.1/002	Magnitude of SC-0224 residues in Eggs and Poultry	[redacted] 1987 Report no. [redacted]87-43	Valid
CA 6.4.1/003	Residue determination of Glyphosate and AMPA in laying hen tissues and eggs following a 28 day feeding study	[redacted] 1987 Report no. [redacted]-6676	Valid
CA 6.4.2/001	Magnitude of residues of N-Acetylglyphosate and degradates in dairy cow tissues and milk	[redacted] 2007 Report no. 28210	Valid
CA 6.4.2/002	Residue determination of Glyphosate and AMPA in dairy cow tissues and milk following a 28 day feeding study	[redacted], 1987 Report no [redacted]-6729	Valid
CA 6.4.2/003	Magnitude of SC-0224 Residues in Meat and Milk	[redacted]1987 Report no. [redacted] 87-44	Valid
CA 6.4.3/001	Residue determination of Glyphosate and AMPA in swine tissues following a 28-day feeding study	[redacted] 1987 Report no. [redacted]-6627	Valid

CA 6.4.1 Poultry

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.1/001
Report author	[redacted]
Report year	2007
Report title	Magnitude of Residues of *N*-Acetylglyphosate and Degradates in Laying Hen Tissues and Eggs
Report No	28212
Document No	[redacted]20088
Guidelines followed in study	EPA Pesticide Assessment Guidelines (Residue Chemistry Test Guidelines OPPTS 806.1480), EU Guidelines (Document 1607/VI/97 rev. 2, 10/6/1999; Appendix G, 7031/VI/95 rev. 4, 22/7/96), Guidance Document on Overview of Residue Chemistry Studies, OECD Environment, Health and Safety Publication, Series of Testing and Assessment No. 64 and Series on Pesticides No. 32, ENV/JM/MONO(2006)32, October 10, 2006
Deviations from current test guideline	A review of this study indicates no deviations from OECD Guideline for the Testing of Chemicals, 505
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes,
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of N-acetyl glyphosate and metabolites in eggs and tissues of laying hens dosed with N-acetyl glyphosate for a period of 35 consecutive days, and at 6, 14, and 20 days after dosing ended (i.e. after a withdrawal period of 6, 14, and 20 days).

N-acetylglyphosate was administered to hens through an aqueous solution for a period of 35 consecutive days on a body weight (bw) basis at target levels of 1.5 mg (Group 1), 5 mg (Group 2), 15 mg (Group 3), and 50 mg (Group 4) N-acetyl glyphosate/kg bw (corresponding to 1.2, 4.0, 12.0 and 40 mg glyphosate equivalents/kg bw. An additional group (Group 5) was also dosed at 50 mg N-acetyl glyphosate/kg bw (40 mg glyphosate equivalents/kg bw), and was used to study depuration of N-acetyl glyphosate residues once dosing had stopped. Each group of hens was divided into three pooled subgroups and the amount of N-acetyl glyphosate required per hen per subgroup was calculated based on the mean body weight (kg bw) per subgroup recorded on Days -1, 7, 14, 21, and 28. These mean weekly dose levels were equivalent to 19.29–24.27, 65.00–92.35, 174.84–246.05, and 596.43-933.33 mg *N*-acetyl glyphosate/kg of diet consumed (dry weight) based upon the actual average daily diet consumption over the 5-week dosing period in this study**.** The equivalent dose levels of glyphosate for Groups 1 through 4 were 15.43–19.42, 52.00–73.88, 139.87–196.84, and 477.14–746.66 mg/kg bw, respectively.

The analytical method LOQ for *N*-acetyl glyphosate and each relevant analyte was 0.025 mg/kg in egg and muscle matrices, and 0.050 mg/kg in liver and fat matrices, expressed as glyphosate equivalents. All analyte and total residue concentration values were also expressed as mg/kg glyphosate equivalents.

Residues were not detected in Day 1 whole eggs in any dose group. The maximum daily total residue levels observed in whole eggs throughout the study were 0.062, 0.116, 0.20, and 0.68 mg/kg glyphosate equivalents at the 1.5, 5, 15, and 50 mg/kg bw dose levels, respectively. The predominant residue in eggs was *N*-acetyl glyphosate. Glyphosate residues were below the LOQ (<0.025 mg/kg) at the 1.5, 5, 15, and 50 mg/kg bw dose levels except for one subgroup on Day 34 at the 1.5 mg/kg bw dose level and two subgroups on Day 34 at the 50 mg/kg bw dose level. Day 31 whole egg sample extracts were screened for AMPA and *N*-acetyl AMPA and no detectable residues were found in the high dose group (50 mg/kg bw). Mean total residues declined quickly during the depuration period from 0.76 to <0.025 mg/kg glyphosate equivalents 10 days after termination of dosing.

Day 21 egg samples were separated into yolk and white subsamples for comparative analysis of residue in egg fractions. Residue levels in both egg fractions were predominantly *N*-acetyl glyphosate. Mean total residue levels of *N*-acetyl glyphosate in egg yolks increased from 0.078 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 1.38 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate in egg whites ranged from <LOQ (<0.025 mg/kg) to 0.045 mg/kg glyphosate equivalents. Across all dose groups, glyphosate residues were below the LOQ in egg yolks and ranged from <LOQ to 0.047 mg/kg glyphosate equivalents in the egg whites.

In tissue samples obtained within *ca* 6 hours of dose completion, residue levels were highest in liver, followed by fat then muscle. *N*-acetyl glyphosate was the predominant residue in all tissue matrices.

In liver, glyphosate, AMPA, and *N*-acetyl AMPA residues were below the LOQ (<0.050 mg/kg) in all dose groups. Mean total residue levels of *N*-acetyl glyphosate increased from 0.19 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 4.3 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 14 days after the end of dosing.

In fat, glyphosate and *N*-acetyl AMPA residues were below the LOQ (<0.050 mg/kg) in all dose groups. AMPA residues were below the LOQ in the 50 mg/kg bw dose group. Mean total residue levels of *N*-acetyl glyphosate increased from 0.11 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 1.33 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 20 days after the end of dosing.

In muscle, glyphosate and *N*-acetyl AMPA residues were below the LOQ (<0.025 mg/kg) in all dose groups. AMPA residues were below the LOQ in the 15 and 50 mg/kg bw dose group. Mean total residue levels of *N*-acetyl glyphosate increased from 0.031 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 0.41 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 6 days after the end of dosing.

Test facilities

Study directory: [redacted]

In-Life phase: [redacted]

Analytical phase: [redacted]

I. Materials and Methods

A. Materials

N-acetyl glyphosate was administered to the treated animals in this study. Further information on the test material is listed in the table below.

1. Test material

Description:	*N*-acetyl glyphosate
Lot number:	002
DuPont SMS Stock No.:	4004911
Active ingredient(s):	*N*-acetyl-N-(phosphonomethyl) glycine
CAS number:	129660-96-4
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	63 % free acid (test item is mixture of disodium and trisodium salts)
Formulation type:	NA
Appearance/colour:	Solid
Certificate of analysis:	25-Apr-2006
Expiry date:	25-Apr-2009
Storage conditions:	Not specified
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Isa Warren laying hens were the test animals used in this study. Details are listed in the table below.

2. Test animals

Species:	Laying hen; Chicken (*Gallus domesticus)*
Gender:	Female
Breed:	*Isa Warren*
Source:	[redacted]
Age:	Point of lay
Weight at dosing, (Day -1):	Ranged from 1.627–1.933 kg per subgroup
Number of animals:	60 hens selected out of a group of 80: (10 in untreated control group, and 10 in each of 4 treated groups (1.5, 5, 15, and 50 mg/kg bw plus 10 additional hens at 50 mg/kg bw for depuration). Each group of 10 hens were divided into three pooled subgroups with subgroups 1 and 2 containing 3 hens and subgroup 3 containing 4 hens.
Animal Identification:	Uniquely numbered and colour coded leg ring
Animal health / observations:	Physical examination of each animal by staff veterinarian during the acclimation period, weekly during the dosing period, and at the end of the withdrawal period (if applicable).
Acclimation period:	>12 days.
Diet:	The non-medicated basal diet was composed of Naturally Pure Layers Pellets (Carrs Billington Agriculture). This diet was fed *ad libitum*.

Water:	Tap water was supplied *ad libitum*.
Housing:	The animals were housed individually in cages with the dimensions of 91 x 45 cm. These were bedded with wood shavings and each cage had an enclosed nest box.

The environmental conditions at the test facility during the in-life phase of the study are summarised in the table below.

3. Environmental conditions

Temperature:	Ambient; ranged from 15–31 °C
Humidity:	Ranged from 29–76 %
Air change:	Not reported
Photoperiod:	16 hours of light/8 hours of darkness

B. Study Design and Methods

The study included 5 treatment groups, an untreated control and 4 treated dose groups (1.5, 5, 15, and 50 mg N-acetyl glyphosate/kg bw, corresponding to 1.2, 4.0, 12 and 40 mg glyphosate equivalents/kg bw). This dosing regime covered a wide range of possible dietary burdens of *N*-acetyl glyphosate depending on regional practices. The animals were assigned to treatment groups during the acclimation period. Each group of 10 hens were divided into three pooled subgroups with subgroups 1 and 2 containing 3 hens and subgroup 3 containing 4 hens. Ten hens were assigned to the untreated control group and each of the four treated groups plus ten additional hens at 50 mg N-acetyl glyphosate/kg bw for depuration.

The control group was administered water while the four treated groups were dosed orally using calibrated positive displacement pipettes. The maximum single dose was 250 µL and therefore the dose was administered to Groups 4 and 5 as two equal volumes. Hens within the same subgroup received the same dose volume of aqueous *N*-acetyl glyphosate solution daily within each dose week. Target dose volumes changed between dose weeks as pooled subgroup body weight means changed at subsequent weekly weighings. The amount of *N*-acetyl glyphosate required per hen per subgroup was calculated based on the mean body weight (kg bw) per subgroup recorded on Days -1, 7, 14, 21, and 28. Dosing of treated animals continued for 35 consecutive days. Upon completion of dosing, hens from Groups 1–4 were sacrificed and tissue samples were collected. The hens within Group 5 subgroups were sacrificed 6, 14, and 20 days after dose termination to evaluate reduction in any residues in eggs or tissues after dosing ended.

Further details on the dosing regimen, including target dose levels, are summarised in the table below.

4. Dosing regimen

Route:	Oral by pipette
Vehicle:	Water
Timing/ frequency per day:	Once daily
Duration:	35 consecutive days
Treatment groups (dose levels):	5 treatment groups; untreated control and 4 dose levels: 1.5, 5, 15, and 50 mg *N*-acetyl glyphosate equivalents/kg bw, corresponding to 1.2, 4.0, 12 and 40 mg glyphosate equivalents/kg bw

The appropriate weight of supplied *N*-acetyl glyphosate (calculated based on the supplied concentration) was dispensed into each dose flask. Each dose solution was then made to target volume by adding pure water. One dose solution was prepared per dose level and each was sufficient for the entire 35-day dosing phase. Each dose solution was divided into five bottles and one fresh bottle was used for dosing per week.

Accuracy was monitored after preparation of dose solutions and throughout the dosing phase of the study. On the first day of each dose week, preceding dose administration, three aliquots of each new bottle for each dose level were collected for analysis. In addition, at the end of the dosing phase, samples were analysed from each dose group to confirm storage stability.

5. Daily observations and animal data collection

The appearance and behaviour of the hens was assessed throughout the study period for general health at least twice daily at *ca.* 1630 h and *ca.* 0830 h the following day prior to dosing. The amount of feed consumed by each subset was determined daily. Body weight was recorded at the beginning and end of the acclimation period, weekly during the test and withdrawal periods, and on the day of sacrifice.

6. Egg and tissue sample collection

Eggs were collected twice daily on Days -3, 1, 3, 5, 7, 10, 14, 17, 21, 24, 28, 31, and 34 and the number of eggs produced by each hen recorded. Eggs collected in the afternoon of the sampling day were stored refrigerated overnight until processing with the eggs collected on the following morning. These eggs were collected prior to dosing and were therefore within the same study day. Before processing, any excrement adhering to the eggs was removed and each egg was weighed. Whole eggs within pooled subgroups were cracked and placed into containers, weighed and homogenised, except on Day 21 when yolk and white pooled samples were processed separately. Three subsamples weighing *ca.* 2 g of each pooled subsample were placed into 50 mL polypropylene centrifuge tubes, diluted with aqueous formic acid (0.1 %, v/v), capped, mixed, and stored frozen at *ca.* -20 °C. In the depuration group (Group 5) eggs were collected on Days 35, 36, 38, 40, 42, 45, 48, 51, and 54 and processed within pooled subgroups as described above.

Within 6 hours after the last dose administration on Day 35, the hens in Groups 1–4 and control Group 6 were sacrificed by dislocation of the neck. The carcasses were washed and the ventral surface plucked to avoid contamination of organs and tissues with excrement. The entire liver, leg and breast muscle in approximately equal portions, and abdominal fat pad with skin attached were retained from each hen. The samples were then pooled within subgroups and frozen prior to homogenizing. In the depuration group (Group 5), tissues were collected on Days 41, 49, and 55 and processed within pooled subgroups as described above.

Egg and tissue samples were initially stored frozen (<-20 °C) in polyethylene containers at the In-life facility, Charles River Laboratories, and then shipped to the Analytical Phase facility (E. I. du Pont de Nemours and Company, Newark, Delaware, USA) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table 6.4.1-1: Egg and tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Egg	Dosing phase: 1, 3, 5, 7, 10, 14, 17, 21, 24, 28, 31, and 34 Withdrawal phase: 35, 36, 38, 40, 42, 45, 48, 51, and 54	Eggs were pooled from each subset within each treatment group.
Muscle[1]	End of dosing: Study Day 35 Withdrawal phase: Study days 41, 49, and 55	~ 600 g/each subset within each treatment group
Fat[2]		~ 75 g/each subset within each treatment group
Liver		~ 100 g/each subset within each treatment group

1 Composite of equal amounts of breast and thigh muscle.
2 Abdominal fat pad with skin attached.

7. Analytical phase

Analysis of dose solutions as well as egg and tissue samples was conducted at the Analytical Phase facility, E. I. du Pont de Nemours and Company, Newark, Delaware, USA.

Following preparation, three aliquots containing *ca.* 200 µL of each dose solution were taken for dose determination analysis, which was performed by HPLC-UV using a standard curve produced using standard solutions of *N*-acetyl glyphosate. Dose solutions were diluted with water to concentrations within the calibration range. These analyses confirmed that the actual concentrations of each solution ranged from 94.75 to 104.63 % of the theoretical concentration.

Eggs, liver, fat, and muscle test samples were analyzed using analytical method DuPont-20009 for *N*-acetyl glyphosate and relevant degradates. The method was applied for quantitative analysis of N-acetyl glyphosate and glyphosate in all matrices as well as AMPA and N-acetyl AMPA in liver. The method was applied for qualitative analysis for AMPA and N-acetyl AMPA in egg, fat, and muscle matrices.

The method of analysis for eggs (including separate analysis of yolks and whites) involved sample dilution in aqueous 0.1 % formic acid/methanol (96/4, v/v). The dilute sample was partitioned with hexane and the hexane layer discarded. The remaining aqueous fraction was partitioned with methylene chloride and the aqueous layer was collected. The methylene chloride fraction was back extracted with additional 0.1 % formic acid/methanol (96/4, v/v) for quantitative recovery of analytes. The aqueous fractions were combined and diluted to 50 mL final volume. An aliquot of the aqueous fraction was filtered through a C_{18} SPE cartridge. The C_{18} purified extract was further purified by solid phase extraction using a polymeric anion exchange (MAX) SPE cartridge and/or polymeric cation exchange (MCX) SPE cartridge, depending on matrix and analytes examined.

The method of analysis for liver, muscle, and fat matrices involved solid phase dispersion of the sample on C_{18} sorbent packing, followed by extraction in 0.1 N HCl solution (96 % water/4 % methanol). Samples were extracted again in water to complete the quantitative transfer of the analytes from matrix to final extract. An aliquot of the extract was diluted in acetonitrile and methanol to precipitate proteins, purified by solid phase extraction using a polymeric anion exchange (MAX) SPE cartridge, and/or polymeric cation exchange (MCX) SPE cartridge, depending on matrix and analytes examined.

Glyphosate and/or AMPA stable isotope standards used as internal standards were added to extracts prior to ion exchange SPE purification. Final extracts were filtered prior to LC/MS/MS analysis. The analytes were resolved by HPLC reverse-phase chromatography using a phenyl-hexyl column coupled to electrospray ionisation in with MS/MS detection to acquire 2 molecular ion transitions (only 1 ion transition was monitored for AMPA in positive ion mode). Quantitative analysis was accomplished using

a single molecular ion transition. The relative abundance of the 2 MS/MS fragment ions provided confirmatory evidence for *N*-acetyl glyphosate.

All analyte and total residue concentration values were expressed as mg/kg glyphosate equivalents. The validated limit of quantitation (LOQ) for *N*-acetyl glyphosate and each relevant analyte was 0.025 mg/kg in egg and muscle matrices, and 0.050 mg/kg in liver and fat matrices, expressed as glyphosate equivalents. The limit of detection (LOD) was estimated during method validation to be less than or equal to 0.009 mg/kg in egg, 0.011 mg/kg in liver matrices, 0.014 mg/kg in fat matrices, and 0.007 mg/kg in muscle matrices for each analyte. Recovery results with samples of egg, fat, muscle, and liver fortified with *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA are summarised in the table below.

Table 6.4.1-2: Recovery results: N-acetyl glyphosate, glyphosate, AMPA, and N-acetyl AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)[1]	Recovery				
			Results/Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
N-acetyl glyphosate	Egg	0.025	82, 93, 96, 112, 109, 91, 72, 72, 81[2], 80[3], 73[4], 115, 79, 78, 81, 73, 99, 103[2], 75	88	14	16	19
		0.050	91, 86, 73, 78[3], 78[4]	81	7	9	5
		0.25	93, 92, 85, 72, 81	85	9	10	5
		0.50	74, 77, 67, 79, 61, 73	72	7	9	6
		1.0	88, 84, 82, 84, 86[2], 80[2], 79[3], 82[4], 69, 82, 82, 82, 66, 66, 76[2], 65	78	8	10	16
		Overall	61–115	82	12	14	51
	Fat	0.050	99, 93, 91	94	4	5	3
		0.50[6]	93, 97, 83	91	7	8	3
		2.0	74, 72, 71	72	2	2	3
		Overall	71–99	86	11	13	9
	Muscle	0.025	90, 93, 78	87	8	9	3
		0.25[6]	84, 92, 70	82	11	13	3
		0.50	68	-	-	-	1
		Overall	68–93	82	10	12	7
	Liver	0.050	82, 87, 87	85	3	3	3
		0.50[6]	89, 109, 90	96	11	12	3
		2.0	75	-	-	-	1
		6.0	85, 80	83	-	-	2
		Overall	75–109	87	10	11	9

Table 6.4.1-2: Recovery results: N-acetyl glyphosate, glyphosate, AMPA, and N-acetyl AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)[1]	Recovery				
			Results/Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Glyphosate	Egg	0.025	99, 108, 100, 85, 76, 86, 82, 96, 87, 54, 123, 164[5], 109, 106, 106, 99[2], 107, 119[2], 103	97	17	17	18
		0.050	84, 79, 79, 72, 97	82	9	11	5
		0.25	99, 93, 80, 71, 83	85	11	13	5
		0.50	41[5], 82, 86, 91, 91,107	91	10	11	5
		1.0	84, 84, 78, 83, 75, 68, 71, 85, 87, 107, 89, 106, 98[2], 95, 88[2], 94	87	11	13	16
		Overall	54–123	91	14	15	49
	Fat	0.050	98, 110, 88	99	11	11	3
		0.50[6]	92, 86, 96	92	5	5	3
		2.0	93	-	-	-	1
		Overall	86–110	95	8	8	7
	Muscle	0.025	96, 102, 82	93	10	11	3
		0.25[6]	82, 88, 78	82	5	6	3
		0.50	77	-	-	-	1
		Overall	77–102	86	10	11	7
	Liver	0.050	93, 98, 74	88	13	15	3
		0.50[6]	86, 85, 79	83	4	4	3
		2.0	86	-	-	-	1
		Overall	74–98	86	8	9	7
AMPA	Egg	0.025	97, 106, 89	98	9	9	3
		0.25	82, 83	83	-	-	2
		Overall	82–106	92	10	11	5
	Fat	0.050	109, 105, 109	107	2	2	3
		0.50[6]	89, 94, 91	91	3	3	3
		Overall	89–109	99	9	9	6
	Muscle	0.025	101[2], 101[2], 84	95	10	10	3
		0.25[6]	91[2], 95[2], 85	90	5	6	3
		0.50	85	-	-	-	1
		Overall	84–101	92	7	8	7
	Liver	0.050	95[2], 92[2], 86	91	5	5	3
		0.50[6]	105[2], 110[2], 91	102	10	10	3
		Overall	86–110	96	9	10	6

Table 6.4.1-2: Recovery results: N-acetyl glyphosate, glyphosate, AMPA, and N-acetyl AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)[1]	Recovery				
			Results/Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
N-acetyl AMPA	Egg	0.025	94, 92, 93	93	1	1	3
		0.25	99, 95	97	-	-	2
		0.50	72	-	-	-	1
		1.0	89	-	-	-	1
		Overall	72–99	91	9	10	7
	Fat	0.050	82, 85, 85	84	2	2	3
		0.50[6]	90, 92, 71	84	11	14	3
		2.0	66	-	-	-	1
		Overall	66-–90	81	10	12	7
	Muscle	0.025	77, 77, 69	74	5	6	3
		0.25[6]	80, 88, 50	72	20	28	3
		0.50	47	-	-	-	1
		Overall	47–88	70	15	22	7
	Liver	0.050[6]	96, 100, 58	85	23	27	3
		0.50[6]	81, 94, 85	87	6	7	3
		2.0	64	-	-	-	1
		Overall	58–100	83	16	19	7

1 mg/kg glyphosate equivalents.
2 Average of two analyses of final extract.
3 Egg yolk sample.
4 Egg white sample.
5 Glyphosate recovery outlier not included in recovery statistics.
6 These values were taken from Appendix 8 of the study report.

Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

II. Results and Discussion

A. Dose levels

As indicated previously, *N*-acetyl glyphosate was administered orally using positive displacement pipettes to hens in the treated dose group. The nominal dose level of *N*-acetyl glyphosate was 1.5, 5, 15, and 50 mg *N*-acetyl glyphosate free acid equivalents/kg bw.

Analysis of dosing solution prepared during the dosing phase of the study confirmed that actual concentrations were close to the theoretical concentrations. A summary of dosing solution analysis results to determine actual concentrations of *N*-acetyl glyphosate is shown in the table below.

Table 6.4.1-3: Actual concentration of N-acetyl glyphosate in dosing solutions

Dose Level (mg/kg)	Theoretical *N*-acetyl glyphosate conc. (mg/mL)	Study Day	Actual *N*-acetyl glyphosate conc. (mg/mL)[1]	% of theoretical conc.
1.5	18.713	-7	18.559	99.18
		-1	17.821	95.23
		7	19.287	103.07
		14	19.055	101.83
		21	19.194	102.57
		28	19.479	104.09
		35	19.025	101.71
		Overall average[2]:	**18.917**	**101.10**
5	62.496	-7	59.602	95.37
		-1	59.216	94.75
		7	63.440	101.51
		14	60.215	96.35
		21	61.861	98.98
		28	63.192	101.11
		35	61.145	97.84
		Overall average[2]:	**61.239**	**97.99**
15	187.431	-7	184.272	98.31
		-1	179.845	95.95
		7	185.736	99.10
		14	190.125	101.44
		21	189.025	100.85
		28	187.954	100.28
		35	196.101	104.63
		Overall average[2]:	**187.580**	**100.08**
50	199.876	-7	195.588	97.85
		-1	192.24	96.18
		7	204.327	102.23
		14	203.138	101.63
		21	206.442	103.29
		28	199.935	100.03
		35	197.824	98.97
		Overall average[2]:	**199.928**	**100.03**

1 Determined by HPLC-UV.
2 Average values calculated for this summary.

Results showed that actual levels of *N*-acetyl glyphosate in each of the four dose levels were close to theoretical levels.

Additionally, in a second table below, the dose level and average dietary burden (mg/kg feed consumed) was calculated (in *N*-acetyl glyphosate and glyphosate equivalents) for each subgroup. These results were calculated using the subgroup average daily dose of *N*-acetyl glyphosate, the subgroup average daily dry feed consumption, and the average body weight of each subgroup during the dosing phase of the study.

Table 6.4.1-4: Actual dose level of *N*-acetyl glyphosate (NAG) administered to laying hens for 35 days expressed on basis of body weight (bw) and concentration in total diet (dry feed)

Nominal dose level[1] (mg/kg bw)	Sub-group	Average body weight during dosing (kg)[1]	Average daily dry feed consumption (kg)[1]	Average daily dose NAG per hen (mg)[1]	*N*-acetyl glyphosate[1,2]		Glyphosate[1,3]	
					mg/kg bw	mg/kg dry feed	mg/kg bw	mg/kg dry feed
1.5	1	*1.80*	*0.126*	*2.71*	*1.50*	*21.58*	*1.20*	*17.26*
	2	*1.90*	*0.127*	*2.85*	*1.50*	*22.48*	*1.20*	*17.98*
	3	*1.62*	*0.115*	*2.44*	*1.50*	*21.34*	*1.20*	*17.07*
	Average:	*1.77*	*0.123*	*2.66*	***1.50***	***21.80***	***1.20***	***17.44***
5.0	1	*1.70*	*0.111*	*8.48*	*5.00*	*77.05*	*4.00*	*61.64*
	2	*1.57*	*0.106*	*7.84*	*5.00*	*74.11*	*4.00*	*59.29*
	3	*1.60*	*0.103*	*7.98*	*5.00*	*78.00*	*4.00*	*62.40*
	Average:	*1.62*	*0.107*	*8.10*	***5.00***	***76.38***	***4.00***	***61.10***
15	1	*1.84*	*0.123*	*27.66*	*15.00*	*225.87*	*12.00*	*180.70*
	2	*1.82*	*0.145*	*27.30*	*15.02*	*189.08*	*12.02*	*151.26*
	3	*1.83*	*0.122*	*27.51*	*15.00*	*224.85*	*12.00*	*179.88*
	Average:	*1.83*	*0.130*	*27.49*	***15.01***	***213.27***	***12.01***	***170.62***
50	1	*1.68*	*0.115*	*84.15*	*49.98*	*743.95*	*39.98*	*595.16*
	2	*1.66*	*0.102*	*83.79*	*49.99*	*817.79*	*39.99*	*654.23*
	3	*1.79*	*0.115*	*89.64*	*50.02*	*783.19*	*40.02*	*626.55*
	Average:	*1.71*	*0.110*	*85.53*	***50.00***	***781.64***	***40.00***	***625.31***
50 Depuration	1	*1.62*	*0.096*	*81.13*	*50.02*	*852.03*	*40.02*	*681.62*
	2	*1.73*	*0.124*	*86.30*	*50.00*	*699.50*	*40.00*	*559.60*
	3	*1.89*	*0.117*	*93.33*	*49.38*	*804.77*	*39.50*	*643.82*
	Average:	*1.75*	*0.113*	*86.92*	***49.80***	***785.44***	***39.84***	***628.35***

1 Expressed as *N*-acetyl glyphosate.
2 All values were averaged for this summary across 5 dosing weeks and are thus shown in italics.
3 N-acetyl glyphosate expressed as glyphosate acid equivalents. Glyphosate acid equivalents were calculated using the ratio of molecular weights of *N*-acetyl glyphosate to glyphosate, 0.8.

B. Animal health and daily observations

Feed consumption for all animals in each test group remained essentially stable during the test period. It was noted on Day 15 that water consumption had increased by those hens receiving the higher concentrations of *N*-acetyl glyphosate (Groups 4 and 5). The most likely reason for the increased thirst was the high salt concentration in these dosing solutions. Body weight fluctuations seen during the study were considered normal for adult animals. Following animal sacrifice, all of the whole organs and tissues collected for analysis and the remaining tissues and organs in general appeared normal except for one hen that had a fluid filled growth on its abdominal fat pad, which was not noted as treatment related. There were no findings concerning animal health or behavior that were considered to be test related, except for four of the hens in the higher dose groups (Groups 4 and 5), which had lower egg production, which implies that the test item at this concentration may have affected egg production in these hens.

C. Residue levels in eggs and tissues

The residue values presented in the study report were determined as mg/kg in glyphosate equivalents.

Egg samples were maintained frozen and analyzed within 30 days of collection and, therefore, no storage stability testing was required for this matrix. Storage stability data for residues in tissue matrices were determined concurrently within this feeding study. After collection, all samples were maintained in frozen condition when stored in freezer at target temperature of -20 °C or shipped on dry ice. The maximum storage intervals for liver, fat, and muscle were 76, 77 and 80 days, respectively. Additional, liver, fat, and muscle samples fortified at 0.25 mg/kg or 0.50 mg/kg glyphosate equivalents were prepared with the initial sample extraction sets and stored frozen for future analysis at 2-time intervals. Analytical sets for storage stability testing consist of two stored fortified samples with a control and two fresh fortified samples and for analysis at two time intervals (a mid point interval and final interval exceeding the longest storage interval for the respective matrix).

Residues of *N*-acetyl glyphosate and glyphosate in eggs collected from untreated control animals were below the LOQ (<0.025 mg/kg). *N*-acetyl glyphosate and glyphosate were detected in the control fat sample (0.017 and 0.006 mg/kg glyphosate equivalents, respectively). AMPA was detected in control liver and muscle (0.011 and 0.003 mg/kg glyphosate equivalents, respectively). *N*-acetyl AMPA was not detected in any control liver, fat, or muscle sample.

Residues were not detected in Day 1 whole eggs in any dose group. The maximum daily total residue levels (calculated as the sum of glyphosate and N-acetyl glyphosate) in whole eggs observed throughout the study were 0.062, 0.116, 0.20, and 0.68 mg/kg glyphosate equivalents at the 1.5, 5, 15, and 50 mg/kg bw dose levels, respectively. The predominant residue in eggs was *N*-acetyl glyphosate. Glyphosate residues were below the LOQ (<0.025 mg/kg) in the 1.5, 5, 15, and 50 mg/kg bw dose levels except for one subgroup on Day 34 in the 1.5 mg/kg bw dose level and two subgroups on Day 34 in the 50 mg/kg bw dose level. Day 31 whole egg sample extracts were screened for AMPA and *N*-acetyl AMPA and no detectable residues were found in the high dose group (50 mg/kg bw). Mean total residues declined quickly during the depuration period from 0.76 to <0.025 mg/kg glyphosate equivalents 10 days after termination of dosing.

Table 6.4.1-5: Residues of *N*-acetyl glyphosate and glyphosate in eggs in the 1.5 mg/kg bw dose group during dosing days 1–35

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
1.5	1	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	3	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	5	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	7	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	10	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	0.025		<0.025		0.050	
	14	1	<0.025	0.026	<0.025	<0.025	<0.050	0.051
		2	<0.025		<0.025		<0.050	
		3	0.028		<0.025		0.053	
	17	1	0.028	0.028	<0.025	<0.025	0.053	0.053
		2	<0.025		<0.025		<0.050	
		3	0.030		<0.025		0.055	
	24	1	0.045	0.034	<0.025	<0.025	0.070	0.059
		2	0.026		<0.025		0.051	
		3	0.030		<0.025		0.055	
	28	1	0.050	0.033	<0.025	<0.025	0.075	0.058
		2	<0.025		<0.025		<0.050	
		3	0.025		<0.025		0.050	
	31	1	0.050	0.037	<0.025	<0.025	0.075	0.062
		2	0.025		<0.025		0.050	
		3	0.035		<0.025		0.060	
	34	1	0.044	0.034	0.030	0.027	0.074	0.061
		2	0.030		<0.025		0.055	
		3	0.029		<0.025		0.054	

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

Table 6.4.1-6: Residues of *N*-acetyl glyphosate and glyphosate in eggs in the 5.0 mg/kg bw dose group during dosing days 1–35

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
5.0	1	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	3	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	5	1	0.044	0.040	<0.025	<0.025	0.069	0.065
		2	0.030		<0.025		0.055	
		3	0.046		<0.025		0.071	
	7	1	0.045	0.053	<0.025	<0.025	0.070	0.078
		2	0.064		<0.025		0.089	
		3	0.051		<0.025		0.076	
	10	1	0.066	0.065	<0.025	<0.025	0.091	0.090
		2	0.060		<0.025		0.085	
		3	0.069		<0.025		0.094	
	14	1	0.066	0.074	<0.025	<0.025	0.091	0.099
		2	0.076		<0.025		0.101	
		3	0.079		<0.025		0.104	
	17	1	0.081	0.079	<0.025	<0.025	0.106	0.104
		2	0.079		<0.025		0.104	
		3	0.078		<0.025		0.103	
	24	1	0.094	0.091	<0.025	<0.025	0.119	0.116
		2	0.087		<0.025		0.112	
		3	0.091		<0.025		0.116	
	28	1	0.093	0.081	<0.025	<0.025	0.118	0.107
		2	0.072		<0.025		0.097	
		3	0.080		<0.025		0.105	
	31	1	0.087	0.080	<0.025	<0.025	0.112	0.105
		2	0.090		<0.025		0.115	
		3	0.064		<0.025		0.089	
	34	1	0.102	0.087	<0.025	<0.025	0.127	0.112
		2	0.093		<0.025		0.118	
		3	0.065		<0.025		0.090	

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

Table 6.4.1-7: Residues of *N*-acetyl glyphosate and glyphosate in eggs in the 15 mg/kg bw dose group during dosing days 1–35

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
15	1	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	3	1	0.044	0.036	<0.025	<0.025	0.069	0.062
		2	0.041		<0.025		0.066	
		3	0.025		<0.025		0.050	
	5	1	0.10	0.08	<0.025	<0.025	0.13	0.11
		2	0.089		<0.025		0.114	
		3	0.051		<0.025		0.076	
	7	1	0.13	0.10	<0.025	<0.025	0.16	0.13
		2	0.10		<0.025		0.13	
		3	0.07		<0.025		0.10	
	10	1	0.11	0.12	<0.025	<0.025	0.14	0.14
		2	0.15		<0.025		0.18	
		3	0.092		<0.025		0.117	
	14	1	0.12	0.18	<0.025	<0.025	0.15	0.20
		2	0.30		<0.025		0.33	
		3	0.11		<0.025		0.14	
	17	1	0.16	0.16	<0.025	<0.025	0.19	0.18
		2	0.19		<0.025		0.22	
		3	0.12		<0.025		0.15	
	24	1	0.18	0.15	<0.025	<0.025	0.21	0.18
		2	0.17		<0.025		0.20	
		3	0.10		<0.025		0.13	
	28	1	0.18	0.14	<0.025	<0.025	0.21	0.16
		2	0.13		<0.025		0.16	
		3	0.10		<0.025		0.13	
	31	1	0.18	0.14	<0.025	<0.025	0.21	0.17
		2	0.16		<0.025		0.19	
		3	0.086		<0.025		0.111	
	34	1	0.17	0.14	<0.025	<0.025	0.20	0.16
		2	0.15		<0.025		0.18	
		3	0.097		<0.025		0.122	

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

Table 6.4.1-8: Residues of *N*-acetyl glyphosate and glyphosate in eggs in the 50 mg/kg bw dose group during dosing days 1–35

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
50	1	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
	3	1	0.12	0.13	<0.025	<0.025	0.15	0.16
		2	0.15		<0.025		0.18	
		3	0.12		<0.025		0.15	
	5	1	0.29	0.26	<0.025	<0.025	0.32	0.29
		2	0.23		<0.025		0.26	
		3	0.26		<0.025		0.29	
	7	1	0.48	0.43	<0.025	<0.025	0.51	0.45
		2	0.50		<0.025		0.53	
		3	0.30		<0.025		0.33	
	10	1	0.56	0.50	<0.025	<0.025	0.59	0.52
		2	0.59		<0.025		0.62	
		3	0.34		<0.025		0.37	
	14	1	0.84	0.66	<0.025	<0.025	0.87	0.68
		2	0.71		<0.025		0.74	
		3	0.42		<0.025		0.45	
	17	1	0.69	0.55	<0.025	<0.025	0.72	0.58
		2	0.53		<0.025		0.56	
		3	0.43		<0.025		0.46	
	24	1	0.72	0.57	<0.025	<0.025	0.75	0.59
		2	0.75		<0.025		0.78	
		3	0.23		<0.025		0.26	
	28	1	0.71	0.53	<0.025	<0.025	0.74	0.55
		2	0.56		<0.025		0.59	
		3	0.30		<0.025		0.33	
	31	1	0.69	0.61	<0.025	<0.025	0.72	0.63
		2	0.70		<0.025		0.73	
		3	0.43		<0.025		0.46	
	34	1	0.65	0.63	0.033	0.032	0.68	0.66
		2	0.83		0.037		0.87	
		3	0.41		<0.025		0.44	

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

Table 6.4.1-9: Residues of *N*-acetyl glyphosate and glyphosate in eggs in the 50 mg/kg bw depuration dose group during the withdrawal phase

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
50 Depuration	34	1	0.63	0.73	0.044	0.035	0.67	0.76
		2	0.78		0.035		0.82	
		3	0.78		<0.025		0.81	
	35	1	0.51	0.69	<0.025	<0.025	0.54	0.71
		2	0.74		<0.025		0.77	
		3	0.80		<0.025		0.83	
	36	1	0.59	0.62	<0.025	<0.025	0.62	0.65
		2	0.73		<0.025		0.76	
		3	0.55		<0.025		0.58	
	38	1	0.37	0.53	0.034	0.028	0.40	0.56
		2	0.71		<0.025		0.74	
		3	0.52		<0.025		0.55	
	40	1	0.10	0.19	<0.025	<0.025	0.13	0.21
		2	0.23		<0.025		0.26	
		3	0.23		<0.025		0.26	
	42	2	0.064	0.048	<0.025	<0.025	0.089	0.073
		3	0.032		<0.025		0.057	
	45	2	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		3	<0.025		<0.025		<0.050	
	48	2	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		3	<0.025		<0.025		<0.050	
	51	3	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
	54	3	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

Egg yolk and whites samples were produced from Day 21 whole egg samples for each dose group. Residue levels in both egg fractions were predominantly *N*-acetyl glyphosate. Mean total residue levels of *N*-acetyl glyphosate in egg yolks increased from 0.078 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 1.38 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate in egg whites ranged from <LOQ (<0.025 mg/kg) to 0.045 mg/kg glyphosate equivalents. Across all dose groups, glyphosate residues were below the LOQ in egg yolks and ranged from <LOQ to 0.047 mg/kg glyphosate equivalents in the egg whites.

Table 6.4.1-10: Residues of *N*-acetyl glyphosate and glyphosate in eggs yolks

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
1.5	21	1	0.074	0.078	<0.025	<0.025	0.099	0.103
		2	0.049		<0.025		0.074	
		3	0.11		<0.025		0.135	
5.0		1	0.20	0.28	<0.025	<0.025	0.23	0.31
		2	0.22		<0.025		0.25	
		3	0.42		<0.025		0.45	
15		1	0.17	0.23	<0.025	<0.025	0.20	0.25
		2	0.23		<0.025		0.26	
		3	0.28		<0.025		0.31	
50		1	1.7	1.38	<0.025	<0.025	1.7	1.4
		2	1.6		<0.025		1.6	
		3	0.85		<0.025		0.88	

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

Table 6.4.1-11: Residues of *N*-acetyl glyphosate and glyphosate in eggs whites

Treatment Group (mg/kg bw)	Study Day	Sub-group	Residues (mg/kg)[1, 2, 3]					
			N-acetyl Glyphosate	Mean N-acetyl Glyphosate	Glyphosate	Mean Glyphosate	Total	Mean Total
1.5	21	1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
5.0		1	<0.025	<0.025	<0.025	<0.025	<0.050	<0.050
		2	<0.025		<0.025		<0.050	
		3	<0.025		<0.025		<0.050	
15		1	<0.025	<0.025	0.030	0.027	0.055	0.052
		2	<0.025		<0.025		0.050	
		3	<0.025		<0.025		0.050	
50		1	0.057	0.045	0.025	0.032	0.082	0.078
		2	0.054		<0.025		0.079	
		3	<0.025		0.047		0.072	

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

In liver, glyphosate, AMPA, and *N*-acetyl AMPA residues were below the LOQ (<0.050 mg/kg) in all dose groups. Mean total residue levels of *N*-acetyl glyphosate increased from 0.19 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 4.3 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 14 days after the end of dosing.

In fat, glyphosate and *N*-acetyl AMPA residues were below the LOQ (<0.050 mg/kg) in all dose groups. AMPA residues were below the LOQ in the 50 mg/kg bw dose group (not analyzed in other dose groups). Mean total residue levels of *N*-acetyl glyphosate increased from 0.11 mg/kg glyphosate equivalents in the

1.5 mg/kg bw dose group to 1.33 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 20 days after the end of dosing.

In muscle, glyphosate and *N*-acetyl AMPA residues were below the LOQ (<0.025 mg/kg) in all dose groups. AMPA residues were below the LOQ in the 15 and 50 mg/kg bw dose group (not analyzed in other dose groups). Mean total residue levels of *N*-acetyl glyphosate increased from 0.031 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 0.41 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 6 days after the end of dosing.

Table 6.4.1-12: Residues of N-acetyl glyphosate, glyphosate, AMPA, and N-acetyl AMPA in liver

Treatment Group (mg/kg bw)	Sub-group	Residues (mg/kg)[1, 2, 3]									
		N-acetyl glyphosate	Mean N-acetyl glyphosate	Glyphosate	Mean Glyphosate	AMPA	Mean AMPA	N-acetyl AMPA	Mean N-acetyl AMPA	Total	Mean Total
1.5	1	0.21	0.19	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.36	0.34
	2	0.20		<0.050		<0.050		<0.050		0.35	
	3	0.16		<0.050		<0.050		<0.050		0.31	
5.0	1	0.76	0.62	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.91	0.77
	2	0.67		<0.050		<0.050		<0.050		0.82	
	3	0.43		<0.050		<0.050		<0.050		0.58	
15	1	0.84	0.79	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.99	0.94
	2	0.94		<0.050		<0.050		<0.050		1.09	
	3	0.59		<0.050		<0.050		<0.050		0.74	
50	1	4.3	4.3	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	4.5	4.5
	2	5.2		<0.050		<0.050		<0.050		5.4	
	3	3.4		<0.050		<0.050		<0.050		3.6	
50 (6-day depuration)	1	0.053	0.053	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.203	0.203
50 (14-day depuration)	2	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.200	0.200
50 (20-day depuration)	3	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	0.200	0.200

1 LOQ (limit of quantitation):0.050 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.050 mg/kg were assigned a value of 0.050 mg/kg if being averaged with a value of 0.050 mg/kg or greater.

Table 6.4.1-13: Residues of N-acetyl glyphosate, glyphosate, AMPA, and N-acetyl AMPA in fat

Treatment Group (mg/kg bw)	Sub-group	Residues (mg/kg)[1, 2, 3]									
		N-acetyl glyphosate	Mean N-acetyl glyphosate	Glyphosate	Mean Glyphosate	AMPA	Mean AMPA	N-acetyl AMPA	Mean N-acetyl AMPA	Total	Mean Total
1.5	1	0.13	0.11	<0.050	<0.050	Not Analysed	Not Analysed	<0.050	<0.050	Not Calculated	Not Calculated
	2	0.089		<0.050				<0.050			
	3	0.11		<0.050				<0.050			
5.0	1	0.48	0.49	<0.050	<0.050	Not Analysed	Not Analysed	<0.050	<0.050	Not Calculated	Not Calculated
	2	0.60		<0.050				<0.050			
	3	0.38		<0.050				<0.050			
15	1	0.22	0.25	<0.050	<0.050	Not Analysed	Not Analysed	<0.050	<0.050	Not Calculated	Not Calculated
	2	0.39		<0.050				<0.050			
	3	0.15		<0.050				<0.050			
50	1	1.2	1.3	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	1.4	1.5
	2	1.9		<0.050		<0.050		<0.050		2.1	
	3	0.88		<0.050		<0.050		<0.050		1.03	
50 (6-day depuration)	1	0.071	0.071	<0.050	<0.050	Not Analysed	Not Analysed	<0.050	<0.050	Not Calculated	Not Calculated
50 (14-day depuration)	2	0.051	0.051	<0.050	<0.050	Not Analysed	Not Analysed	<0.050	<0.050	Not Calculated	Not Calculated
50 (20-day depuration)	3	<0.050	<0.050	<0.050	<0.050	Not Analysed	Not Analysed	<0.050	<0.050	Not Calculated	Not Calculated

1 LOQ (limit of quantitation):0.050 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.050 mg/kg were assigned a value of 0.050 mg/kg if being averaged with a value of 0.050 mg/kg or greater.

Table 6.4.1-14: Residues of N-acetyl glyphosate, glyphosate, AMPA, and N-acetyl AMPA in muscle

Treatment Group (mg/kg bw)	Sub-group	Residues (mg/kg)[1, 2, 3]									
		N-acetyl glyphosate	Mean N-acetyl glyphosate	Glyphosate	Mean Glyphosate	AMPA	Mean AMPA	N-acetyl AMPA	Mean N-acetyl AMPA	Total	Mean Total
1.5	1	0.036	0.031	<0.025	<0.025	Not Analysed	Not Analysed	<0.025	<0.025	Not Calculated	Not Calculated
	2	<0.025		<0.025				<0.025			
	3	0.032		<0.025				<0.025			
5.0	1	0.14	0.13	<0.025	<0.025	Not Analysed	Not Analysed	<0.025	<0.025	Not Calculated	Not Calculated
	2	0.16		<0.025				<0.025			
	3	0.10		<0.025				<0.025			
15	1	0.078	0.08	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.153	0.16
	2	0.13		<0.025		<0.025		<0.025		0.21	
	3	0.042		<0.025		<0.025		<0.025		0.117	
50	1	0.39	0.41	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.47	0.49
	2	0.58		<0.025		<0.025		<0.025		0.66	
	3	0.26		<0.025		<0.025		<0.025		0.34	
50 (6-day depuration)	1	<0.025	<0.025	<0.025	<0.025	Not Analysed	Not Analysed	<0.025	<0.025	Not Calculated	Not Calculated
50 (14-day depuration)	2	<0.025	<0.025	<0.025	<0.025	Not Analysed	Not Analysed	<0.025	<0.025	Not Calculated	Not Calculated
50 (20-day depuration)	3	<0.025	<0.025	<0.025	<0.025	Not Analysed	Not Analysed	<0.025	<0.025	Not Calculated	Not Calculated

1 LOQ (limit of quantitation):0.025 mg/kg
2 Residue values are in glyphosate equivalents.
3 All values calculated for this summary. For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

III. Conclusion

The magnitude of the residues of *N*-acetyl glyphosate and metabolites were determined in eggs and tissues of laying hens dosed with *N*-acetyl glyphosate for a period of 35 consecutive days, and at 6, 14, and 20 days after dosing ended (i.e. after a withdrawal period of 6, 14, and 20 days).

Residues were not detected in Day 1 whole eggs in any dose group. The maximum daily total residue levels (sum of glyphosate and N-acetyl glyphosate) observed were 0.062, 0.116, 0.20, and 0.68 mg/kg glyphosate equivalents at the 1.5, 5, 15, and 50 mg/kg bw dose levels, respectively. The predominant residue in eggs was *N*-acetyl glyphosate. Glyphosate residues were below the LOQ (<0.025 mg/kg) in the 1.5, 5, 15, and 50 mg/kg bw dose levels except for one subgroup on Day 34 in the 1.5 mg/kg bw dose level and two subgroups on Day 34 in the 50 mg/kg bw dose level. Day 31 whole egg sample extracts were screened for AMPA and *N*-acetyl AMPA and no detectable residues were found in the high dose group (50 mg/kg bw). Mean total residues declined quickly during the depuration period from 0.76 to <0.025 mg/kg glyphosate equivalents 10 days after termination of dosing.

Day 21 egg samples were separated into yolk and whites subsamples for comparative analysis of residue in egg fractions. Residue levels in both egg fractions were predominantly *N*-acetyl glyphosate. Mean total residue levels of *N*-acetyl glyphosate in egg yolks increased from 0.078 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 1.38 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate in egg whites ranged from <LOQ (<0.025 mg/kg) to 0.045 mg/kg glyphosate equivalents. Across all dose groups, glyphosate residues were below the LOQ in egg yolks and ranged from <LOQ to 0.047 mg/kg glyphosate equivalents in the egg whites.

In tissue samples obtained within *ca* 6 hours of dose completion, residue levels were highest in liver, followed by fat then muscle. Residue levels in liver, fat, and muscle generally increased with dose, except for fat and muscle, where the residues at the 15 mg/kg bw/d level are less than at the 5 mg/kg bw/d level, which be attributed to natural experimental variability. *N*-acetyl glyphosate was the predominant residue in all tissue matrices.

In liver, glyphosate, AMPA, and *N*-acetyl AMPA residues were below the LOQ (<0.050 mg/kg) in all dose groups. Mean total residue levels of *N*-acetyl glyphosate increased from 0.19 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 4.3 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 14 days after the end of dosing.

In fat, glyphosate and *N*-acetyl AMPA residues were below the LOQ (<0.050 mg/kg) in all dose groups. AMPA residues were below the LOQ in the 50 mg/kg bw dose group. Mean total residue levels of *N*-acetyl glyphosate increased from 0.11 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 1.33 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 20 days after the end of dosing.

In muscle, glyphosate and *N*-acetyl AMPA residues were below the LOQ (<0.025 mg/kg) in all dose groups. AMPA residues were below the LOQ in the 15 and 50 mg/kg bw dose group. Mean total residue levels of *N*- acetyl glyphosate increased from 0.031 mg/kg glyphosate equivalents in the 1.5 mg/kg bw dose group to 0.41 mg/kg glyphosate equivalents in the 50 mg/kg bw dose group. *N*-acetyl glyphosate levels were below the LOQ within 6 days after the end of dosing.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the residues of *N*-acetyl glyphosate and metabolites in poultry (hen) eggs and tissues (fat, muscle, and liver) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA residues that may transfer to eggs and edible poultry tissues. It was performed under GLP, and it is considered to be scientifically valid. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 505 and OECD Guidance Document on Residues in Livestock (Series on Pesticides No. 73).

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.1/002
Report author	[redacted]
Report year	1987
Report title	Magnitude of SC-0224 residues in eggs and poultry
Report No	[redacted] 87-43
Document No	Not available
Guidelines followed in study	US EPA: Subdivision O, Pesticide Assessment Guidelines for Residue Chemistry
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 505: • Hens were slaughtered approximately 24 hours after last daily dosing instead of within 6 hours. • More than 4 hens combined to derive one sample => only 1 sample per sampling interval and feeding level • Sample weights after slaughter not reported • For meat 50 % white and 50 % dark meat was sampled instead of 50 % leg and 50 % breast • Depuration phase only 1 interval instead of 3 intervals • Storage stability data not collected on hen tissues
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues in eggs and tissues of laying hens dosed with of glyphosate-trimesium (SC-0224), the trimethylsulfonium salt of glyphosate, for a period of 28 consecutive days, and at 7 days after dosing ended (i.e., after a withdrawal period of 7 days).

Residue analysis was conducted for the N-phosphonomethyl glycine anion (PMG) (also known as carboxymethylaminomethyl phosphonate, or CMP), the trimethylsulfonium cation (TMS), and AMPA (aminomethylphosphonic acid). TMS is not a relevant analyte in this dossier, therefore data with respect to this analyte is not presented in the following summary.

Glyphosate-trimesium was administered to hens through oral gavage for a period of 28 consecutive days at nominal concentrations of glyphosate-trimesium of 0.5 ppm (mg of glyphosate-trimesium/kg of feed consumed), 5 mg/kg feed, and 50 mg/kg feed (corresponding to 0.344, 3.44 and 34.36 mg glyphosate/kg feed). Measured levels of glyphosate-trimesium in the dosing solutions were near nominal values. Actual levels of glyphosate-trimesium in the treatment groups averaged 0.52, 5.1 and 50 mg/kg feed corresponding to 0.357, 3.50 and 34.36 mg glyphosate/kg feed, respectively. Expressed on a body weight basis, the average dose levels of glyphosate-trimesium were 0.036, 0.37 and 3.65 mg/kg bw/day, corresponding to 0.025, 0.25 and 2.5 mg glyphosate/kg bw/day, respectively.

The analytical method LOD in eggs was 0.010 mg/kg for PMG and 0.02 mg/kg for AMPA. The analytical method LOD in fat, muscle, liver, and kidney was 0.05 mg/kg for PMG and AMPA.

Residues of PMG in all egg samples (days 1–35) from the 0.5 and 5.0 mg/kg treatment groups were below the LOD (<0.010 mg/kg). In the 50 mg/kg treatment group, PMG residues were detected at treatment days 7 through 28 with a maximum residue level of 0.015 mg/kg on day 21, returning to below the LOD by day 35. Residues of AMPA were below the LOD in all egg samples (days 1–35).

Residues of PMG and AMPA in all fat samples (days 1–35) from the 0.5, 5.0, and 50 mg/kg treatment groups were below the LOD (<0.05 mg/kg).

Residues of PMG and AMPA in all muscle samples (days 1–35) from the 0.5, 5.0, and 50 mg/kg treatment groups were below the LOD (<0.05 mg/kg).

Residues of PMG and AMPA were below the LOD in all liver samples (days 1–35).

Residues of PMG in all kidney samples (days 1–35) from the 0.5 mg/kg treatment group were below the LOD (<0.05 mg/kg). In the 5.0 mg/kg treatment group, PMG residues were detected at treatment day 28 with a residue level of 0.072 mg/kg. PMG residues in kidney collected after a 7-day withdrawal period were below the LOD. In the 50 mg/kg treatment group, PMG residues were detected at treatment day 28 with a residue level of 0.30 mg/kg. PMG residues in kidney collected after a 7-day withdrawal period were 0.11 mg/kg. Residues of AMPA were below the LOD in all kidney samples (days 1–35).

Residues of PMG were detected in control kidney (0.07 and 0.08 mg/kg), likely due to low-level interferences, and muscle (0.08 mg/kg). All other residue results in control samples were below the limit of quantitation.

Test facilities

Study directory:	Stauffer Chemical Company, Richmond, California 94804, USA
In-Life phase:	Hazleton Laboratories America, Inc., Madison, Wisconsin 53704, USA
Analytical phase:	Stauffer Chemical Company, Richmond, California 94804, USA

I. Materials and Methods

A. Materials

One test material, glyphosate-trimesium (SC-0224), was administered to the treated animals in this study. Further information on the test materials is listed in the tables below.

1. Test materials

Description:	Glyphosate-trimesium technical
Batch number:	8289-35-1
HLA sample number:	789030
Active ingredient(s):	Glyphosate (N-phosphonomethyl glycine)
CAS number:	81591-81-3
Content of a.s. nominal:	56.29 %
Content of a.s. analysed:	Not provided
Formulation type:	NA
Appearance/colour:	Not provided
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in glass container
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Single-comb white Leghorn laying hens were the test animals used in this study. Details are listed in the table below.

2. Test animals

Species:	Laying hen; Chicken (*Gallus gallus domesticus)*
Gender:	Female
Breed:	White Leghorn
Source:	[redacted]
Age:	23 weeks
Weight at dosing, (Day 0):	Ranged from 1.190–1.865 kg
Number of animals:	40 hens selected out of a group of 60: (10 in untreated control group, and 10 in each of 3 treated groups)
Animal Identification:	Uniquely numbered leg band
Animal health / observations:	Physical examination of each animal by staff veterinarian during of acclimation period (Day -3) and just before sacrifice (Days 28 and 35). The animals were approved for use in the study by the staff veterinarian on 27-Jul-1983.
Acclimation period:	10 days
Diet:	The basal diet was composed of Ralston Purina Layena (Lot Nos. 473921, 4731681, and 4732091). This diet was fed *ad libitum*. There were no known contaminants in the basal diet which would interfere with the conduct or outcome of this study.
Water:	Water was supplied *ad libitum* from stainless steel troughs.
Housing:	The animals were individually housed in 28 cm x 43 cm x 38 cm laying cages with roll-away floors. The cages were located in three-deck laying batteries with 5 birds/deck. Each deck was fitted with a communal feeder and waterer.

The environmental conditions at the test facility during the in-life phase of the study are summarised in the table below.

3. Environmental conditions

Temperature:	Ambient; ranged from 20–24 °C
Humidity:	Ranged from 61–74 %
Air change:	Not reported
Photoperiod:	16 hours of light/8 hours of darkness

B. Study Design and Methods

The study included 4 treatment groups, an untreated control and 3 treated groups (dose levels of 0.5, 5.0, and 50 mg glyphosate-trimesium /kg feed, corresponding to 0.34, 3.44 and 34.36 mg glyphosate/kg feed) . The levels were selected to adequately define a residue spectrum broad enough to include all residue levels which might possibly be observed in raw or processed commodities used for poultry feed. The animals were assigned to treatment groups late in the acclimation period. Animals were randomly assigned to treatment groups based on a computer-generated stratified randomisation scheme. Ten hens were assigned to the untreated control group and 10 hens were assigned to each of the three treated groups.

The control group was given vehicle while the three treated groups were given a dosing solution containing glyphosate-trimesium. Dosing of treated animals continued for 28 consecutive days. Upon completion of dosing, 7 animals from each treatment group were sacrificed and tissue samples were collected. The remaining hens were retained for use in a withdrawal phase of the study to evaluate reduction in any residues in eggs or tissues after dosing ended. The remaining 12 hens (3 control and 3 hens in each of the 3 treated groups) were sacrificed at 7 days after the end of the dosing period (i.e. Study Day 35).

Further details on the dosing regimen, including target dose levels, are summarised in the table below.

1. Dosing regimen

Route:	Oral via gavage
Vehicle:	Deionised water
Timing / frequency per day:	Daily at approximately 1:00 pm
Duration:	28 consecutive days
Treatment groups (dose levels):	4 treatment groups; untreated control and 3 dose levels: 0.5, 5.0, and 50 mg glyphosate-trimesium/kg feed, corresponding to 0.34, 3.44 and 34.36 mg glyphosate/kg feed

The test item was prepared in deionised water for each dose level. The test solutions for the 0.5, 5.0, and 50 mg glyphosate acid/kg dose levels contained 0.057, 0.57, and 5.7 mg glyphosate-trimesium per 2 mL dose, respectively. The calculation of the daily dose was based upon average feed consumption (114 g/hen/day) of all the hens during the acclimation period with no correction for individual hen consumption. The daily dose was delivered to each hen by syringe and Teflon® intubation needle.

Diluted test item samples were collected and sent to the sponsor to confirm the concentration of glyphosate-trimesium. The batches of dosing solution used to administer the test materials to the hens in this study were prepared weekly and stored no longer than 7 days before use. The stability of glyphosate-trimesium in the testing solution was not evaluated as part of this report.

Samples (500 g each) were collected from each batch of feed provided to the hens. Samples (500 g each) of drinking water were collected at the beginning and end of the test period. These feed and drinking water samples were retained and stored frozen.

2. Daily observations and animal data collection

All animals were observed daily for general condition and behaviour. At weekly intervals, the amount of feed consumed by each subset of five birds was determined, and the average individual consumption calculated. Body weight was recorded at the beginning and end of the acclimation period and weekly thereafter.

3. Egg and tissue sample collection

Eggs were collected daily from Day -9 through Day 35 and the number of eggs produced by each hen recorded. Egg weights were recorded from Day -2 through Day 35. Eggs collected on Days -1, 1, 2, 4, 7, 14, 21, and 28 of the treatment period and Day 7 of the withdrawal period were pooled by treatment group for analytical evaluation. These eggs were wiped with a damp towel and allowed to dry. The contents of each egg were put into a clean polyethylene container and frozen; shells were discarded. Eggs not required for analysis were incinerated intact.

At the time of tissue sample collection, specified animals were euthanised (using carbon dioxide gas). Samples of abdominal fat, muscle (50 % white meat: 50 % dark meat), liver, and kidney were collected from animals individually upon completion of the 28-day dosing period (within 24 hours of administration of the final dose) or during the withdrawal phase of the study at 7 days after the end of the dosing period (Study Day 35). Gross necropsy was performed on sacrificed animals. Tissue samples were stored frozen in polyethylene containers.

Egg and tissue samples were stored frozen initially at the In-life facility, Hazleton Laboratories, and then shipped to the Analytical Phase facility (Stauffer Chemical Company, Richmond, California, USA) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table 6.4.1-15: Egg and tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Egg	Dosing phase: -1, 1, 2, 4, 7, 14, 21, 28 Withdrawal phase: 35	Eggs were pooled from each treatment group
Muscle	End of dosing: Study Day 28 Withdrawal phase: Study day 35	Equal amount of white and dark muscle /animal[1]
Fat		Abdominal fat/animal[1]
Liver		Entire liver/animal[1]
Kidney		Both kidneys/animal[1]

1 Duplicate samples were collected; one shipped for analysis and one held as a reserve sample.

4. Analytical phase

Analysis of feed samples as well as egg and tissue samples was conducted at the Analytical Phase facility, Stauffer Chemical Company, Richmond, California, USA.

An analytical method (RRC 83-40) was developed for the determination of PMG and TMS in technical glyphosate-trimesium. Quantitation was achieved by using an HPLC equipped with UV detection.

An analytical method (RRC 87-41) was developed for the determination of PMG and AMPA in hen eggs, as well as fat, muscle, liver, and kidney tissues. In eggs, fat, and muscle, the procedure used an acidic modifier solution (KH_2PO_4, methanol, and HCl) followed by cation exchange resin cleanup. After concentration to dryness, PMG and AMPA were derivatised with 9-fluorenylmethyl chloroformate and

analysed by HPLC with UV detection. In liver and kidney, the procedure used an aqueous/organic partition extraction (1:1 deionised water and chloroform) prior to the addition of the acidic modifier solution.
Calculated background concentrations of the analytes were below the detection limit of the methods for most control samples; therefore, residue concentrations are listed as less than the detection limit. The limit of detection (LOD) was 0.05 mg/kg each for PMG and AMPA in fat, muscle, liver, and kidney. In eggs, the LOD was 0.010 mg/kg for PMG and 0.02 mg/kg for AMPA. The lowest fortification levels for glyphosate and AMPA in fat, muscle, lover and kidney were 0.2 mg/kg and 0.01 mg/kg in egg.
Recovery results with samples of egg, fat, muscle, liver, and kidney fortified with PMG and AMPA are summarised in the table below. The recoveries were all within the acceptable range of 70-110 %, with a few exceptions: recovery of glyphosate in liver at fortification level 0.5 mg/kg, in kidney at 0.4 mg/kg were 64 and 67 %, recovery of AMPA in muscle, liver and kidney 66, 66 and 58 %, respectively. Taking into account, that only single recoveries were measured and overall recoveries are all within the acceptable range of 70-110 %, the recoveries are considered to be acceptable. RSDs were all below 20 %, except for overall recovery of glyphosate in kidney of 26 %.

Table 6.4.1-16: Recovery results: PMG and AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results/Range[1] (%)	Mean[2] (%)	Standard deviation[2] (%)	Relative standard deviation[2] (%)	Number analyses (n)
PMG	Egg	0.010	90	-	-	-	1
		0.020	100, 108	*104*	-	-	2
		0.030	77	-	-	-	1
		0.050	79, 92, 93, 104	*92.0*	*10*	*11*	4
		0.40	89, 71	*80.0*	-	-	2
		Overall	71–108	*90.3*	*12*	*13*	10
	Fat	0.2	114	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90–114	*102*	-	-	2
	Muscle	0.2	71	-	-	-	1
		0.5	73, 78	*75.5*	-	-	2
		Overall	71–78	*74.0*	*3.6*	*4.9*	3
	Liver	0.2	73	-	-	-	1
		0.5	64	-	-	-	1
		1.0	75	-	-	-	1
		Overall	64–75	*70.7*	*5.9*	*8.3*	3
	Kidney	0.2	108, 66	*87.0*	-	-	2
		0.4	67	-	-	-	1
		0.5	99	-	-	-	1
		Overall	66–108	*85.0*	*22*	*26*	4
AMPA	Egg	0.010	100	-	-	-	1
		0.020	75, 80	*77.5*	-	-	2
		0.030	73	-	-	-	1
		0.050	70	-	-	-	1
		0.40	73, 89, 60	*74.0*	*15*	*20*	3
		Overall	60–100	*77.5*	*12*	*16*	8

Table 6.4.1-16: Recovery results: PMG and AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results/Range[1] (%)	Mean[2] (%)	Standard deviation[2] (%)	Relative standard deviation[2] (%)	Number analyses (n)
	Fat	0.2	85	-	-	-	1
		0.5	86	-	-	-	1
		Overall	85–86	*85.5*	-	-	2
	Muscle	0.2	66	-	-	-	1
		0.5	87, 68	*77.5*	-	-	2
		Overall	66–87	*73.7*	*12*	*16*	3
	Liver	0.2	66	-	-	-	1
		0.5	82	-	-	-	1
		1.0	93	-	-	-	1
		Overall	66–93	*80.3*	*14*	*17*	3
	Kidney	0.2	58	-	-	-	1
		0.4	71	-	-	-	1
		0.5	70	-	-	-	1
		Overall	58–71	*66.3*	*7.2*	*11*	3

1 Recoveries compensated for background level found in unfortified samples.
2 Mean values, standard deviations, and relative standard deviations were calculated for this summary and are shown in italics

II. Results and Discussion

A. Dose levels

As indicated previously, glyphosate-trimesium was administered through oral gavage to hens in the treated dose group. The nominal concentration of glyphosate-trimesium in feed was 0.5 mg/kg, 5 mg/kg, and 50 mg/kg (expressed as glyphosate-trimesium salt).

Analysis of dosing solution prepared during the dosing phase of the study confirmed that actual dose levels were close the nominal/targeted dose levels. A summary of dosing solution analyses is shown in the table below. The actual dose levels of glyphosate-trimesium were estimated based on the analyses of the dosing solutions. The data are summarised in the table below.

Table 6.4.1-17: Actual dose levels of glyphosate-trimesium based on the analysis of the dosing solutions

Nominal dose level	Week number	Subset 1	Subset 2	Average
0.5 mg/kg feed	1	0.57	0.67	0.62
	2	0.44	0.53	0.49
	3	0.45	0.51	0.48
	4	0.45	0.53	0.49
	Overall average ± standard deviation[1]:			0.52 ± *0.07*
5.0 mg/kg feed	1	5.2	7.0	6.1
	2	4.4	5.1	4.8
	3	4.3	5.0	4.6
	4	4.6	5.3	5.0
	Overall average ± standard deviation[1]:			5.1 ± *0.7*
50 mg/kg feed	1	56	65	60
	2	45	49	47
	3	43	49	46
	4	44	50	47
	Overall average ± standard deviation[1]:			50 ± *6.7*

1 Standard deviations were calculated for this summary and are reported in italics.

The results showed that the actual levels of glyphosate-trimesium for each of the 3 dose levels were close to nominal/target levels. The overall average glyphosate-trimesium dose levels were 0.52 mg/kg, 5.1 mg/kg, and 50 mg/kg for the nominal 0.5, 5.0, and 50 mg/kg treatments groups, respectively.

Additionally, in a second table below, dosage was calculated for this summary and expressed with respect to the average animal body weight (i.e., mg test material / kg bw/day). These results were calculated using the average body weight of each dose level during the dosing phase of the study. The overall averages for glyphosate-trimesium dosage on a body weight basis in the 0.5, 5.0, and 50 mg/kg treatments groups were 0.036 mg/kg bw/day, 0.37 mg/kg bw/day, and 3.65 mg/kg bw/day, respectively.

Table 6.4.1-18: Actual dose levels of glyphosate-trimesium administered to laying hens for 28 days expressed on basis of basis of body weight (bw)

Nominal dose level	Actual average daily dose (mg/kg feed)	Average body weight during dosing[1] (kg)	Average daily dry feed consumption[1] (kg)	mg/kg bw/day[1]
0.5 mg/kg feed	0.52	*1.602*	*0.112*	*0.036*
5.0 mg/kg feed	5.1	*1.569*	*0.114*	*0.37*
50 mg/kg feed	50	*1.589*	*0.116*	*3.65*

1 Values were calculated for this summary and are reported in italics.

B. Animal health and daily observations

There were no findings concerning animal health or behavior that were considered to be test related. Feed consumption for all animals in each test group remained essentially stable during the test period. Body weight fluctuations seen during the study were considered normal for adult animals. Egg production was high and uniform across treatments. Egg weight was also uniform, and no remarkable differences were noted between treatments. Following animal sacrifice, necropsy/pathology evaluation indicated no macroscopic or microscopic observations that appeared treatment related.

C. Residue levels in eggs and tissues

Residues of PMG and AMPA in eggs collected from untreated control animals were below the LOD. Residues of PMG were detected in control kidney (0.07 and 0.08 mg/kg), likely due to low-level

interferences, and muscle (0.08 mg/kg). All other residue results in control tissue samples were below the LOD (<0.05 mg/kg).

Frozen storage stability of PMG and AMPA in eggs was determined by analysis of samples from a local grocery. No significant degradation of PMG and AMPA in eggs was observed for 683 days, which was the maximum period of frozen storage evaluated. Frozen storage stability of PMG and AMPA in hen matrices (fat, muscle, liver and kidney) was not evaluated as part of this feeding study. All samples in this study were analysed within 69 days of collection.

Residues of PMG in all egg samples (days 1–35) from the 0.5 and 5.0 mg/kg treatment groups were below the LOD (<0.010 mg/kg). In the 50 mg/kg treatment group, PMG residues were detected at treatment days 7 through 28 with a maximum residue level of 0.015 mg/kg on day 21, returning to below the LOD by day 35. Residues of AMPA were below the LOD in all egg samples (days 1–35).

Table 6.4.1-19: Residues of PMG in eggs for Dosing Days 1–28

	PMG residue (mg/kg)[1, 2, 3]							
	Study Day							
Treatment Group	**1**	**2**	**4**	**7**	**14**	**21**	**28[4]**	**Average[5]**
50 mg/kg feed (*3.65 mg/kg bw*)	<0.010	<0.010	<0.010	0.010	0.011	0.015	0.014	*0.011*

1 LOQ (limit of quantitation):0.010 mg/kg.
2 Residue values are uncorrected for recovery.
3 For purposes of calculating averages, residue values of <0.010 mg/kg were assigned a value of 0.010 mg/kg if being averaged with a value of 0.010 mg/kg or greater.
4 Study Day 28 was the end of the 28-day dosing period.
5 Average value was calculated for this summary and is reported in italics.

Residues of PMG and AMPA in all fat samples (days 1–35) from the 0.5, 5.0, and 50 mg/kg treatment groups were below the LOD (<0.05 mg/kg).

Residues of PMG and AMPA in all muscle samples (days 1–35) from the 0.5, 5.0, and 50 mg/kg treatment groups were below the LOD (<0.05 mg/kg).

Residues of PMG and AMPA were below the LOD in all liver samples (days 1–35).

Residues of PMG in all kidney samples (days 1–35) from the 0.5 mg/kg treatment group were below the LOD (<0.05 mg/kg). In the 5.0 mg/kg treatment group, PMG residues were detected at treatment day 28 with a residue level of 0.072 mg/kg. PMG residues in kidney collected after a 7-day withdrawal period were below the LOD. In the 50 mg/kg treatment group, PMG residues were detected at treatment day 28 with a residue level of 0.30 mg/kg. PMG residues in kidney collected after a 7-day withdrawal period were 0.11 mg/kg. Residues of AMPA were below the LOD in all kidney samples (days 1–35).

Table 6.4.1-20: Residues of PMG in kidney

Treatment Group	Study Day[1]	Analysis replicate	Residue found[2, 3] (mg/kg)	
			PMG	PMG, average
5.0 mg/kg feed (*0.37 mg/kg bw*)	28	1	0.072	0.072
		2	0.071	
	35	1	<0.05	<0.05
		2	<0.05	
50 mg/kg feed (*3.65 mg/kg bw*)	28	1	0.31	0.30
		2	0.29	
	35	1	0.11	-

1 Study Day 28 is at the end of the 28-day dosing period; Study Day 35 is during the withdrawal period, 7 days after the end of dosing.
2 LOD (limit of quantitation):0.05 mg/kg
3 Residue values are uncorrected for recovery.

III. Conclusion

The results from this study indicate that for laying hens, a direct relationship exists between the level of glyphosate-trimesium in the diet and the concentration of residues in eggs and tissues. At dosage levels of 5.0 mg/kg in feed or less, no residues of any of the two analytes were detected in any sample of eggs or tissues (except PMG residues of 0.072 mg/kg in kidney), and no residues of AMPA were observed even at the highest (50 mg/kg feed) dosage level.

At the 50 mg/kg feed dosage level, PMG was observed in eggs at 0.010 to 0.015 mg/kg and in kidneys at 0.30 mg/kg in samples from hens dosed for 28 days. Residue concentrations decreased rapidly after glyphosate-trimesium dosing ceased.

The results show that glyphosate-trimesium, when fed continuously for 28 days at 50 mg/kg feed to laying hens, produced residues in eggs and edible tissues (muscle, fat and liver) below LOD. The residue concentrations decreased rapidly when dosing was discontinued, indicating that glyphosate and AMPA do not accumulate irreversibly under the conditions tested. In addition, no treatment-related effects on feed consumption, body weight, or egg production were evident at the three dosage levels studied.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the residues of glyphosate and AMPA in poultry (hen) eggs and tissues (fat, muscle, liver and kidney) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of glyphosate and AMPA residues that may transfer from the poultry diet to eggs and edible poultry tissues. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 505 and OECD Guidance Document on Residues in Livestock (Series on Pesticides No. 73) with a few deviations.

Hens were slaughtered approximately 24 hours after last daily dosing instead of within 6 hours. More than 4 hens were combined to derive one sample and hence only 1 sample per sampling interval and feeding level was taken. The sample weights after slaughter were not reported. For meat 50 % white and 50 % dark meat was sampled instead of 50 % leg and 50 % breast. And for the depuration phase only 1 interval instead of 3 intervals was analysed. Nevertheless, increase and decline of the residues in eggs and tissues in the highest dose groups where residues were found can clearly be seen.

Storage stability of glyphosate and AMPA within this study was demonstrated on cow tissues (muscle, fat, liver and kidney) and eggs for a period of 683 days (23 months). The results of storage stability of cow tissues could be extrapolated to hen tissues. Additionally, the hen tissue samples (analysed within 69 days of collection) are covered by storage stability data on hen matrices of a different study (refer to CA 6.1) for which 13-25 months of storage stability was demonstrated.

Residue concentrations are listed as less than the detection limit and not less than the quantification limit, as calculated background concentrations of the analytes were below the detection limit of the methods for most control samples. The limit of detection (LOD) was 0.05 mg/kg each for PMG and AMPA in fat, muscle, liver, and kidney. In eggs, the LOD was 0.010 mg/kg for PMG and 0.02 mg/kg for AMPA. The lowest fortification levels for glyphosate and AMPA in fat, muscle, lover and kidney were 0.2 mg/kg and 0.01 mg/kg in egg.

The study is considered valid as these deficits are not expected to significantly impact the quality or reliability of the study.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.1/003
Report author	[redacted]
Report year	1987
Report title	Residue determination of glyphosate and AMPA in laying hen tissues and eggs following a 28 day feeding study
Report No	[redacted]-6676
Document No	M-651048-01-1
Guidelines followed in study	US EPA: Subdivision O, Pesticide Assessment Guidelines for Residue Chemistry
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 505: • Hens were slaughtered on the day of last dosing but within 6 hours of the final dose cannot be confirmed. • Sample weights after slaughter not reported • Only 2 samples per sampling interval and feeding level instead of 3 samples • In eggs plateau was not reached at day 7, hence more than just weekly samplings would be required between 7 and 28 days • Depuration phase only 2 intervals instead of 3 intervals • Insufficient detail provided in the study report to determine the interval of sample frozen storage before extraction and analysis.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate (*N*-phosphonomethyl glycine) and AMPA (aminomethylphosphonic acid) in eggs and tissues of laying hens dosed with glyphosate and AMPA for a period of 28 consecutive days, and at 7 and 28 days after dosing ended (i.e. after a withdrawal period of 7 days and 28 days).

Glyphosate and AMPA (in a 9:1 ratio) were administered to hens through dietary intake for a period of 28 consecutive days with use of a feed diet that was fortified with glyphosate and AMPA at each of three levels (1X, 3X, and 10X treatment groups). The nominal concentration of glyphosate in the diet for the 1X, 3X, and 10X treatment groups was 36 ppm (mg/kg), 108 mg/kg, and 360 mg/kg, respectively. The nominal concentration of AMPA in the diet for the 1X, 3X, and 10X treatment groups was 4.0 mg/kg, 12 mg/kg, and 40 mg/kg, respectively. Measured levels of glyphosate and AMPA attained in the hen diet were near nominal values. Actual levels of glyphosate (not corrected for recovery) in the 1X, 3X, and 10X treatments groups in feed on a dry weight basis averaged 36.3 mg/kg, 104.5 mg/kg, and 345.5 mg/kg, respectively. Expressed on a body weight basis, the average dose levels of glyphosate in the 1X, 3X, and 10X groups was 2.4 mg/kg bw/day, 7.1 mg/kg bw/day, and 23.3 mg/kg bw/day, respectively. The actual level of AMPA (not corrected for recovery) in the 1X, 3X, and 10X treatments groups in feed on a dry weight basis averaged 3.9 mg/kg, 11.2 mg/kg, and 37.1 mg/kg, respectively. Expressed on a body weight basis, the average dose levels of glyphosate in the 1X, 3X, and 10X groups was 0.25 mg/kg bw/day, 0.76 mg/kg bw/day, and 2.50 mg/kg bw/day, respectively.

The analytical method LOQ for glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) in eggs was 0.025 mg/kg, and was 0.05 mg/kg for glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) in fat, muscle, liver, and kidney.

The residue values presented in the summary in the study report had been corrected for recovery. The residue values described below were not corrected for recovery.

Residues of glyphosate and AMPA in all egg samples (days 1–56) from the 1X treatment group were below the LOQ (<0.025 mg/kg). Treatment days 7–28 of the 3X and days 4–28 of the 10X samples contained glyphosate residues above the LOQ and AMPA residues below the LOQ (except 10X-day 21 subgroup R, which contained 0.026 mg/kg AMPA). The glyphosate residue levels ranged from <0.025 mg/kg (3X-days 1–7 and 10X-days 1–4) to 0.026 mg/kg (3X-days 14–28) to 0.091 mg/kg (10X-day 21). Withdrawal days 29–30 of the 3X and days 29–35 of the 10X samples contained glyphosate residues above the LOQ and AMPA residues below the LOQ. The glyphosate level ranged from 0.027 mg/kg (3X-days 29 and 30) to 0.082 mg/kg (10X-day 30).

Residues of glyphosate and AMPA in all fat samples (days 1–56) from the 1X, 3X, and 10X treatment group were below the LOQ (<0.05 mg/kg) except one of the four results from day 28 of the 10X treatment, which was 0.056 mg/kg glyphosate.

Residues of glyphosate and AMPA in all muscle samples (days 1–56) from the 1X, 3X, and 10X treatment group were below the LOQ (<0.05 mg/kg).

The average level of glyphosate in liver samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.055 mg/kg, 0.152 mg/kg, and 0.603 mg/kg, respectively. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA levels were below the LOQ (<0.05 mg/kg) in liver samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average level of AMPA was 0.076 mg/kg, and 0.298 mg/kg, respectively. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

The average level of glyphosate in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.314 mg/kg, 0.986 mg/kg, and 3.82 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for the 1X treatment group, and 0.141 mg/kg, and 0.066 mg/kg in the 3X and 10X treatment groups, respectively. AMPA levels were below the LOQ (<0.05 mg/kg) in kidney samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average level of AMPA was 0.054 mg/kg, and 0.284 mg/kg, respectively. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for all the treatment groups.

Test facilities

Study directory:	Monsanto Company, St. Louis, Missouri 63198, USA
In-Life phase:	Hazleton Laboratories America, Inc., Madison, Wisconsin 53704, USA
Analytical phase:	Monsanto Company, St. Louis, Missouri 63198, USA

I. Materials and Methods

A. Materials

Two test materials, glyphosate and AMPA, were administered to the treated animals in this study. Further information on the test materials is listed in the tables below.

1. Test materials

Test material number 1:

Description:	Glyphosate
Batch number:	Not reported
HLA sample number:	50501936
Active ingredient(s):	Glyphosate (N-phosphonomethyl glycine)
CAS number:	1071-83-6
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	97.6 %
Formulation type:	NA
Appearance/colour:	Powdered solid
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in screw-top glass jar
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Test material number 2:

Description:	AMPA
Batch number:	Not reported
HLA sample number:	50703772
Active ingredient(s):	AMPA (aminomethylphosphonic acid)
CAS number:	1066-51-9
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	97.0 %
Formulation type:	NA
Appearance/colour:	Powdered solid
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in screw-top glass jar
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Single-comb white Leghorn laying hens were the test animals used in this study. Details are listed in the table below.

2. Test animals

Species:	Laying hen; Chicken (*Gallus gallus domesticus)*
Gender:	Female
Breed:	White Leghorn
Source:	[redacted]
Age:	38 weeks
Weight at dosing (Day -1):	Ranged from 1.492–2.007 kg
Number of animals:	100 hens selected out of a group of 130: (40 in untreated control group, and 20 in each of 3 treated groups (1X, 3X, and 10X dose levels)
Animal Identification:	Uniquely numbered leg band
Animal health / observations:	Physical examination of each animal by staff veterinarian at the beginning of acclimation (Day -15), at the beginning of the test period (Day -1), and just before sacrifice (Days 27, 34, and 55). The animals were approved for use in the study by the staff veterinarian on 29-Jul-1985.
Acclimation period:	19 days.
Diet:	The basal diet was composed of Purina Accu-Line Chicken Blend Concentrate® Lot No. 4751761 (25.25 %), ground yellow corn (67.25 %), and ground limestone (7.50 %). This diet was fed *ad libitum*. There were no known contaminants in the basal diet which would interfere with the conduct or outcome of this study.
Water:	Water was supplied *ad libitum* from stainless steel troughs.
Housing:	The animals were individually housed in 28 cm x 43 cm x 38 cm laying cages with roll-away floors. The cages were located in three-deck laying batteries with 10 birds/deck (two sub-sets of five birds). Each subset of five birds ate and drank from a communal feeder and waterer.

The environmental conditions at the test facility during the in-life phase of the study are summarised in the table below.

3. Environmental conditions

Temperature:	Ambient; ranged from 22–25 °C
Humidity:	Ranged from 49–80 %
Air change:	Not reported
Photoperiod:	16 hours of light/8 hours of darkness

B. Study Design and Methods

The study included 4 treatment groups, an untreated control and 3 treated groups (1X, 3X, and 10X dose levels). The 1X dose level was based on the maximum expected level of glyphosate and AMPA residues in the feed diet for chicken based on uses considered at the time the study was conducted. Exaggerated dose levels (3X and 10X) were also included in the study, consistent with guidelines for poultry feeding studies. The animals were assigned to treatment groups late in the acclimation period. Animals were randomly assigned to treatment groups based on body weight and egg production. Forty hens were assigned to the untreated control group and 20 hens were assigned to each of the three treated groups.

The control group was fed a non-treated diet while the three treated groups were fed rations containing both glyphosate and AMPA in a 9:1 ratio. Dosing of treated animals continued for 28 consecutive days. Upon completion of dosing, 10 animals from each treatment group were sacrificed and tissue samples were collected. The remaining hens were retained for use in a withdrawal phase of the study to evaluate reduction in any residues in eggs or tissues after dosing ended. Five hens from each of the 3 treated groups was sacrificed at 7 days after the end of the dosing period (i.e. Study Day 35), and the remaining 25 hens (10 control and 5 hens in each of the 3 treated groups) were sacrificed at 28 days after the end of the dosing period (i.e. Study Day 56).

Further details on the dosing regimen, including target dose levels, are summarised in the table below.

1. Dosing regimen

Route:	Oral via dietary intake
Vehicle:	Corn which was fortified with glyphosate and AMPA
Timing / frequency per day:	Test diet was added to feeders as necessary
Duration:	28 consecutive days
Treatment groups (dose levels):	4 treatment groups; untreated control and 3 dose levels (dry feed basis): 1X: nominal at 36 mg/kg glyphosate + 4 g/kg AMPA in total diet 3X: nominal at 108 mg/kg glyphosate + 12 mg/kg AMPA in total diet 10X: nominal at 360 mg/kg glyphosate + 40 mg/kg AMPA in total diet

The corn was fortified with glyphosate and AMPA for use in dosing the animals in the treated groups by addition of the powdered solid test materials. Glyphosate was pre-ground to a powder prior to use while AMPA was used as received. A series of blending steps achieved a uniform concentration of glyphosate and AMPA. Calcium carbonate and Accu-Line Chicken Blend were then added and the entire batch mixed.

Fortified feed samples were collected and analysed to confirm that the blending procedure produced a uniform concentration of the test materials throughout the treated batch. Samples were collected from the top, bottom, left and right positions of the mixing bowl for the three dose levels. Results from analysis of

the samples confirmed that uniform distribution of the test materials in the feed concentrate was achieved. Additionally, stability of glyphosate and AMPA in the feed diet was evaluated. Analysis of fortified feed indicated no significant decrease in glyphosate or AMPA concentrations when stored for 12 days at 25 °C. The batches of treated diets used to administer the test materials to the hens in this study were stored no longer than 7 days before use. Therefore, the period of demonstrated test material stability in the feed diet covers the maximum period of storage experienced in the study.

Samples (200 g each) were collected from each batch of feed provided to the hens and were analysed to determine levels of glyphosate and AMPA.

2. Daily observations and animal data collection

All animals were observed daily for general condition and behaviour. At weekly intervals, the amount of feed consumed by each subset of five birds was determined, and the average individual consumption calculated. Body weight was recorded weekly during the acclimation, test, and withdrawal periods.

3. Egg and tissue sample collection

Eggs were collected daily and the number of eggs produced by each hen recorded. Egg weights were recorded during the treatment and withdrawal periods. Eggs collected on Days -1, 1, 2, 4, 7, 14, 21, and 28 of the treatment period and Days l, 2, 4, 7, 14, 21, and 28 of the withdrawal period were pooled from each subset of five birds within each treatment group. These eggs were wiped with a damp towel and allowed to dry. The contents of each egg were put into a clean polyethylene container, the container shaken to break yolks, and frozen; shells were discarded. The weight each egg contributed to the pool was recorded. Eggs not required for analysis were incinerated intact.

At the time of tissue sample collection, specified animals were euthanised (using carbon dioxide gas). Samples of abdominal fat, breast and thigh muscle (50:50), liver, and kidney were collected from animals individually upon completion of the 28-day dosing period (on Study Day 28) or during the withdrawal phase of the study at 7 days or 28 days after the end of the dosing period (Study Days 35, and 56, respectively). Tissues were pooled from each subset of five birds within each treatment group. Gross necropsy was performed on sacrificed animals.

Egg and tissue samples were initially stored frozen (<-20 °C) in polyethylene containers at the In-life facility, Hazleton Laboratories, and then shipped to the Analytical Phase facility (Monsanto, St. Louis, Missouri, USA) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table 6.4.1-21: Egg and tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Egg	Dosing phase: -1, 1, 2, 4, 7, 14, 21, 28; Withdrawal phase: 29, 30, 32, 35, 42, 49, 56	Eggs were pooled from each subset of five birds within each treatment group.
Muscle[1]	End of dosing: Study Day 28 Withdrawal phase: Study days 35 and 56	~ 800 g/each subset of five birds within each treatment group[2]
Fat		~ 200 g/each subset of five birds within each treatment group[2]
Liver		~ 250 g/each subset of five birds within each treatment group[2]
Kidney		~ 50 g/each subset of five birds within each treatment group[2]

1 Composite of equal amounts of breast and thigh muscle.
2 Pooled samples were divided into duplicate samples; one shipped for analysis and one held as a reserve sample.

4. Analytical phase

Analysis of feed samples as well as egg and tissue samples was conducted at the Analytical Phase facility, Monsanto, St. Louis, Missouri, USA.

An analytical methodology was developed and validated for the determination of glyphosate and AMPA in the feed diet. The procedure consisted of extracting the feed diets with an aqueous/organic partition extraction (2:1 deionised water and chloroform) on a shaker, centrifuging, and ion exchange resin clean up. Quantitation was achieved by using a liquid chromatograph equipped with an Aminex A-9 analytical column, an o-phthalaldehyde (OPA) post-column reactor and a fluorescence detector.

The limit of validation/quantitation (LOQ) of the method was 4 mg/kg. Each feed diet was analysed in duplicate.

Recovery results with feed fortified with glyphosate and AMPA demonstrate that the intended dose concentration was achieved and are summarised in the table below.

Table 6.4.1-22: Recovery results: glyphosate and AMPA in feed

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Results/Range (%)	Mean[1] (%)	Standard deviation[1] (%)	Relative standard deviation[1] (%)	Number analyses (n)
Feed	Glyphosate	36 (1X)	99.8, 92.2, 88.5, 93.6, 102, 97.7, 87.0, 90.1, 99.1, 98.6	94.9	*5.2*	*5.5*	10
		108 (3X)	98.2, 94.4, 88.8, 90.9	93.1	*4.1*	*4.4*	4
		360 (10X)	95.3, 93.3, 93.9, 94.7, 92.5, 92.3, 95.6, 98.1	94.5	*1.9*	*2.0*	8
		Overall	87.0–102	94.4	4.0	*4.2*	22
	AMPA	4 (1X)	97.0, 90.4, 88.0, 90.8, 102, 97.6, 90.2, 93.0, 91.1, 92.7	93.3	*4.3*	*4.6*	10
		12 (3X)	92.0, 89.2, 86.3, 85.0	88.1	*3.1*	*3.5*	4
		40 (10X)	93.5, 92.6, 93.2, 93.2, 89.4, 91.2, 90.6, 93.5	92.2	*1.6*	*1.7*	8
		Overall	85.0–102	91.9	3.7	*4.0*	22

1 Standard deviations for individual fortification levels and relative standard deviations were calculated for this summary and are shown in italics.

Another analytical methodology was developed and validated for the determination of glyphosate and AMPA in hen eggs, as well as fat, muscle, liver, and kidney tissues. All samples were analysed using the analytical method based on the well-established method DFG 405 (refer to CA 4.1.2). The procedure used an aqueous/organic partition extraction (2:1 deionised water and chloroform). Glyphosate and AMPA were isolated from hen fat, muscle, liver, kidney and egg extracts by elution through Chelex 100 resin in the Fe(III) form. Glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron was removed using an ion exchange resin. After concentration to dryness to remove the hydrochloric acid, samples were analysed using a two column switching high pressure liquid chromatograph equipped with an OPA post-column reactor and a fluorescence detector.

The limit of validation/quantitation (LOQ) was 0.05 mg/kg each for glyphosate and AMPA in a fat, muscle, liver and kidney, and is 0.025 mg/kg each for glyphosate and AMPA in egg. Each tissue and egg sample was analysed in duplicate with a typical analytical set consisting of 2 control samples, 2 fortified

controls and 8 treated samples. Recovery results with samples of egg, fat, muscle, liver, and kidney fortified with glyphosate and AMPA are summarised in the table below.

Table 6.4.1-23: Recovery results: glyphosate and AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results/Range (%)	Mean[1] (%)	Standard deviation[1] (%)	Relative standard deviation[1] (%)	Number analyses (n)
Glyphosate	Egg	0.025	77.1, 78.3, 89.6, 91.7, 89.6, 100, 94.4, 104, 86.8, 83.0, 95.7, 94.0, 100, 102, 100, 94.4, 94.1, 97.4, 101, 98.5, 98.4, 96.4, 89.6, 86.3, 92.9, 89.4, 85.0, 81.4, 96.3, 91.1, 95.6, 81.4, 87.7, 80.0, 98.8, 91.8, 74.1, 77.6, 65.8, 70.8, 77.1, 75.1, 85.3, 84.2, 92.3, 88.4, 94.1, 92.4, 84.5, 89.9, 74.1, 75.8	*88.8*	*9.0*	*10*	52
		0.050	88.7, 80.2, 79.9, 87.1, 97.2, 96.0, 86.9, 91.9	*88.5*	*6.4*	*7.3*	8
		0.100	91.9, 89.4, 96.1, 89.7, 88.6, 90.3	*91.0*	*2.7*	*3.0*	6
		Overall	65.8–104	88.9	8.3	9.4	66
	Fat	0.05	84.5, 85.0, 87.4, 83.2	*85.0*	*1.8*	*2.1*	4
		0.10	85.7, 85.5	*85.6*	-	-	2
		Overall	83.2–87.4	85.2	1.4	1.6	6
	Muscle	0.05	104, 99.8, 84.6, 96.1, 96.0, 98.2	96.5	6.5	6.7	6
	Liver	0.05	75.1, 77.8	*76.5*	-	-	2
		0.25	74.3, 78.8	*76.6*	-	-	2
		1.0	83.0, 77.9	*80.5*	-	-	2
		Overall	74.3–83.0	77.8	3.1	4.0	6
	Kidney	0.05	68.6, 71.6	*70.1*	-	-	2
		1.0	91.3, 88.5	*89.9*	-	-	2
		5.0	91.7, 92.7	*92.2*	-	-	2
		Overall	68.6–92.7	84.1	10.9	13.0	6
AMPA	Egg	0.025	70.3, 75.4, 77.8, 84.2, 88.7, 94.7, 93.4, 96.8, 73.2, 72.8, 92.9, 91.6, 97.6, 93.8, 94.3, 87.0, 88.0, 91.2, 99.1, 97.0, 96.5, 93.8, 93.8, 96.8, 96.4, 92.6, 82.6, 80.2, 85.6, 84.3, 94.9, 95.0, 97.2, 78.2, 89.7, 87.2, 69.7, 70.7, 77.3, 72.4, 69.5, 71.4, 85.2, 84.2, 88.1, 90.1, 91.6, 90.0, 83.0, 85.2, 76.9, 80.6	*86.4*	*8.9*	*10*	52
		0.050	83.2, 76.9, 77.8, 82.3, 94.5, 93.6, 80.6, 84.4	*84.2*	*6.6*	*7.9*	8
		0.100	89.8, 87.0, 89.0, 86.7, 83.6, 84.2	*86.7*	*2.5*	*2.9*	6
		Overall	69.5–99.1	86.1	8.2	9.5	66
	Fat	0.05	84.0, 89.5, 86.8, 84.0	*86.1*	*2.6*	*3.1*	4
		0.10	82.4, 83.6	*83.0*	-	-	2
		Overall	82.4–89.5	85.1	2.6	3.1	6

Table 6.4.1-23: Recovery results: glyphosate and AMPA in eggs and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results/Range (%)	Mean[1] (%)	Standard deviation[1] (%)	Relative standard deviation[1] (%)	Number analyses (n)
	Muscle	0.05	94.8, 97.8, 93.3, 93.7, 94.3, 95.7	94.9	1.6	1.7	6
	Liver	0.05	73.8, 81.4	*77.6*	-	-	2
		0.25	76.0, 81.0	*78.5*	-	-	2
		1.0	83.9, 78.7	*81.3*	-	-	2
		Overall	73.8–83.9	79.1	3.7	4.7	6
	Kidney	0.05	83.3, 87.9	*85.6*	-	-	2
		1.0	89.1, 86.6	*87.9*	-	-	2
		5.0	87.2, 86.7	*87.0*	-	-	2
		Overall	83.3–89.1	86.8	1.9	2.2	6

1 Mean, standard deviation, and relative standard deviation values for individual fortification levels was calculated for this summary and are shown in italics.

II. Results and Discussion

A. Dose levels

As indicated previously, corn was fortified with glyphosate and AMPA at specified levels as the vehicle to administer the test materials through dietary intake to hens in the treated dose group. The nominal concentration of glyphosate in the diet for the 1X, 3X, and 10X treatment groups was 36 mg/kg, 108 mg/kg, and 360 mg/kg, respectively. The nominal concentration of AMPA in the diet for the 1X, 3X, and 10X treatment groups was 4.0 mg/kg, 12 mg/kg, and 40 mg/kg, respectively.

Analysis of samples of feed collected during the dosing phase of the study confirmed that actual dose levels were close the nominal/targeted dose levels. A summary results of analysis of feed to determine actual dose levels of glyphosate and AMPA (not corrected for recovery) is shown in the table below.

Table 6.4.1-24: Actual dose levels of glyphosate and AMPA in feed (not corrected for recovery)

Nominal dose level	Week number	Average Glyphosate (mg/kg)	Average AMPA (mg/kg)
1X Glyphosate: 36 mg/kg AMPA: 4 mg/kg	1	35.0	4.1
	2	38.3	3.5
	3	37.1	4.0
	4	35.0	3.9
	Overall average[1]:	*36.3±1.6*	*3.9±0.3*
3X Glyphosate: 108 mg/kg AMPA: 12 mg/kg	1	100.2	11.0
	2	102.4	10.5
	3	109.9	11.3
	4	105.5	12.0
	Overall average[1]:	*104.5±4.2*	*11.2±0.6*
10X Glyphosate: 360 mg/kg AMPA: 40 mg/kg	1	358.2	38.9
	2	318.4	36.9
	3	355.5	37.0
	4	350.1	35.7
	Overall average[1]:	*345.5±18*	*37.1±1.3*

1 Average and standard deviation values were calculated for this summary and are shown in italics. Standard deviations are calculated from the four weekly average values.

Results showed that actual levels of glyphosate and AMPA in each of the 3 dose levels were close to nominal/target levels. The overall average for glyphosate in the total diet on a dry feed basis (not corrected for recovery) in the 1X, 3X, and 10X treatments groups was 36.3 mg/kg, 104.5 mg/kg, and 345.5 mg/kg, respectively. The overall average for AMPA in the total diet on a dry feed basis (not corrected for recovery) in the 1X, 3X, and 10X treatments groups was 3.9 mg/kg, 11.2 mg/kg, and 37.1 mg/kg, respectively.

Additionally, in a second table below, dosage was calculated and expressed on the basis of subgroup average animal body weight (i.e. mg test material / kg bw/day). These results were calculated using the subgroup average intake of glyphosate and AMPA and average body weight of each subgroup during the dosing phase of the study. The overall average for glyphosate dosage on a body weight basis in the 1X, 3X, and 10X treated groups was 2.4 mg/kg bw/day, 7.1 mg/kg bw/day, and 23.3 mg/kg bw/day, respectively. The overall average for AMPA dosage on a body weight basis in the 1X, 3X, and 10X treated groups was 0.25 mg/kg bw/day, 0.76 mg/kg bw/day, and 2.50 mg/kg bw/day, respectively.

Table 6.4.1-25: Actual dose levels of glyphosate and AMPA administered to laying hens for 28 days expressed on basis of basis of body weight (bw) and concentration in total diet (dry feed)

Nominal dose level	Subset Number	Average body weight during dosing (kg)[1]	Average daily dry feed consumption (kg)[1]	Glyphosate dose/day[1]		AMPA dose/day[1]	
				mg/kg bw	mg / animal	mg/kg bw	mg / animal
1X[2] [36 mg/kg glyphosate + 4 mg/kg AMPA in dry feed (total diet)]	I	*1.84*	*0.12*	*2.3*	*4.27*	*0.25*	*0.45*
	L	*1.76*	*0.12*	*2.5*	*4.45*	*0.27*	*0.47*
	J	*1.73*	*0.11*	*2.3*	*3.98*	*0.24*	*0.42*
	K	*1.84*	*0.13*	*2.4*	*4.41*	*0.25*	*0.47*
	Average:	*1.79*	*0.12*	*2.4*	*4.28*	*0.25*	*0.45*
3X[3] [108 mg/kg glyphosate + 12 mg/kg AMPA in dry feed (total diet)]	M	*1.72*	*0.12*	*7.5*	*13.0*	*0.81*	*1.39*
	P	*1.72*	*0.11*	*7.0*	*12.0*	*0.75*	*1.28*
	N	*1.72*	*0.11*	*6.7*	*11.5*	*0.72*	*1.24*
	O	*1.72*	*0.12*	*7.3*	*12.5*	*0.78*	*1.34*
	Average:	*1.72*	*0.12*	*7.1*	*12.2*	*0.76*	*1.31*
10X[4] [360 mg/kg glyphosate + 40 mg/kg AMPA in dry feed (total diet)]	Q	*1.83*	*0.12*	*22.2*	*40.6*	*2.39*	*4.36*
	R	*1.80*	*0.13*	*24.0*	*43.2*	*2.58*	*4.64*
	S	*1.67*	*0.12*	*24.6*	*41.1*	*2.64*	*4.42*
	T	*1.89*	*0.12*	*22.6*	*42.7*	*2.43*	*4.58*
	Average:	*1.80*	*0.12*	*23.3*	*41.9*	*2.50*	*4.50*

1 All values were calculated for this summary and are thus shown in italics.
2 Average of weekly mg glyphosate/kg feed was 36.33 mg/kg and average mg AMPA/kg feed was 3.86 mg/kg.
3 Average of weekly mg glyphosate/kg feed was 104.49 mg/kg and average mg AMPA/kg feed was 11.21 mg/kg.
4 Average of weekly mg glyphosate/kg feed was 345.54 mg/kg and average mg AMPA/kg feed was 37.10 mg/kg.

B. Animal health and daily observations

There were no findings concerning animal health or behavior that were considered to be test related. Feed consumption for all animals in each test group remained essentially stable during the test period. Body weight fluctuations seen during the study were considered normal for adult animals. Following animal sacrifice, necropsy/pathology evaluation indicated no macroscopic or microscopic observations that appear treatment related.

C. Residue levels in eggs and tissues

The residue values presented in the summary in the study report had been corrected for recovery. The residue values in the tables below were not corrected for recovery.

Residues of glyphosate and AMPA in eggs collected from untreated control animals were below the LOQ (<0.025 mg/kg). Residues of glyphosate and AMPA in tissues (fat, muscle, liver, and kidney) collected from untreated control animals were below the LOQ (<0.05 mg/kg).

Frozen storage stability of glyphosate and AMPA in hen matrices (eggs, fat, muscle, liver, and kidney) was evaluated in a separate study completed subsequent to this feeding study. No significant degradation of glyphosate or AMPA in hen fat, muscle, liver, or eggs was observed for 430 days, which was the maximum period of frozen storage evaluated. No significant degradation of hen kidney was observed for a period of 130 days, which was the maximum period of frozen storage evaluated.

Residues of glyphosate and AMPA in all egg samples (days 1-56) from the 1X treatment group were below the LOQ (<0.025 mg/kg). Treatment days 7–28 of the 3X and days 4–28 of the 10X samples contained glyphosate residues above the LOQ and AMPA residues below the LOQ (except 10X-day 21 subgroup R which contained 0.026 mg/kg AMPA). The glyphosate residue levels ranged from <0.025 mg/kg (3X-days 1–7 and 10X-days 1–4) to 0.026 mg/kg (3X-days 14–28) to 0.091 mg/kg (10X-day 21). The plateau for glyphosate was reached approximately at days 14-21.
Withdrawal days 29–30 of the 3X and days 29–35 of the 10X samples contained glyphosate residues above the LOQ and AMPA residues below the LOQ. The glyphosate level ranged from 0.027 mg/kg (3X-days 29 and 30) to 0.082 mg/kg (10X-day 30).

Table 6.4.1-26: Residues of glyphosate in eggs for dosing days 1–28

Treatment Group	Subgroup No.	Glyphosate residue (mg/kg)[1, 2, 3] Study Day 1	2	4	7	14	21	28[4]	Average
3X Glyphosate (average): *104.5 mg/kg in feed; 7.1 mg/kg bw*	M	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*0.026*	*0.025*	*0.026*	*0.025*
	P	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*0.026*	*<0.025*	*0.025*
	N	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*0.026*	*0.027*	*0.027*	*0.026*
	O	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*0.026*	*<0.025*	*<0.025*	*0.025*
	Average:	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*0.026*	*0.026*	*0.026*	*0.025*
10X Glyphosate (average): *345.5 mg/kg in feed; 23.3 mg/kg bw*	Q	*<0.025*	*<0.025*	*<0.025*	*0.063*	*0.097*	*0.080*	*0.074*	*0.056*
	R	*<0.025*	*<0.025*	*<0.025*	*0.078*	*0.063*	*0.116*	*0.095*	*0.061*
	S	*<0.025*	*<0.025*	*<0.025*	*0.062*	*0.091*	*0.085*	*0.076*	*0.055*
	T	*<0.025*	*<0.025*	*<0.025*	*0.067*	*0.076*	*0.085*	*0.085*	*0.055*
	Average:	*<0.025*	*<0.025*	*<0.025*	*0.067*	*0.082*	*0.091*	*0.082*	*0.057*

5 LOQ (limit of quantitation):0.025 mg/kg
6 All values calculated for this summary. Residue values are uncorrected for recovery.
7 For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.
8 Study Day 28 is at the end of the 28-day dosing period

Table 6.4.1-27: Residues of glyphosate in eggs for withdrawal phase days 29–56

Treatment Group	Subgroup No.	Glyphosate residue (mg/kg)[1, 2, 3] Study Day						
		29	30	32	35	42	49	56
3X Glyphosate (average): *104.5 mg/kg in feed; 7.1 mg/kg bw*	N	*0.029*	*0.026*	*<0.025*	*<0.025*	-	-	-
	O	*<0.025*	*0.028*	*<0.025*	*<0.025*	*<0.025*	*<0.025*	*<0.025*
	Average:	*0.027*	*0.027*	*<0.025*	*<0.025*	-	-	-
10X Glyphosate (average): *345.5 mg/kg in feed; 23.3 mg/kg bw*	S	*0.078*	*0.083*	*0.056*	*<0.025*	-	-	-
	T	*0.078*	*0.081*	*0.063*	*0.025*	*<0.025*	*<0.025*	*<0.025*
	Average:	*0.078*	*0.082*	*0.060*	*0.025*	-	-	-

1 LOQ (limit of quantitation):0.025 mg/kg
2 All values calculated for this summary. Residue values are uncorrected for recovery.
3 For purposes of calculating averages, residue values of <0.025 mg/kg were assigned a value of 0.025 mg/kg if being averaged with a value of 0.025 mg/kg or greater.

The residues of glyphosate and AMPA in all fat samples (days 1–56) from the 1X, 3X, and 10X treatment group were below the LOQ (<0.05 mg/kg) except one of the four results from day 28 of the 10X treatment, which was 0.056 mg/kg glyphosate.

Table 6.4.1-28: Residues of glyphosate and AMPA in fat

Treatment Group	Subgroup No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
10X Glyphosate (average): *345.5 mg/kg in feed; 23.3 mg/kg bw* AMPA (average): *37.1 mg/kg in feed; 2.50 mg/kg bw*	Q	28	1	0.056	*0.053*	<0.050	<0.050
			2	<0.050		<0.050	
	R	28	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
	Study Day 28, 10X treatment group average:				*0.051*		*<0.050*

1 Study Day 28 is at the end of the 28-day dosing period.
2 LOQ (limit of quantitation):0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages for this summary, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater.

The residues of glyphosate and AMPA in all muscle samples (days 1–56) from the 1X, 3X, and 10X treatment group were below the LOQ (<0.05 mg/kg).

The average level of glyphosate in liver samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.055 mg/kg, 0.152 mg/kg, and 0.603 mg/kg, respectively. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA levels were below the LOQ (<0.05 mg/kg) in liver samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average level of AMPA was 0.076 mg/kg, and 0.298 mg/kg, respectively. AMPA residues in liver were below the LOQ (<0.05 mg/kg)

in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

The average level of glyphosate in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.314 mg/kg, 0.986 mg/kg, and 3.82 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for the 1X treatment group, and 0.141 mg/kg, and 0.066 mg/kg in the 3X and 10X treatment groups, respectively. AMPA levels were below the LOQ (<0.05 mg/kg) in kidney samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average level of AMPA was 0.054 mg/kg, and 0.284 mg/kg, respectively. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for all the treatment groups.

Table 6.4.1-29: Residues of glyphosate and AMPA in liver

Treatment Group	Subgroup No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
1X	I	28	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
Glyphosate (average): *36.3 mg/kg in feed; 2.4 mg/kg bw*	L	28	1	0.060	0.059	<0.050	<0.050
			2	0.058		<0.050	
	Study Day 28, 1X treatment group average:				*0.055*		*<0.050*
AMPA (average): *3.9 mg/kg in feed; 0.25 mg/kg bw*	J	35	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
	K	56	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
3X	M	28	1	0.139	0.138	0.069	0.070
			2	0.137		0.070	
Glyphosate (average): *104.5 mg/kg in feed; 7.1 mg/kg bw*	P	28	1	0.169	0.166	0.083	0.082
			2	0.163		0.080	
	Study Day 28, 3X treatment group average:				*0.152*		*0.076*
AMPA (average): *11.2 mg/kg in feed; 0.76 mg/kg bw*	N	35	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
	O	56	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
10X	Q	28	1	0.621	0.614	0.331	0.329
			2	0.607		0.327	
Glyphosate (average): *345.5 mg/kg in feed; 23.3 mg/kg bw*	R	28	1	0.538	0.592	0.248	0.267
			2	0.646		0.286	
	Study Day 28, 10X treatment group average:				*0.603*		*0.298*
AMPA (average): *37.1 mg/kg in feed; 2.50 mg/kg bw*	S	35	1	0.107	0.113	0.108	0.113
			2	0.119		0.119	
	T	56	1	<0.05	<0.05	<0.050	<0.050
			2	<0.05		<0.050	

1 Study Day 28 is at the end of the 28-day dosing period; Study Days 35 and 56 are during the withdrawal period, 7 days and 28 days after the end of dosing, respectively.
2 LOQ (limit of quantitation):0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater.

Table 6.4.1-30: Residues of glyphosate and AMPA in kidney

Treatment Group	Subgroup No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
1X Glyphosate (average): *36.3 mg/kg in feed; 2.4 mg/kg bw* AMPA (average): *3.9 mg/kg in feed; 0.25 mg/kg bw*	I	28	1	0.257	0.274	<0.050	<0.050
			2	0.291		<0.050	
	L	28	1	0.341	0.354	<0.050	<0.050
			2	0.367		<0.050	
	Study Day 28, 1X treatment group average:				*0.314*		*<0.050*
	J	35	1	0.050	0.050	<0.050	<0.050
			2	<0.050		<0.050	
	K	56	1	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
3X Glyphosate (average): *104.5 mg/kg in feed; 7.1 mg/kg bw* AMPA (average): *11.2 mg/kg in feed; 0.76 mg/kg bw*	M	28	1	0.682	0.731	<0.050	<0.050
			2	0.780		<0.050	
	P	28	1	1.23	1.24	0.060	0.058
			2	1.25		0.056	
	Study Day 28, 3X treatment group average:				*0.986*		*0.054*
	N	35	1	0.222	0.192	<0.050	<0.050
			2	0.161		<0.050	
	O	56	1	0.232	0.141	<0.050	<0.050
			2	<0.050		<0.050	
10X Glyphosate (average): *345.5 mg/kg in feed; 23.3 mg/kg bw* AMPA (average): *37.1 mg/kg in feed; 2.50 mg/kg bw*	Q	28	1	3.26	3.32	0.262	0.266
			2	3.38		0.269	
	R	28	1	4.76	4.32	0.337	0.302
			2	3.87		0.266	
	Study Day 28, 10X treatment group average:				*3.82*		*0.284*
	S	35	1	0.292	0.292	<0.050	<0.050
	T	56	1	0.067	0.066	<0.050	<0.050
			2	0.065		<0.050	

1 Study Day 28 is at the end of the 28-day dosing period; Study Days 35 and 56 are during the withdrawal period, 7 days and 28 days after the end of dosing, respectively.
2 LOQ (limit of quantitation): 0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater.

III. Conclusion

Residues of glyphosate and AMPA in all egg samples (days 1–56) from the 1X treatment group were below the LOQ (<0.025 mg/kg). Treatment days 7–28 of the 3X and days 4–28 of the 10X samples contained glyphosate residues above the LOQ and AMPA residues below the LOQ (except 10X-day 21 subgroup R which contained 0.026 mg/kg AMPA). The glyphosate residue levels ranged from <0.025 mg/kg (3X-days 1–7 and 10X-days 1–4) to 0.026 mg/kg (3X-days 14–28) to 0.091 mg/kg (10X-day 21). Withdrawal days 29–30 of the 3X and days 29–35 of the 10X samples contained glyphosate residues above the LOQ and AMPA residues below the LOQ. The glyphosate level ranged from 0.027 mg/kg (3X-days 29 and 30) to 0.082 mg/kg (10X-day 30).

Residues of glyphosate and AMPA in all fat samples (days 1–56) from the 1X, 3X, and 10X treatment group were below the LOQ (<0.05 mg/kg) except one of the four results from day 28 of the 10X treatment, which was 0.056 mg/kg glyphosate.

Residues of glyphosate and AMPA in all muscle samples (days 1–56) from the 1X, 3X, and 10X treatment group were below the LOQ (<0.05 mg/kg).

The average level of glyphosate in liver samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.055 mg/kg, 0.152 mg/kg, and 0.603 mg/kg, respectively. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA levels were below the LOQ (<0.05 mg/kg) in liver samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average level of AMPA was 0.076 mg/kg, and 0.298 mg/kg, respectively. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

The average level of glyphosate in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.314 mg/kg, 0.986 mg/kg, and 3.82 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for the 1X treatment group, and 0.141 mg/kg, and 0.066 mg/kg in the 3X and 10X treatment groups, respectively. AMPA levels were below the LOQ (<0.05 mg/kg) in kidney samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average level of AMPA was 0.054 mg/kg, and 0.284 mg/kg, respectively. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for all the treatment groups.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the residues of glyphosate and AMPA in poultry (hen) eggs and tissues (fat, muscle, liver and kidney) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of glyphosate and AMPA residues that may transfer from the poultry diet to eggs and edible poultry tissues. It was performed under GLP, and it is considered to be scientifically valid. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 505 and OECD Guidance Document on Residues in Livestock (Series on Pesticides No. 73) with a few deviations.

Hens were slaughtered on the day of last dosing but within 6 hours of the final dose cannot be confirmed. The sample weights after slaughter were not reported. Only 2 samples per sampling interval and feeding level were taken instead of 3 samples. In eggs plateau was not reached at day 7, hence more than just weekly samplings would be required between 7 and 28 days. For the depuration phase only 2 intervals instead of 3 intervals were analysed, however depuration of residues was investigated at all dose levels. After 35 days of withdrawal residues of glyphosate above LOQ could be detected in liver at 10X dose (0.15 mg/kg) and in kidney at 0.06, 0.23 and 0.35 mg/kg at 1X, 3X and 10X dose, respectively. AMPA was detected only in liver at 0.15 mg/kg at 10X dose. After 56 days of withdrawal glyphosate residues could be detected only in kidney at 3X and 10X dose (0.17 and 0.08 mg/kg, respectively). AMPA was not detected in any tissue after 56 days of withdrawal.

The period for which samples were stored frozen before extraction/analysis is not provided. The date of analysis is not specified. However, the dates of sacrifice are given as 27.08.1985, 3.09.1985 and 24.09.1985 for the first, second and the final sacrifice. The date of draft final report and raw data inspection is on 02.10.1887. Thus, the maximum storage time is 766 days (about 25 months). For poultry, residues of glyphosate and AMPA in fat, muscle and liver were shown to be stable for at least 25 months, for kidney and egg 13 and 14 months, respectively (CA 6.1, [redacted] 1988). Additionally, in a feeding study presented in this chapter (CA 6.4.1/002, [redacted] 1987) residues of glyphosate and AMPA in eggs and liver were proven to be stable for 683 days (23 months). The storage time calculated based on the date of finalisation of the report is likely to be a huge overestimation.

The study is considered valid as these deficits are not expected to significantly impact the quality or reliability of the study.

Assessment and conclusion by RMS:

Relevant published articles from Literature Search Report

1. Information on the study

Data point	CA 6.4.1/004
Report author	Shehata, A.A. *et al.*
Report year	2014
Report title	Distribution of Glyphosate in Chicken Organs and its Reduction by Humic Acid Supplementation
Document No.	DOI 10.2141/ jpsa.0130169 ISSN 1346-7395
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/ reliable with restrictions

2. Full summary of the study according to OECD format

Executive Summary

Glyphosate (N-(phosphonomethyl) glycine) is a most popular **herbicide in agricultural practices** throughout the world. It is possible that glyphosate spread in the ecosystems can reach plants, animals. The present work was directed to investigate the glyphosate residue in different organs of broiler chickens using ELISA and to study the possibility of its neutralisation using humic acid, *Chlorella vulgaris* and *Saccharomyces boulardii*. Results showed that glyphosate residues could be detected in the animal feed and different organs as liver, spleen, lung, intestine, heart, muscles and kidney. Humic acid, *Chlorella vulgaris* and *Saccharomyces boulardii* showed neutralisation of the antimicrobial effect of glyphosate in vitro. Also, feed supplementation of commercial broiler with humic acid (0.2 %) leads to a significant decrease in the glyphosate content, i.e. by 53 %, 28 %, 44 %, 50 %, 56 %, 16 %, 63 % and 0 % in serum, liver, spleen, lung, gastro-intestinal tract, heart, muscles and kidney, respectively. There were no significant effects of humic acid on the production parameters. This enlightenment will help to overcome the negative effect of glyphosate residues on gastrointestinal microbiota and protect consumers from glyphosate residues in chicken meat.

Materials and Methods

Distribution of Glyphosate in Feed and Tissues

A total of one hundred commercial broiler chickens collected from different farms were slaughtered at 30-day-old. Different organs as liver, spleen, lung, intestine, heart, muscles and kidney were collected and tested for presence of glyphosate using ELISA. Briefly, samples were collected from 10 chickens per farm at 39-day-old after slaughtering and cut to small pieces. In relation to its ability to retain water specimens were suspended in aqua distilled (Braun, Germany) at the rate of 1:1 (low water retention), 1:5 or 1:10 (high water retention). The specimens were heated at 100℃ for 10 min, homogenised with ULTRA-TURRAX® (IKA, Wilmington, Germany) and frozen at minus 80℃ for eight hours. Homogenised specimens were thawed at 40℃ and centrifuged at 10000 x g for 10 min. The supernatant

was filtered with an ultracentrifugal filter (3000 Da) to remove proteins and peptides. Filtrates were centrifuged again at 10000 x g for 10 min and the supernatant was tested for glyphosate concentration by ELISA using Glyphosate ELISA kits (Abraxis, Warminister, PA, USA) according to the manufacturer's protocol. Test validation was done with Gas Chromatography-Mass Spectroscopy (GC-MS) by Medizinische Labor (Bremen, Germany), the correlation coefficient between the two tests was 98 %.

In vitro Neutralisation of Glyphosate

The minimal inhibitory concentration (MIC) of glyphosate (Roundup UltraMax®, Monsanto, USA) on *E. faecalis*, *Bacillus badius* (isolated from algae *Chlorella vulgaris*, Ökologische Produkte Altmark Co., Germany) and *Bifidobacterium adolescentis* (isolated from chickens), as indicators, was determined according to the National Committee for Clinical Laboratory Standards (NCCLS). Briefly, the lowest concentration of glyphosate which shows bactericidal or bacteriostatic effects was determined in a 24-well micro-titre plate. Serial dilutions of glyphosate (5, 2.5, 1.2, 0.6, 0.3, 0.15 and 0.075 mg/ml) were made in reinforced clostridial medium (RCM, Sifin, Germany). Tested bacteria was added at a final concentration of 10^4 CFU/ml and the test plates containing diluted glyphosate and tested bacteria were incubated overnight at 37℃. The MIC value was evaluated by quantitative analysis of bacterial growth on Citrat-Azid-Tween-Carbonat Agar (CATC, Oxoid, Germany). The neutralizing effect of humic acid RB4, composed of different molecular weights molecules ranged from 1500 Da to 200000 Da, (WH Pharmawerk Weinböhla GmbH, Weinböhla, Germany), was tested. The MIC value of glyphosate on *E. faecalis*, *Bacillus badius* and *Bifidobacterium adolescentis* in the presence of humic acid RB4 (1 mg/ml), *Chlorella vulgaris* extract (Ökologische Produkte Altmark Co., Germany) at a concentration of 1 mg/ml and *Saccharomyces boulardii* at a concentration of 10^9 CFU/ml (UCB Pharma GmbH, Monheim, Germany) determined.

In vivo Neutralisation of Glyphosate Using Humic Acid

The experiment was performed in two chicken broiler barns, designated A and B, each barn accommodated for 22000 broiler chicks. Chickens kept in house A were fed the basic diet without supplementation of humic acid, while chickens kept in house B were fed the same diet with humic acid RB4 (WH Pharmawerk Weinböhla GmbH, Weinböhla, Germany) supplementation (0.2 %) from the first day till slaughtering. The ration was formulated as follow: starter (21 % corn, 40 % wheat, 29 % soya bean and 4.5 % fat), grower (22 % corn, 47 % wheat, 19 % soya bean and 5 % fat), and finisher (17 % corn, 48 % wheat, 17 % soya bean and 4.9 % fat). Chickens were allowed to have free access to feed and water until the end of experiment. All chickens were vaccinated against infectious bronchitis (IB) at 12-day-old, Newcastle disease (ND) and infectious bursal disease at 18-days-old. The total mortality and body weight (BW) were calculated at the end of the experiments. Glyphosate residues were determined in serum, liver, spleen, lung, GIT, heart, muscles and kidney using ELISA as mentioned above.

Statistical Analysis

The statistical analysis was carried out with GraphPad Prism 4 (GaphPad Software, La Jolla, USA). Two-way analysis of variance followed by unpaired Student t-test was used to identify significant differences between means.

Results

Distribution of Glyphosate in Feed and Tissues

The glyphosate residues could be detected in feed, liver, spleen, lung, intestine, heart, muscles and kidney using ELISA in the concentrations of 370, 9.8, 21.1, 24.2, 98.3, 20.4, 5.0 and 16.0 ng/gm, respectively (Table 1).

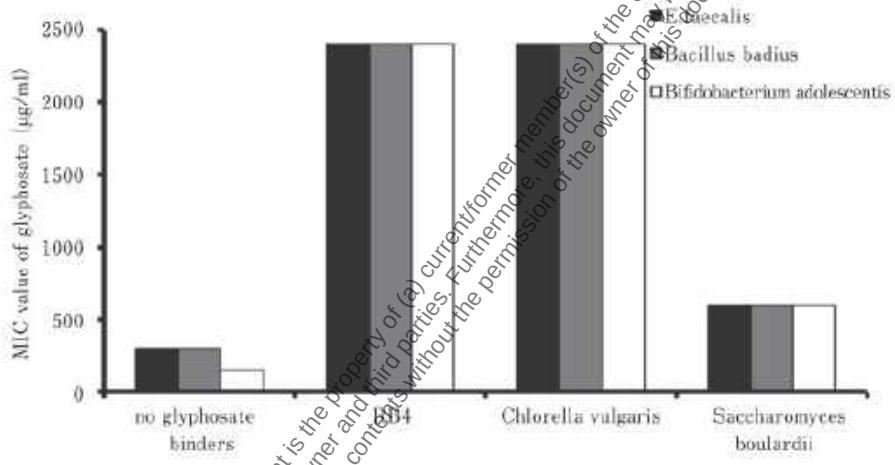


Table 1: Distribution of glyphosate in feed and chickens tissues.

Sample N=30	Glyphosate (ng/gm)		
	Minimum	Maximum	Mean±SD
Feed	190.0	400.0	370.0±92.0
Liver	6.0	13.6	9.8±3.0
Spleen	11.8	25.0	21.1±17.0
Lung	12.0	25.0	24.2±9.0
Intestine	20.0	120.0	98.3±42.0
Heart	17.0	20.0	20.4±0.6
Muscles	3.6	4.9	5.0±0.3
Kidney	0.4	17.6	16.0±13.0

Neutralisation of Glyphosate in vitro

The MIC value of glyphosate for *E. faecalis*, *Bacillus badius* and *Bifidobacterium adolescentis* were 300, 300 and 150 µg/ml, respectively. The RB4 and *Chlorella vulgaris* in concentrations of 1 mg/ml showed the higher neutralisation of the antimicrobial effect of glyphosate. The MIC-values of glyphosate for *E. faecalis*, *Bacillus badius* and *Bifidobacterium adolescentis* in the presence of humic acid or *Chlorella vulgaris* were 2400 µg/ml (Fig. 1). However, the MIC-value of glyphosate for *E. faecalis*, *Bacillus badius* and *Bifidobacterium adolescentis* in the presence Saccharomyces boulardii was 600 µg/ml (Fig. 1).

Figure 1: Changes in the MIC values of glyphosate on *E. faecalis*, *Bacillus badius* and *Bifidobacterium adolescentis* using different glyphosate binders.

In vivo Neutralisation of Glyphosate Using Humic Acid

In untreated chickens, the glyphosate concentrations in serum, liver, spleen, lung, GIT, heart, muscles and kidney were 2, 14, 21, 24, 101, 20, 6 and 6 ng/gm, respectively, however, in humic acid treated chickens, glyphosate residues were 0.88, 9.78, 11.79, 12.20, 43.6, 17.4, 1.9 and 6. 2 ng/gm, respectively. Supplementation of humic acid caused a significant decrease in the glyphosate content, i.e. by 53 %, 28 %, 44 %, 50 %, 56 %, 16 %, 63 % and 0 % in serum, liver, spleen, lung, GIT, heart, muscles and kidney, respectively (Fig. 2). At 30-day-old, there is no significant improvement of body weight and total mortalities between humic acid-treated and untreated chickens (Table 2), the average body weight of both was 1.69 Kg. However at 39-day-old, the average body weight of 2.456 Kg while it was 2.339 Kg in untreated chickens (Table 2).

Figure 2: Effect of humic acid supplementation on glyphosate accumulation in chickens. Glyphosate was measured using ELISA and expressed as ng/gm. Asterisks denote significant decrease of glyphosate in humic acid treated chickens (* $P = 0.05$, ** $= P < 0.001$).

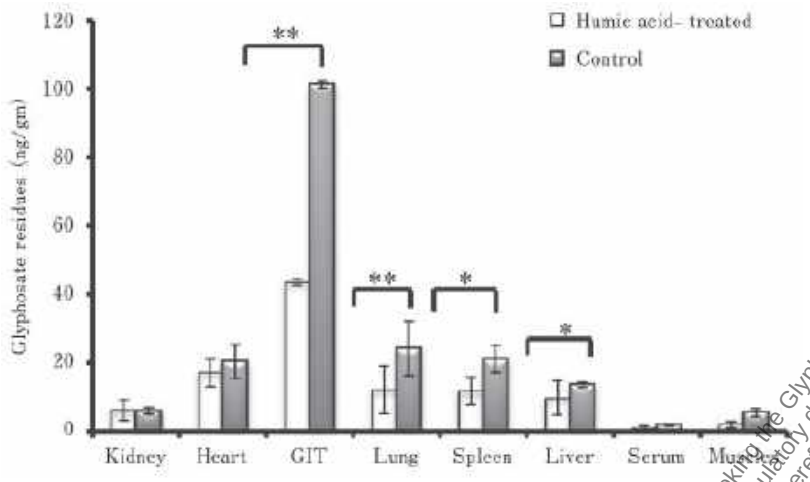


Table 2: Effect of humic acid supplementation on the production parameters.

Parameter	Humic acid-treated chickens	Non-treated chickens
Total number	22500	22100
Slaughtered number at 30-day-old	6509	6509
Slaughtered number at 39-day-old	14581	13573
Body weight at 30-day-old (average/kg)	1.69	1.69
Body weight at 39-day-old (average/kg)	2.453	2.339
Total feed intake (kg)	76530	78690
Food conversion ratio	1.64	1.66

Discussion

Distribution of Glyphosate in Feed and Tissues

Glyphosate residues in food and feed have been on the rise, due to higher rates and frequency of application, which in turn is due to increasing weed resistance (Samsel and Seneff, 2013). In the present study glyphosate residues could be detected in liver, spleen, lung, intestine, heart, muscles, kidney and animal feed (Table 1). The maximum residue levels (MRLs) of glyphosate in soya bean, maize, cereal grains, cotton seed, alfalfa, hay, sorghum straw, wheat and wheat straw were agreed by the United Nations Food and Culture Organisation's to be 20, 5.0, 30, 40, 500, 500, 50, 200 and 300 mg/kg (WHO, 1994). Data on the real presence of glyphosate and its metabolite in feed from glyphosate sprayed crops are sparse. A now common practice of crop desiccation through herbicide administration shortly before the harvest assures an increased glyphosate residues in food sources as well (Baig *et al.*, 2003; Ellis *et al.*, 1998). Also, the maximum daily intake (MDI) of glyphosate depends on the ration composition and the percent of each component in the ration. Glyphosate residues concentrate in approximately 80 % genetically modified plants grown for food and feed up to 400 ppm, maximal residual levels.

Neutralisation of Glyphosate in vitro

Many studies have reported that glyphosate can be sorbed to humic acids (Piccolo *et al.*, 1996; Banta *et al.*, 2009; Mazzei and Piccolo, 2012). In the present study the humic acid RB4 neutralised the antimicrobial effect of glyphosate in vitro. The MIC-value of glyphosate for *E. faecalis*, *Bacillus badius* and *Bifidobacterium adolescentis* in the presence of RB4 humic acids or Clorella vulgaris were 2.4 mg/ml.

Chlorella has also useful detoxifying properties. The use of oral supplements of *Chlorella pyrenoidosa* has been reported to significantly reduce dioxin levels in breast milk of 35 nursing women in Japan (Nakano *et al.*, 2007). Also Chlorella supplementation significantly reduced liver toxicity and cadmium-accumulation in cadmium poisoned rats (Shim *et al.*, 2008).

Yeast has been used as general performance promoter in poultry feeds and has been shown to have beneficial effects against mycotoxins exposure (Celyk *et al.*, 2003, Santin *et al.*, 2003, Baptista *et al.*, 2004). The absorbent ability of yeast to mycotoxins could be attributed to the presence of innumerable sites on its surface for physical adsorption of molecules (Shetty and Jespersen 2006). In the present study *Saccharomyces boulardii* showed a low absorbent ability to glyphosate (Fig. 1).

Neutralisation of Glyphosate by Humic Acid Supplementation in vivo
The use of humic acids and their sodium salt for the oral treatment of all animals on food production farms is currently permitted. Supplementing animal feeds with non-nutritive adsorbents as humic acid has proven to substantially reduce mycotoxicosis (Sabater-Vilar *et al.*, 2007) and improved the performance, carcass, GIT and meat quality traits (Ozturk *et al.*, 2011). In our study, the mortality was negligible with no difference between control and humic acid-treated group. Also the humic acid-treated chickens showed no improvement in feed conversion in birds and body weight at 30-day-old (Table 2). Kocabagli *et al.* (2002) reported an improvement in feed conversion in birds that were given 0.25 % humic acid either from 0 to 42 d or during grow-out periods only, between d 21 to 42. A similar conclusion was drawn by Yoruk *et al.* (2004), who showed a better feed conversion in hens supplemented with 0.1-0.2 % humic acid, and it did not affect body weight. On the contrary, Rath *et al.* (2006) found that humic acid-treated chickens showed a reduction in body weight, and the feed conversion ratio was numerically higher.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The publication provides information about the levels of parent glyphosate residues in feed and tissues of broiler chicken (including edible tissues such as muscle and liver). This may allow to estimate residue transfer factors from poultry feed to poultry meat. Therefore, the publication is considered relevant. The authors further investigated the impact of a feed supplementation with humic acid on the transfer of glyphosate residues in poultry tissues. It was concluded that the supplementation with humic acid allows to significantly decrease the residues of glyphosate in poultry tissues (-63 % in muscle and -28 % in liver). Thus, the control group (which received feed without humic acid supplementation) represents a worst case in terms of residues and is more relevant from a regulatory perspective. The highest residues found in chicken muscle and liver were extremely low (ca. 0.005 mg/kg and 0.018 mg/kg, respectively). This is consistent with the results of the submitted poultry feeding studies (which were conducted at dose levels far above the dietary exposure of the broiler chickens in the publication). However, both the experimental procedures and the obtained results are not described with a sufficient level of accuracy and it is difficult to figure out exactly what was done and how the presented results were generated. The sample preparation procedure (with consecutive steps at 100°C and -80°C) is quite unusual and no method validation data are presented. Because of that, the publication is reliable with restrictions.

CA 6.4.2 Ruminants

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.2/001
Report author	[redacted]
Report year	2007

Report title	Magnitude of residues of *N*-acetyl glyphosate and degradates in dairy cow tissues and milk
Report No	28210
Document No	[redacted]20087
Guidelines followed in study	U.S. EPA Residue Chemistry Test Guidelines, OPPTS 860.1480, Meat/Milk/Poultry/Eggs (1996) OECD Guidelines for the Testing of Chemicals (505), Residues in Livestock, 8 January 2007 EU Guidance Appendix G: Livestock Feeding Studies, 7031/VI/95 Revision 4 (22/7/1996)
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 505: • Depuration phase includes only 2 intervals instead of 3 intervals
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of this study was to determine the magnitude of the residues in milk and tissues of lactating dairy cattle dosed orally with of *N*-acetyl glyphosate for a period of 28 consecutive days, and at 4 to 8 days after dosing ended (i.e. after a depuration withdrawal period of 4 to 8 days).
N-acetyl glyphosate was administered orally as an aqueous solution *via* drench gun to four groups of lactating Holstein/Friesian cows (3 cows/group) twice daily for 28 consecutive days. Dosing was conducted at target treatment levels of 1.25, 3.75, 12.5, and 37.5 mg *N*-acetyl glyphosate/kg bodyweight (equivalent to 1, 3, 10, and 30 mg of glyphosate/kg bodyweight). Additional two cows were dosed at 37.5 mg/kg bodyweight for 28 days followed by a 4 to 8-day depuration period. Two control cows were dosed with dose vehicle only (containing no *N*-acetyl glyphosate) for the 28-day treatment period. The actual mean weekly dose levels were 1.268–1.287, 3.780–3.831, 12.59–12.69, and 38.33–38.94 mg *N*-acetyl glyphosate/kg bodyweight (equivalent to 1.015–1.029, 3.024–3.065, 10.07–10.16, and 30.66–31.15 mg of glyphosate/kg bodyweight). The mean weekly dose levels were equivalent to 43.39-45.28, 129.1–130.0, 419.5–451.7, and 1153–1200 mg *N*-acetyl glyphosate/kg feed, based upon the actual levels of feed consumption (equivalent to 34.71–36.23, 103.3–104.0, 335.6–361.4, and 922.2–960.0 mg of glyphosate/kg daily feed).

All cows used in the study were in good general health throughout the acclimation and treatment periods. No treatment-related effects on feed consumption, milk production, or bodyweight were observed during the study.

Whole milk was collected twice daily and samples from afternoon sampling were combined with samples from the next morning. Milk samples were collected from individual cows and samples from Days -1, 1, 3, 5, 7, 10, 14, 21, and 28 were analysed. Milk samples from the depuration group were collected during the dosing period as above and on Days 1, 3, 5, and 7 post-dosing. Skim milk and cream samples were prepared from milk collected on Days 14 and 28 and analysed. Within 24 hours after the final morning dose for Day 28, three cows each from the treated groups plus one control cow were sacrificed. Cows from the depuration phase were sacrificed 4 and 8 days post-last morning dose. Following sacrifice,

samples of kidney, liver, fat (composite sample consisting of renal, omental, and subcutaneous fat), and muscle (composite sample consisting of loin, hind, and diaphragm muscle) were collected for analysis.

Milk and tissue samples were analysed for *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA by LC/MS/MS using DuPont-20009 analytical method.

Method validation was conducted with unfortified controls and controls fortified with *N*-acetyl glyphosate, glyphosate, *N*-acetyl AMPA, and AMPA in animal matrices at LOQ and 10×LOQ levels in this study. The validated limit of quantitation (LOQ) for *N*-acetyl glyphosate and each relevant analyte was 0.025 mg/kg in milk and muscle matrices, and 0.050 mg/kg in liver, kidney, and fat matrices. In addition, unfortified controls and controls fortified at the LOQ and 10×LOQ were analysed concurrently with the treated specimens to verify method performance. Residue levels of *N*-acetyl glyphosate in kidney and liver exceeded 10×LOQ (0.50 mg/kg) and additional fortification recoveries at 5.0 mg/kg and 2.0 mg/kg, respectively, were determined to verify method performance above the maximum found residue level. Mean of the validation and concurrent recoveries per fortification level from fortified control milk and tissue samples for N acetyl glyphosate and relevant analytes were within the acceptable range of 70–110 %. RSD was always below 20 %. Consistently with the analytical method, the results for analysis of *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA in milk commodities and tissues were expressed as glyphosate equivalents and were summarised as such.
After collection, samples were maintained in frozen condition (i.e. stored in freezer at target temperature of -20°C or shipped on dry ice). Storage stability data for residues in milk and tissues were determined concurrently with this feeding study. The results indicate that the residues of *N*-acetyl glyphosate and its degradation products are stable in cattle matrices for the maximum periods of frozen storage encountered in this study.
All milk and tissue samples were analysed for *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA, except for several whole milk, skim milk, cream and muscle samples which were analysed for *N*-acetyl glyphosate and glyphosate only.

In whole milk, residues of *N*-acetyl glyphosate and glyphosate were below the LOQ of 0.025 mg/kg in the samples analyzed from all dose levels and sampling intervals. Although the level of *N*-acetyl glyphosate found did not exceed the LOQ of 0.025 mg/kg in any milk samples, it was detected in a significantly greater number of samples compared to glyphosate. Generally, there were very few detections of residues in the 1.25 mg/kg bw and 3.75 mg/kg bw dose groups. Additionally, screening of all dose group cows in Day 7 and Day 14 whole milk samples and the 12.5 mg/kg bw and 37.5 mg/kg bw dose groups in Day 21 whole milk samples showed no detectable residues of AMPA or *N*-acetyl AMPA. Although residue levels in milk were too low for the samples collected during the 7-day depuration period to provide clear results concerning a rate of residue decline, residues of *N*-acetyl glyphosate were still detectable in milk samples collected at 7 days after dosing at 37.5 mg/kg bw/day was terminated. Glyphosate was not detected in milk during the depuration phase of the study.

In tissue samples obtained within 24 hours of completion of 28 consecutive days of dosing with *N*-acetyl glyphosate, residue levels were highest in kidney followed generally in decreasing order by liver, fat, and muscle. In each tissue, *N*-acetyl glyphosate was found in higher concentrations than concentrations of glyphosate, AMPA, or *N*-acetyl AMPA.

In kidney, *N*-acetyl glyphosate, glyphosate, and *N*-acetyl AMPA were detected in all dose groups. *N*-acetyl glyphosate was found at levels above the LOQ of 0.05 mg/kg bw at all dose levels. *N*-acetyl glyphosate residues in kidney ranged from 0.060 mg/kg glyphosate equivalents in the 1.25 mg/kg bw dose group to 3.2 mg/kg in the 37.5 mg/kg bw dose group. Glyphosate was found at or above the LOQ of 0.05 mg/kg in kidney in the two highest dose groups, 12.5 mg/kg bw and 37.5 mg/kg bw. AMPA and *N*-acetyl AMPA were found at or above the LOQ of 0.05 mg/kg in kidney only in the highest dose group, 37.5 mg/kg bw.

In liver, *N*-acetyl glyphosate was detected at all dose levels and exceeded the LOQ of 0.05 mg/kg in the two highest dose levels (12.5, and 37.5 mg/kg bw). N-acetyl glyphosate residues in liver ranged from 0.010 mg/kg at the 12.5 mg/kg bw dose level to 0.52 mg/kg bw at the 37.5 mg/kg bw dose level. AMPA was detected, but <LOQ of 0.05 mg/kg and only at the 12.5 and 37.5 mg/kg bw dose levels. Glyphosate was detected, but <LOQ of 0.05 mg/kg and only at the highest dose level, 37.5 mg/kg bw. *N*-Acetyl AMPA was not detected in liver at any of the dose levels evaluated.
In fat, concentrations of *N*-acetyl glyphosate ranged from <0.05 mg/kg at the lowest dose level of 1.25 mg/kg bw to 0.22 mg/g at the 37.5 mg/kg bw dose level. Glyphosate was detected in fat samples at all four dose levels, but did not exceed the LOQ of 0.05 mg/kg. *N*-acetyl AMPA was detected in fat at only the two highest dose levels, 12.5 and 37.5 mg/kg bw/day, but did not exceed the LOQ of 0.05 mg/kg bw. AMPA residues were not detected at the highest dose level, 37.5 mg/kg bw.

In muscle, *N*-acetyl glyphosate was not detected in the 1.25 mg/kg bw dose group, and concentrations ranged from <LOQ of 0.025 mg/kg in the 3.75 mg/kg bw dose group to 0.053 mg/kg in the 37.5 mg/kg bw dose group. Glyphosate residues were not detected in muscle from the two highest dose groups, 12.5 and 37.5 mg/kg bw. AMPA and *N*-acetyl AMPA were not detected in muscle samples analysed.

Following cessation of dosing, residues in tissues generally declined during the depuration period. In kidney and liver, residues of *N*-acetyl glyphosate, glyphosate and AMPA were detected, but <LOQ of 0.05 mg/kg after an 8-day depuration period. *N*-Acetyl AMPA residues in kidney were not detected (ND) after a 4-day depuration period. In liver, residues of *N*-acetyl glyphosate, glyphosate, and AMPA were detected, but <LOQ of 0.05 mg/kg after an 8-day depuration period. *N*-Acetyl AMPA residues in liver were not-detected (ND) either at the end of the 28-day dosing period or during the depuration phase. In fat, the level of *N*-acetyl glyphosate appeared to decline slower than in other tissues and was found at 0.14 mg/kg after an 8-day depuration period. Residues of glyphosate and *N*-acetyl AMPA were detected, but remained below the LOQ of 0.05 mg/k at the 4-day and 8-day depuration intervals, although both compounds were found at the same levels at the end of the 28-day dosing period. In muscle, *N*-acetyl glyphosate and glyphosate residues were detected after the 8-day depuration period, but were below the LOQ of 0.025 mg/kg.

Test facilities

Study directory: [redacted]
In-Life phase: [redacted]
Analytical phase: [redacted]

I. Materials and Methods

A. Materials

The test material, *N*-acetyl glyphosate (IN-MCX20, technical test substance), was administered to the treated animals in this study. Further information on the test material is listed in the table below.

1. Test material:

Description:	IN-MCX20, Test substance, technical
Lot number:	003
CRL sample number:	-
Active ingredient(s):	*N*-acetyl glyphosate
CAS number:	129660-96-4
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	19.7 % w/v in sodium acetate solution
Formulation type:	NA; technical grade active substance
Appearance/colour:	Liquid, colour not reported
Analysis date:	25/07/2006
Expiry date:	25/07/2009
Storage conditions:	Store at room temperature (ambient <25 °C)
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Lactating Holstein/Friesian dairy cows were the test animals used in this study. Details are listed in the table below.

2. Test animals

Species:	Lactating dairy cattle; Bovine (*Bos Taurus)*
Gender:	Female
Breed:	Holstein/Friesian
Source:	[redacted]
Age:	Approximately 3 – 9 years old
Weight:	Approximately 480 – 700 kg
Milk production:	All cows were in good milk yielding capacity. During the pre-trial period milk production was approximately 14–23 kg/animal/day.
Number of animals:	16 cows: (2 cows in untreated control group; 3 cows in each of 4 treated groups; additional 2 cows in the highest dose group for the depuration phase of the study)
Animal Identification:	Uniquely numbered ear tag and/or leg band
Animal health / observations:	During the acclimation period, all animals were examined by a veterinarian and certified fit for inclusion in the study. Animals were observed twice daily (a.m. and p.m.) for mortality and moribundity and for general health and appearance during the acclimation and treatment periods of the study. Body weights were recorded weekly during the acclimation and study periods. The animals were also weighed immediately prior to sacrifice. Feed consumption and milk production were recorded daily during the acclimation and study periods.
Acclimation period:	Minimum of 8 days
Diet:	The animals were offered hay *ad libitum* as well as a twice-daily protein concentrate ration (total of 8 kg with 4 kg offered to each cow at each milking occasion; at approximately 0730 h and 1530 h each day).

Water:	Tap water was supplied *ad libitum*.
Housing:	The animals were housed in a traditional large animal unit with straw for bedding.
	All animals were initially group housed. Following allocation to individual groups, the animals were housed in individual group pens for at least 7 days prior to dosing and for the duration of the study period. The layout of the pens within the animal unit was arranged so that there was minimal possibility of contamination between group pens.

3. Environmental conditions

The environmental conditions at the test facility during the in-life phase of the study are summarised in the table below:

Temperature:	Ambient; ranged from -1 to 19°C
Humidity:	Ambient, ranged from 31–98 %
Air change:	Not reported
Photoperiod:	Natural light cycles. Lighting was natural with supplemental lighting provided as needed

B. Study Design and Methods

This study was designed with six groups of cows. Assignment of animals to each group was at random; however, the randomisation was checked to ensure that feed consumption and milk production were similar across treatment groups. Bias was controlled in this study by placing the cows into groups based on feed consumption and milk production.

Four groups of cows were dosed at target treatment levels of *N*-acetyl glyphosate at 1.25, 3.75, 12.5, and 37.5 mg/kg bodyweight. Each of these four groups consisted of 3 cows. The four indicated dose levels were selected to cover a wide range of possible dietary burdens of *N*-acetyl glyphosate, depending on regional use practices. A fifth group, which consisted of 2 cows, was used for the depuration phase of the study to evaluate potential decline in residue levels at up 8 days after completion of the 28-day dosing period. The depuration animals were dosed at 37.5 mg/kg bodyweight. The sixth group of cows was an untreated control group, to which two cows were assigned.

N-acetyl glyphosate (as 19.7 % w/v [free acid] in sodium acetate) was administered orally to the five treated groups of lactating Holstein/Friesian cows at the nominal dose levels indicated above twice daily for 28 consecutive days.

Samples of milk and tissues were collected from each individual treated animal and analysed for residues of *N*-acetyl glyphosate and specified metabolites. Samples of milk and tissue collected from the animals in the untreated control group served as the source of control samples. Milk samples were collected at specified intervals during both the 28-day dosing period and the depuration phase of the study. Tissue samples (liver, kidney, fat, and muscle) were collected within 24 hours of administration of the final dose of test material at the end of the 28-day dosing period. Additionally, tissue samples were collected from animals assigned to the depuration phase of the study at 4 days or 8 after completion of the 28-day dosing period.

Further details on the dosing regimen, including target dose levels, are summarised in the table below as well as text that follows.

1. Dosing regimen

Route:	Oral *via* drench gun
Vehicle:	Aqueous sodium acetate solution (1 % w/w), adjusted to pH 6
Timing / frequency per day:	Twice daily (*ca* 0800 h and 1600 h), following each milking
Duration:	28 consecutive days
Treatment groups (dose levels):	4 dose levels (1.25, 3.75, 12.5, and 37.5 mg *N*-acetylglyphosate/kg bodyweight (equivalent to 1, 3, 10, and 30 mg glyphosate/kg bodyweight):

Treatment Group	Nominal dose level (mg/kg bodyweight)	
	N-acetyl glyphosate	Glyphosate equivalents [1]
1	1.25	1
2	3.75	3
3	12.5	10
4	37.5	30
5 (depuration)	37.5	30
6 (untreated control)	0	0

1 Based on molecular weight of *N*-acetylglyphosate and glyphosate, multiplication of the *N*-acetylglyphosate dose level by a factor of 0.8 results in the expression of the dose level in glyphosate equivalents.

Dose solutions containing *N*-acetyl glyphosate were administered orally, using calibrated drench guns (60 mL capacity), twice daily (*ca* 0800 h and 1600 h) following each milking for 28 consecutive days. The dose vehicle used for dosing solutions was an aqueous sodium acetate solution (1 % w/w), which was adjusted to pH 6 for biological compatibility. Control animals were dosed with dose vehicle only (1 % sodium acetate solution without *N*-acetyl glyphosate) prior to the dosing of treatment animals at each dose occasion. Individual drench guns were assigned to each dose group to avoid the possibility of cross-contamination.

Dose solutions for each group were prepared on a weekly basis during the study period. The test item (*N*-acetyl glyphosate 19.7 % w/v in sodium acetate solution) and all dose solutions were stored at ambient temperature.

The target concentration for each dose solution was calculated by determining the total mass of *N*-acetyl glyphosate required for each dose week based on the mean group bodyweight in relation to the total target dose volume required. The volume of dose solution required was based on use of approximately 50 mL per animal per dose occasion along with a suitable excess quantity of solution.
The dose solutions were prepared by use of the required amount of *N*-acetyl glyphosate (based on concentration in the test material) with addition of the dose vehicle to reach the target weight. The specific gravity of each dose solution was determined to enable doses to be administered by volume. Following preparation, aliquots of each dose solution were taken for dose determination analysis (dose accuracy) performed by HPLC-UV using a standard curve produced using an analytical standard. These analyses were performed prior to dosing and confirmed the theoretical concentrations of each solution (within 10 % of target with the exception of one value which was within 11 % of target). Additional aliquots were also taken from week 1 dose solutions to allow the stability of each dose solution to be determined over a 14-day storage period (at ambient temperature as per dose solution storage conditions). Analytical results demonstrated stability of *N*-acetyl glyphosate in dosing solutions for the 14-day storage interval tested. A summary of results of analyses to determine dose accuracy and stability of *N*-acetyl glyphosate in dosing solutions is shown in the table below.

Table 6.4.2-1: Summary of dose accuracy and stability of *N*-acetyl glyphosate in dosing solutions

Group	Dose Solution Number[1]	Preparation Date	Analysis Date	Theoretical Concentration (mg/mL)	Actual Concentration (mg/mL)[2]	% of Theoretical Concentration
1	1.1	27 Mar 07	28 Mar 07	7.328	7.446	101.6
2	2.1			25.054	25.718	102.7
3	3.1			73.417	75.093	102.3
4 and 5	4.1			235.908	241.352	102.3
1	1.2	03 Apr 07	04 Apr 07	7.475	6.644	88.9
2	2.2			24.931	25.513	90.3
3	3.2			79.235	72.415	91.4
4 and 5	4.2			236.203	215.139	91.1
1	1.3	10 Apr 07	11 Apr 07	7.560	7.683	101.6
2	2.3			25.285	25.631	101.4
3	3.3			80.879	79.881	98.8
4 and 5	4.3			235.809	236.190	100.2
1	1.1	27 Mar 07 (Dose stability)	10 Apr 07	7.328	7.746	105.7
2	2.1			25.054	26.047	104.0
3	3.1			73.417	72.988	99.4
4 and 5	4.1			235.908	233.822	99.1
1	1.4	17 Apr 07	18 Apr 07	7.601	7.376	97.0
2	2.4			25.527	24.615	96.4
3	3.4			81.854	76.402	93.3
4 and 5	4.4			235.612	227.192	96.4

1 = Each dose solution was numbered based on group and study week (e.g. Group 1 week 4 dose solution = 1.4)
2 = Determined by HPLC-UV

Individual volumes of dose solution required to deliver the target amount of *N*-acetyl glyphosate for each cow were calculated based on individual bodyweights recorded on the day prior to each dose week commencing (Day -1, 7, 14, and 21). Dose volumes were calculated based on the target dose level for each Treatment Group [1.25, 3.75, 12.5, or 37.5 mg/kg bw] and the concentration of each dose solution.

Calibration of the drench guns used to administer the dosing solutions was performed on a daily basis by dispensing the maximum and minimum expected dose volumes (3 aliquots of each) and recording the weight of each aliquot. Water was used to perform these calibrations and results were always within 5 % of the expected value (with the CV also being less than 5 %).

2. Daily observations and animal data collection

Animals were observed twice daily (a.m. and p.m.) for mortality and moribundity and for general health and appearance during the acclimation and treatment periods of the study.

Feed consumption and milk production were recorded daily during the acclimation and study periods. Body weights were recorded weekly during the acclimation and study periods. The animals were also weighed immediately prior to sacrifice.

1. Milk and tissue sample collection

Samples of milk and tissues were collected for residue analysis.

Samples of whole milk were collected for residue analysis on Study Days -1, 1, 3, 5, 7, 10, 14, 17, 21, 24, and 28 as well as Days 1, 3, 5, and 7 post dose for depuration animals. All animals were milked at *ca* 0730 h and *ca* 1530 h throughout the study using individual stainless steel vacuum operated milking machines. During the dosing period, cows were milked in an appropriate order to minimize the possibility of contamination between dose groups. Milk was collected from animals individually in the afternoon of

the indicated sampling day and stored refrigerated at *ca* +4°C overnight prior to being combined with the next morning milk to comprise a single daily milk sample. Subsamples of the combined afternoon and following morning milk by individual animal were collected (2 × *ca* 100 mL) and retained for residue analysis.

After retaining subsamples of whole milk on Study Days 14 and 28, the remaining bulk milk samples were used to prepare cream and skim milk (*ca* 100 mL of each) using a Clair® Milky electronic cream separator.

Whole milk, skim milk, and cream samples were aliquoted for analysis (3 × 2 g aliquots taken into 50 mL centrifuge tubes). Each aliquot was diluted with 24 mL of aqueous formic acid (0.1 %, v/w) capped and mixed (by shaking) prior to frozen storage. All milk, cream, and skimmed milk aliquots were stored in a freezer (*ca* -20°C) alongside the remaining subsamples. Two aliquots of each whole milk, cream, and skim milk sample were shipped frozen on dry ice to the Analytical Test Site as soon as practical after collection for analysis.

Within 24 hours (*ca* 23–24 hours after the final morning dose for Day 28, cows from Groups 1–4 (plus a control animal from Group 6) were sacrificed by captive bolt followed by exsanguination. Cows from the depuration phase were sacrificed 4 and 8 days post last morning dose.

Following sacrifice, the following whole organs and tissue samples were collected from each animal and weighed: Liver, kidney, muscle (composite sample consisting of loin, hind, and diaphragm muscle [*ca* 350 g of each type of muscle]) and fat (composite sample consisting of renal, omental and subcutaneous fat [*ca* 350 g of each type of fat]). Both kidneys from each individual animal were cut into slices and stored frozen. For liver, a 2 kg representative sample (10 × *ca* 200 g sections taken from several areas considered to be representative of the entire organ) was cut into slices and retained. The remainder of each liver sample was also retained and stored frozen alongside the sample taken for analysis (retained for potential analysis in the event of unequivocal results being obtained). All samples were stored at *ca* -20°C prior to and after being homogenised.

In preparation for residue analysis, the tissue samples were homogenised. Where possible, control samples were homogenised first, followed by samples in ascending order of treatment. Appropriate precautions and cleaning between samples were employed to avoid cross contamination.

All tissue samples were homogenised using a Hobart VPU 250 followed by a VCB62 in the presence of copious amounts of dry ice to generate a fine, homogeneous sample. Samples were then returned to freezer storage (*ca* -20°C) to allow any remaining dry ice to dissipate (>24 h). Aliquots (3 × *ca* 2 g) of each powdered tissue sample were then taken into centrifuge tubes (50 mL capacity). Subsamples of each homogenised liver and kidney sample (4 × 100 g) were taken and stored alongside the remaining bulk samples (which were also divided into two samples) at ca -20°C. The remaining muscle and fat samples were divided into four sub-samples and returned to storage at -20°C.

Sub-samples (2 × 2 g) of tissue samples were shipped frozen on dry ice to the Analytical Test Site for residue analysis, where they were stored frozen (*ca* -20°C).
A summary of the sampling information is shown in the table below.

Table 6.4.2-2: Milk and tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Whole milk, Cream, and Skim milk	Dosing phase: -1, 1, 3, 5, 7, 10, 14, 17, 21, 24, and 28 Depuration phase: 1, 3, 5, and 7 (post dosing)	Subsamples (2 × *ca* 100 mL) of combined afternoon and following morning milk by individual animal were collected to comprise a single daily milk sample for each animal. Additionally, after retaining subsamples of whole milk on Study Days 14 and 28, the remaining bulk milk samples were used to prepare cream and skim milk by mechanical separation (*ca* 100 mL of each). Whole milk, skim milk, and cream samples were aliquoted for analysis (3 × 2 g aliquots taken into 50 mL centrifuge tubes). Each aliquot was diluted with 24 mL of aqueous formic acid (0.1 %, v/w) capped and mixed (by shaking) prior to frozen storage. All samples were stored frozen (*ca* -20°C).
Muscle[1]	End of dosing period: (within 24 hours of the final morning dose for Study Day 28) Depuration phase: At 4 days and 8 days post last morning dose	*ca* 1050 g composite;
Fat[2]		*ca* 1050 g composite
Liver		*ca* 2 kg (10 x 200 g slices representative of entire organ)
Kidney		Both kidneys (sliced before freezing and homogenisation)

1 Composite of equal amounts (*ca* 350 g each) of loin, hind, and diaphragm muscle
2 Composite of equal amounts of omental, subcutaneous, and renal fat.

1. Analytical phase

Analysis of milk and tissue samples was conducted at the Analytical Test Site, DuPont Crop Protection, Newark, Delaware, USA.

Results obtained from the ruminant (goat) metabolism study conducted with *N*-acetyl glyphosate supported inclusion of the following as analytes in this cattle feeding study: *N*-acetyl glyphosate and glyphosate in all milk and tissues matrices, AMPA in liver and kidney, and *N*-acetyl AMPA in kidney. In addition to the above defined analytes, selected milk, muscle, and fat samples were also analysed for AMPA and *N*-acetyl AMPA.

Milk, liver, kidney, muscle, and fat study samples were analysed using DuPont-20009 analytical method for *N*-acetyl glyphosate and relevant degradates. The method was applied for quantitative analysis of *N*-acetyl glyphosate and glyphosate in all matrices, AMPA in liver and kidney, and *N*-acetyl AMPA in kidney. The method was applied for qualitative analysis of AMPA in milk, fat, and muscle and for qualitative analysis of *N*-acetyl AMPA in milk, liver, fat, and muscle.

The method of analysis for milk, including skim milk and cream, involved sample dilution in aqueous 0.1 % formic acid/methanol (96/4, v/v). The dilute sample was partitioned with hexane and the hexane layer discarded. The remaining aqueous fraction is partitioned with methylene chloride and the aqueous layer was collected. The methylene chloride fraction was back extracted with additional 0.1 % formic acid/methanol (96/4, v/v) for quantitative recovery of analytes. The aqueous fractions were combined and diluted to final volume 50 mL. An aliquot of the aqueous fraction was filtered through a C_{18} SPE cartridge. The C_{18} purified extract was further purified by solid phase extraction using polymeric anion

exchange (MAX) SPE cartridge and/or polymeric cation exchange (MCX) SPE cartridge, depending on matrix and analytes examined.

The method of analysis for liver, kidney, muscle, and fat matrices involved solid phase dispersion of the sample in C_{18} sorbent packing, followed by extraction in 0.1N HCl solution (96 % water/4 % methanol). Samples were extracted again in water to complete the quantitative transfer of the analytes from matrix to final extract. An aliquot of the extract was diluted in acetonitrile and methanol to precipitate proteins, then purified by solid phase extraction using polymeric anion exchange (MAX) SPE cartridge and/or polymeric cation exchange (MCX) SPE cartridge, depending on matrix and analytes examined.

Glyphosate and/or AMPA stable isotope standards used as internal standards were added to extracts prior to ion exchange SPE purification. Final extracts were filtered prior to LC/MS/MS analysis.

All analyte concentration values were expressed as mg/kg glyphosate equivalents.

Method validation was conducted with unfortified controls and controls fortified with *N*-acetyl glyphosate, glyphosate, N-acetyl AMPA, and AMPA in animal matrices at LOQ and 10×LOQ levels in this study. The validated limit of quantitation (LOQ) for *N*-acetyl glyphosate and each relevant analyte was 0.025 mg/kg in milk and muscle matrices, and 0.050 mg/kg in liver, kidney, and fat matrices. In addition, unfortified controls and controls fortified at the LOQ and 10×LOQ were analysed concurrently with the treated specimens to verify method performance. Residue levels of *N*-acetyl glyphosate in kidney and liver exceeded 10×LOQ (0.50 mg/kg) and additional fortification recoveries at 5.0 mg/kg and 2.0 mg/kg, respectively, were analysed to verify method performance above the maximum found residue level. Mean of the validation and concurrent recoveries per fortification level from fortified control milk and tissue samples for *N*-acetyl glyphosate and relevant analytes were within the acceptable range of 70–110 %. RSD was always below 20 %.

A summary of validation and concurrent recovery results are shown in the table below.

Table 6.4.2-3: Validation and concurrent recovery results: *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA in milk commodities, and tissues (liver, kidney, fat, and muscle)

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
N-acetylglyphosate	Whole Milk	0.025	83, 91, 81, 75, 98, 81, 91, 106, 78, 89, 94, 97, 91, 105, 97, 85	90	9	10	16
		0.050	78, 82, 101, 84, 87, 106, 82	89	10	12	7
		0.25	85, 90, 87, 81, 89, 87, 89, 100, 86, 81, 94, 101, 90, 86	89	6	7	14
		0.50	98	-	-	-	1
		Overall	75–106	90	8	9	38
	Skim Milk	0.025	86, 92, 74	84	9	11	3
		0.25	79, 83, 67	77	8	11	3
		Overall	67–92	80	9	11	6
	Cream	0.025	73, 87, 90	83	9	11	3
		0.25	82, 88, 83	84	4	4	3

Table 6.4.2-3: Validation and concurrent recovery results: *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA in milk commodities, and tissues (liver, kidney, fat, and muscle)

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	73–90	84	6	7	6
	Liver	0.050	98, 80, 105, 93, 75, 92, 84, 112	92	13	14	8
		0.50	92, 94, 81, 86, 78, 76, 85, 73, 83, 86	84	7	8	10
		1.0	75, 75	75	-	-	2
		2.0	82, 77	80	-	-	2
		Overall	73–112	86	10	12	22
	Kidney	0.050	97, 106, 103, 94	100	5	5	4
		0.50	82, 84, 85, 88, 73, 81, 87, 77,85	83	5	6	9
		5.0	84, 82	83	-	-	2
		Overall	73–106	87	9	10	15
	Fat	0.050	107, 104, 107, 104	105	2	2	4
		0.25	86, 86, 90, 84, 93	88	4	4	5
		0.50	86, 87, 96	90	5	6	3
		Overall	84–107	94	9	10	12
	Muscle	0.025	102, 93, 113, 76	96	16	17	4
		0.25	81, 87, 78, 73, 68, 72, 75, 69	75	7	9	8
		Overall	68–113	82	14	17	12
Glyphosate	Whole Milk	0.025	90, 119, 81, 114, 104, 100, 75, 87, 70, 85, 96, 79, 86, 100, 115	93	15	16	15
		0.050	80, 95, 72, 93, 92, 99, 70	86	12	14	7
		0.25	88, 83, 74, 87, 73, 72, 85, 88, 80, 106, 118, 106, 106, 109	91	15	16	14
		0.50	82	-	-	-	1
		Overall	70–119	91	14	15	37
	Skim Milk	0.025	99, 114, 88	100	13	13	3
		0.25	102, 96, 94	97	4	5	3
		Overall	88–114	99	9	9	6
	Cream	0.025	86, 95, 101	94	8	8	3
		0.25	96, 104, 102	101	4	4	3
		Overall	86–104	97	7	7	6
	Liver	0.050	105, 92, 90, 78, 80, 102, 84, 92	90	10	11	8
		0.50	86, 85, 88, 82, 71, 76, 88, 87, 74, 76	81	6	8	10
		1.0	81, 83	82	-	-	2

Table 6.4.2-3: Validation and concurrent recovery results: *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA in milk commodities, and tissues (liver, kidney, fat, and muscle)

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		Overall	71–105	85	9	11	20
	Kidney	0.050	116, 78, 96, 113	101	18	18	4
		0.50	81, 84, 92, 91, 86, 95,99, 95, 90	90	6	6	9
		Overall	78–116	93	11	12	13
	Fat	0.050	113, 86, 100, 91	98	12	12	4
		0.25	91, 89, 95, 94, 99	94	4	4	5
		0.50	95, 97, 98	97	1	2	3
		Overall	86–113	96	7	7	12
	Muscle	0.025	89, 77, 94, 103	91	11	12	4
		0.25	91, 81, 82, 86, 79, 77, 99, 90	86	7	9	8
		Overall	77–103	87	9	10	12
AMPA	Whole Milk	0.025	82, 90, 115, 109	99	16	16	4
		0.050	103, 90	96	-	-	2
		0.25	83, 71, 95, 89	85	10	12	4
		Overall	71–117	93	13	14	10
	Liver	0.050	77, 101, 103, 118, 94, 95, 111	100	13	13	7
		0.50	85, 86, 97, 92, 81, 98, 84, 88, 99, 90	90	6	7	10
		1.0	90, 91	91	-	-	2
		Overall	77–118	95	10	11	19
	Kidney	0.050	76, 83, 100, 113	93	17	18	4
		0.50	77, 71, 95, 88, 84, 109, 93, 98, 95	90	12	13	9
		Overall	71–113	91	13	14	13
	Fat	0.050	97, 95, 115	102	11	11	3
		0.25	82, 91, 92, 115	95	14	15	4
		0.50	92, 97	95	-	-	2
		Overall	82–115	98	11	11	9
	Muscle	0.025	84, 88, 103	92	10	11	3
		0.25	101, 99, 81, 87, 94, 85	91	8	9	6
		Overall	81–103	91	8	9	9
N-acetyl AMPA	Whole Milk	0.025	72, 89, 82, 79	80	7	9	4
		0.050	93, 83	88	-	-	2
		0.25	73, 67, 80, 86	77	8	11	4
		Overall	67–93	80	8	10	10

Table 6.4.2-3: Validation and concurrent recovery results: *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA in milk commodities, and tissues (liver, kidney, fat, and muscle)

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
	Liver	0.050	93, 107, 76, 71, 72, 71, 86	82	14	17	7
		0.50	95, 93, 73, 81, 63, 68, 86, 73, 99	81	13	16	9
		1.0	66, 72	69		-	2
		Overall	63–107	80	13	16	18
	Kidney	0.050	93, 94, 69, 80	84	12	14	4
		0.50	87, 93, 71, 75, 79, 95, 75, 92	83	9	11	8
		Overall	69–95	84	10	12	12
	Fat	0.050	95, 94, 108, 86	96	9	10	4
		0.25	74, 78, 84, 81, 85	80	4	6	5
		0.50	90, 93, 89	91	2	2	3
		Overall	74–108	88	9	10	12
	Muscle	0.025	93, 96, 84	91	6	7	3
		0.25	86, 80, 64, 81, 73, 106, 66	80	14	18	7
		Overall	64–106	83	13	16	10

II. Results and Discussion

A. Dose levels

Dosing was conducted at target (nominal) levels of 1.25, 3.75, 12.5, and 37.5 mg *N*-acetyl glyphosate/kg bodyweight/day (equivalent to 1, 3, 10, and 30 mg of glyphosate/kg bodyweight/day) for 28 consecutive days. As shown below, actual dose levels attained during the study were in good agreement with the target (nominal) levels.

The actual mean weekly dose levels over the 4-week study period ranged as follows: 1.268–1.287, 3.780-3.831, 12.59–12.69, and 38.33–38.94 mg N-acetyl glyphosate/kg bodyweight/day (equivalent to 1.015–1.029, 3.024–3.065, 10.07–10.16, and 30.66–31.15 mg of glyphosate/kg bodyweight/day). The mean weekly dose levels were equivalent to 43.39–45.28, 129.1–130.0, 419.5–451.7, and 1153-1200 mg *N*-acetyl glyphosate/kg dry feed based upon the actual average daily feed consumption (equivalent to 34.71–36.23, 103.3–104.0, 335.6–361.4, and 922.2–960.0 mg of glyphosate/kg dry feed). The control cows (Treatment group 6) were dosed with dose vehicle only (containing no *N*-acetyl glyphosate) for the 28-day treatment period.

A summary of the actual dose levels attained for the study treatment groups is shown in the table below.

Table 6.4.2-4: Mean actual dosing levels of N-acetyl glyphosate and mean actual dosing levels expressed as glyphosate equivalents

				N-acetyl glyphosate [2, 4]		Glyphosate equivalents [2, 5]	
Trt. group	Target dose level (mg/kg bw) [1]	Mean administered dose (mg/cow/day) [1, 2]	Mean feed consumption (kg/cow/day) [2, 3]	Actual mean dose level (mg/kg bw)	Actual mean dietary burden (mg/kg feed)	Actual mean dose level (mg/kg bw)	Actual mean dietary burden (mg/kg feed)
1	1.25	743.3–760.1	16.8–17.5	1.268–1.287	43.39–45.28	1.015–1.029	34.71–36.23
2	3.75	2452–2510	19.0–19.3	3.780–3.831	129.1–130.0	3.024–3.065	103.3–104.0
3	12.5	7342–7776	17.0–17.7	12.59–12.69	419.5–451.7	10.07–10.16	335.6–361.4
4 and 5	37.5	20996–21441	17.7–18.2	38.33–38.94	1153–1200	30.66–31.15	922.2–960.0
6	0	0	18.6-20.1	0	0	0	0

1 Target dose level = mg *N*-acetyl glyphosate / kg bodyweight / day
2 The range of values given for administered dose, feed consumption, and dose levels reflect the range of average values over the 4-week study period
3 Feed consumption is expressed on a dry weight basis
4 Actual dosing levels for *N*-acetyl glyphosate are provided as mg/kg bodyweight/day and mg/kg dry feed (dietary burden)
5 Actual dosing levels for *N*-acetyl glyphosate, expressed as glyphosate acid equivalents, are provided as mg/kg bodyweight/day and mg/kg dry feed (dietary burden)

B. Animal health and daily observations

All cows used in the study were in good general health throughout the acclimation and treatment periods.

Feed consumption was found to remain relatively constant for the duration of the acclimation and study periods. This indicates that dosing with *N*-acetyl glyphosate did not have an adverse effect on feed consumption. No notable differences in milk production patterns were observed in cows between the acclimation and study periods, indicating that dosing with *N*-acetyl glyphosate did not adversely impact milk production. Additionally, body weights for individual animals remained relatively constant throughout the treatment period. This is typical of mature lactating cows and indicated that there were no meaningful effects on the cows from ingestion of N-acetyl glyphosate or the dose vehicle.

C. Residue levels in milk and tissues

Storage stability data for residues in milk and tissues were determined concurrently with this feeding study. The results indicate residues are stable in cattle matrices for the maximum periods of frozen storage encountered in this study. After collection, samples were maintained in frozen condition when stored in freezer at target temperature of -20°C or shipped on dry ice. Milk, skim milk, and cream samples were analyzed within 30 days following collection with the exception of Day 14 cream (34 days) and the whole milk depuration samples (maximum of 37 days). Additional extractions of the whole milk depuration samples (1 and 5 days post dose) were made after a maximum storage interval of 61 days. To demonstrate analyte stability in milk for the samples exceeding 30 days of frozen storage, the Day 14 whole milk sample was re-extracted 76 days after collection and showed consistent residues (0.019 mg/kg and 0.021 mg/kg, respectively). The maximum storage intervals for liver, kidney, muscle, and fat were 71, 74, 83, and 74 days, respectively. Hence, liver, kidney, muscle, and fat samples fortified at 0.25 mg/kg or 0.50 mg/kg glyphosate equivalents (10 × LOQ) were prepared with the initial sample extraction sets and stored frozen for analysis at 2 time intervals extending to longer than the maximum storage interval for each matrix. The storage stability was tested for N-acetyl glyphosate, glyphosate, AMPA and N-acetyl AMPA in liver, kidney, fat, and muscle. The results support analyte stability in

tissue matrices for 90–91 days, thus covering the maximum period of frozen storage for these matrices encountered in this study.

The results for analysis of *N*-acetyl glyphosate, glyphosate, AMPA, and *N*-acetyl AMPA in milk commodities and tissues were all reported as glyphosate equivalents and are summarised as such below.

A summary of residue analysis results for whole milk as well as skim milk and cream is provided in the two tables below. Whole milk residues were less than the LOQ of 0.025 mg/kg in glyphosate equivalents for *N*-acetyl glyphosate and glyphosate in the samples analyzed from all dose levels and sampling intervals. Although the level of *N*-acetyl glyphosate found did not exceed the LOQ of 0.025 mg/kg in any milk samples, it was detected in a significantly greater number of samples compared to glyphosate. Generally, there were very few detections of residues in the 1.25 mg/kg bw and 3.75 mg/kg bw dose groups. Residue analyses of samples from the 1.25 mg/kg bw and 3.75 mg/kg bw dose group samples were discontinued at 14 and 21 study days, respectively, each following 2 consecutive milk sampling intervals with no detection of residues. Screening of all dose group cows in Day 7 and Day 14 whole milk samples and the 12.5 mg/kg bw and 37.5 mg/kg bw dose groups in Day 21 whole milk samples showed no detectable residues of AMPA or *N*-acetyl AMPA. Results of these analyses for AMPA and *N*-acetyl AMPA are not included in the table below.

Although residue levels in milk were too low for the samples collected during the 7-day depuration period to provide clear results concerning a rate of residue decline, residues of *N*-acetylglyphosate were still detectable in milk samples collected at 7 days after dosing at 37.5 mg/kg bw/day was terminated.

In skim milk, *N*-acetyl glyphosate was found at 0.032 mg/kg in one sample from the highest dose group (37.5 mg/kg bw). However, in all other samples of skim milk and in all cream samples residues *N*-acetyl glyphosate and glyphosate residues were below the LOQ (<0.025 mg/kg) or not detected (<LOD).

Table 6.4.2-5: Whole milk residue level results

Nominal dose level (mg/kg bw) [1]	Sample collection day	Animal ID	Residue levels mg/kg (glyphosate equivalents) [2, 3, 4, 5]	
			***N*-acetyl glyphosate**	**Glyphosate**
0.0 (Control)	-1	015F	ND	ND
	1		ND	ND
	3		ND	ND
	5		ND	ND
	7		ND	ND
	10		ND	ND
	14		ND	ND
	21		ND	ND
	28		ND	ND
1.25	-1	001F	ND	ND
		002F	ND	ND
		003F	ND	ND
	1	001F	ND	ND
		002F	ND	ND
		003F	ND	ND
	3	001F	ND	ND
		002F	ND	ND
		003F	ND	ND
	5	001F	<0.025	ND
		002F	ND	<0.025
		003F	ND	<0.025
		Mean =	<0.025	*<0.025*

Table 6.4.2-5: Whole milk residue level results

Nominal dose level (mg/kg bw) [1]	Sample collection day	Animal ID	Residue levels mg/kg (glyphosate equivalents) [2, 3, 4, 5]	
			N-acetyl glyphosate	Glyphosate
	7	001F	ND	ND
		002F	ND	ND
		003F	ND	ND
	10	001F	ND	ND
		002F	ND	ND
		003F	ND	ND
3.75	-1	004F	ND	ND
		005F	ND	ND
		006F	ND	ND
	1	004F	<0.025	ND
		005F	ND	ND
		006F	<0.025	ND
		Mean =	*<0.025*	ND
	3	004F	ND	ND
		005F	ND	ND
		006F	ND	ND
	5	004F	ND	<0.025
		005F	ND	ND
		006F	ND	<0.025
		Mean =	ND	*<0.025*
	7	004F	<0.025	ND
		005F	<0.025	ND
		006F	<0.025	ND
		Mean =	*<0.025*	ND
	10	004F	ND	ND
		005F	ND	ND
		006F	ND	ND
	14	004F	ND	ND
		005F	ND	ND
		006F	ND	ND
12.5	-1	007F	ND	ND
		008F	ND	ND
		009F	ND	ND
	1	007F	ND	ND
		008F	ND	ND
		009F	<0.025	ND
		Mean =	*<0.025*	ND
	3	007F	<0.025	ND
		008F	ND	ND
		009F	ND	ND
		Mean =	*<0.025*	ND
	5	007F	ND	<0.025
		008F	<0.025	ND
		009F	<0.025	<0.025
		Mean =	*<0.025*	*<0.025*
	7	007F	<0.025	ND
		008F	<0.025	ND
		009F	<0.025	ND
		Mean =	*<0.025*	ND
	10	007F	ND	<0.025
		008F	ND	ND
		009F	<0.025	<0.025

Table 6.4.2-5: Whole milk residue level results

Nominal dose level (mg/kg bw) [1]	Sample collection day	Animal ID	Residue levels mg/kg (glyphosate equivalents) [2, 3, 4, 5]	
			N-acetyl glyphosate	Glyphosate
		Mean =	*<0.025*	*<0.025*
	14	007F	ND	ND
		008F	<0.025	ND
		009F	<0.025	ND
		Mean =	*<0.025*	ND
	21	007F	<0.025	<0.025
		008F	<0.025	<0.025
		009F	<0.025	<0.025
		Mean =	*<0.025*	*<0.025*
	28	007F	<0.025	ND
		008F	<0.025	ND
		009F	<0.025	ND
		Mean =	*<0.025*	ND
37.5	-1	010F	ND	ND
		011F	ND	ND
		012F	ND	ND
		013F	ND	ND
		014F	ND	ND
	1	010F	<0.025	ND
		011F	<0.025	ND
		012F	<0.025	<0.025
		013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	<0.025
	3	010F	<0.025	ND
		011F	<0.025	ND
		012F	<0.025	ND
		013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	ND
	5	010F	<0.025	<0.025
		011F	<0.025	<0.025
		012F	<0.025	ND
		013F	<0.025	<0.025
		014F	<0.025	<0.025
		Mean =	*<0.025*	*<0.025*
	7	010F	<0.025	ND
		011F	<0.025	ND
		012F	<0.025	ND
		013F	<0.025	<0.025
		014F	<0.025	ND
		Mean =	*<0.025*	<0.025
	10	010F	<0.025	ND
		011F	<0.025	<0.025
		012F	<0.025	ND
		013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	<0.025
	14	010F	<0.025	ND
		011F	<0.025	ND
		012F	<0.025	ND
		013F	<0.025	ND

Table 6.4.2-5: Whole milk residue level results

Nominal dose level (mg/kg bw) [1]	Sample collection day	Animal ID	Residue levels mg/kg (glyphosate equivalents) [2,3,4,5]	
			N-acetyl glyphosate	Glyphosate
		014F	<0.025	ND
		Mean =	*<0.025*	ND
	21	010F	<0.025	ND
		011F	<0.025	<0.025
		012F	<0.025	<0.025
		013F	<0.025	<0.025
		014F	<0.025	<0.025
		Mean =	*<0.025*	*<0.025*
	28	010F	<0.025	ND
		011F	<0.025	ND
		012F	<0.025	ND
		013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	ND
Depuration phase of the study				
37.5	28 (0-day depuration)	013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	ND
	29 (1-day depuration)	013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	ND
	31 (3-day depuration)	013F	<0.025	ND
		014F	<0.025	ND
		Mean =	*<0.025*	ND
	33 (5-day depuration)	014F	<0.025	ND
	35 (7-day depuration)	014F	<0.025	ND

1 Nominal dose level, mg *N*-acetyl glyphosate / kg bodyweight / day
2 Results for each analyte are reported as glyphosate equivalents
3 LOQ for both analytes in milk commodities = 0.025 mg/kg. ND = Not Detected, (LODs in milk commodities: *N*-acetyl glyphosate: 0.005 mg/kg (error in report stating 0.05 mg/kg), glyphosate: 0.004 mg/kg)
4 Residues results for detected residues that were below the LOQ (i.e. values ≥ LOD, but <LOQ) are listed as in the table as <LOQ (i.e. <0.025), although in the study report the numerical values between LOD and LOQ were listed
5 For purposes of calculating means, values <LOQ (i.e.<0.025) were assigned a value of the LOQ (i.e.0.025) if being averaged with a result ≥ LOQ or with a ND result. ND results were assigned a value of 0. Means are displayed in italics when the value is different than that listed in the study report due to different handling of residue results ≥ LOD, but <LOQ.

Table 6.4.2-6: Skim milk and cream residue level results

Matrix	Nominal dose level (mg/kg bw) [1]	Sample collection day	Animal ID	Residue levels mg/kg (glyphosate equivalents) [2,3,4,5]	
				N-acetyl glyphosate	Glyphosate
Skim milk	3.75	14	004F	ND	ND
			005F	ND	ND
			006F	ND	ND
	12.5	14	007F	<0.025	ND
			008F	ND	ND
			009F	ND	ND
			Mean =	*<0.025*	ND
		28	007F	ND	ND
			008F	<0.025	ND
			009F	<0.025	ND
			Mean =	*<0.025*	ND
	37.5	14	010F	0.032	<0.025
			011F	<0.025	<0.025
			012F	<0.025	<0.025
			Mean =	*0.027*	<0.025
		28	010F	<0.025	<0.025
			011F	<0.025	ND
			012F	<0.025	ND
			Mean =	*<0.025*	*<0.025*
Cream	3.75	14	004F	<0.025	<0.025
			005F	ND	ND
			006F	ND	ND
			Mean =	*<0.025*	*<0.025*
	12.5	14	007F	ND	ND
			008F	ND	ND
			009F	<0.025	ND
			Mean =	*<0.025*	ND
		28	007F	<0.025	ND
			008F	ND	ND
			009F	<0.025	ND
			Mean =	*<0.025*	ND
	37.5	14	010F	<0.025	ND
			011F	<0.025	ND
			012F	<0.025	ND
			Mean =	*<0.025*	ND
		28	010F	<0.025	ND
			011F	<0.025	ND
			012F	<0.025	ND
			Mean =	*<0.025*	ND

1 Nominal dose level; mg *N*-acetyl glyphosate / kg bodyweight / day
2 Results for each analyte are reported as glyphosate equivalents
3 LOQ for both analytes in milk commodities = 0.025 mg/kg. ND = Not Detected; (LODs in milk commodities: *N*-acetyl glyphosate: 0.005 mg/kg, glyphosate: 0.004 mg/kg).
4 Residues results for detected residues that were below the LOQ (i.e. values ≥ LOD, but <LOQ) are listed as in the table as <LOQ (i.e. <0.025), although in the study report the numerical values between LOD and LOQ were listed
5 For purposes of calculating means, values <LOQ (i.e.<0.025) were assigned a value of the LOQ (i.e.0.025) if being averaged with a result ≥ LOQ or with a ND result. ND results were assigned a value of 0. Means are displayed in italics when the value is different than that listed in the study report due to different handling of residue results ≥ LOD, but <LOQ.

In tissue samples obtained within *ca* 24 hours of completion of 28 consecutive days of dosing with *N*-acetyl glyphosate, residue levels were highest in kidney followed generally in decreasing order by

liver, fat, and muscle. A summary of residue results is provided in the four tables below for kidney, liver, fat, and muscle, respectively.

In kidney, the residue found in highest concentration was *N*-acetyl glyphosate, followed in decreasing order of magnitude by glyphosate, *N*-acetyl AMPA, and AMPA. *N*-Acetyl glyphosate, glyphosate, and *N*-acetyl AMPA were detected in kidney in all dose groups. *N*-Acetyl glyphosate was found at levels above the LOQ of 0.05mg/kg bw at all dose levels. *N*-Acetyl glyphosate residues in kidney ranged from 0.060 mg/kg glyphosate equivalents in the 1.25 mg/kg bw dose group to 3.2 mg/kg in the 37.5 mg/kg bw dose group. Glyphosate was found at or above the LOQ of 0.05 mg/kg in kidney in the two highest dose groups, 12.5 mg/kg bw and 37.5 mg/kg bw. AMPA was not detected in the two lowest dose level groups, 1.25 mg/kg be and 3.75 mg/kg bw, while *N*-acetyl AMPA was found in kidney at all 4 dose levels. AMPA and *N*-acetyl AMPA were found at or above the LOQ of 0.05 mg/kg in kidney only in the highest dose level, 37.5 mg/kg bw.

In liver, the residue found in highest concentration was *N*-acetyl glyphosate, which was detected at all dose levels and exceeded the LOQ of 0.05 mg/kg in the two highest dose levels (12.5, and 37.5 mg/kg bw). *N*-Acetyl glyphosate residues in liver ranged from 0.010 mg/kg at the 12.5 mg/kg bw dose level to 0.52 mg/kg bw at the 37.5 mg/kg bw dose level. AMPA was detected (<LOQ of 0.05 mg/kg) at both the 12.5 and 37.5 mg/kg bw dose levels. Glyphosate was detected, but <LOQ of 0.05 mg/kg and only at the highest dose level, 37.5 mg/kg bw. *N*-Acetyl AMPA was not detected in liver at any of the dose levels evaluated.

In fat, the residue found in highest concentration was *N*-acetyl glyphosate, ranging from <0.05 mg/kg at the lowest dose level of 1.25 mg/kg bw to 0.22 mg/g at the 37.5 mg/kg bw dose level. Glyphosate was detected in fat samples at all four dose levels, but did not exceed the LOQ of 0.05 mg/kg. *N*-Acetyl AMPA was detected in fat at only the two highest dose levels, 12.5 and 37.5 mg/kg bw/day, but did not exceed he LOQ of 0.05 mg/kg bw. AMPA residues were not detected at the highest dose level, 37.5 mg/kg bw.

In muscle, the residue found in highest concentration was *N*-acetyl glyphosate, ranging from <LOQ of 0.025 mg/kg in the 3.75 mg/kg bw dose group to 0.053 mg/kg in the 37.5 mg/kg bw dose group. Glyphosate residues were not detected in muscle from the two highest dose groups, 12.5 and 37.5 mg/kg bw. AMPA and *N*-acetyl AMPA were not detected in muscle samples analysed.

Following cessation of dosing, residues in tissues generally declined during the depuration period. In kidney, residues of *N*-acetylglyphosate, glyphosate and AMPA were detected, but <LOQ of 0.05 mg/kg after an 8-day depuration period. *N*-Acetyl AMPA residues in kidney were not-detected (ND) after a 4-day depuration period. In liver, residues of *N*-acetylglyphosate, glyphosate, and AMPA were detected, but <LOQ of 0.05 mg/kg after an 8-day depuration period. *N*-Acetyl AMPA residues in liver were not-detected (ND) either at the end of the 28-day dosing period or during the depuration phase. In fat, the level of *N*-acetylglyphosate appeared to decline slower than in other tissues and was found at 0.14 mg/kg after an 8-day depuration period. Residues of glyphosate and *N*-acetyl AMPA were detected, but remained below the LOQ of 0.05 mg/k at the 4-day and 8-day depuration intervals, although both compounds were found at the same level at the end of the 28-day dosing period. Analysis for AMPA was not conducted on fat samples during depuration, but it is assumed that the residues would be not-detected (ND) since AMPA residues in fat were ND in the highest dose treatment (37.5 mg/kg bw) at the end of the 28-day doing period. In muscle, *N*-acetylglyphosate and glyphosate residues were detected after the 8-day depuration period, but were below the LOQ of 0.025 mg/kg. Muscle samples were not analysed for AMPA or N-acetyl AMPA during the depuration period, but were assumed to not have residues at detectable levels since residues of both compounds were below the LOD at the end of the 28-day dosing period.

Table 6.4.2-7: Residue level results in kidney

Matrix	*N*-acetyl glyphosate dose level (mg/kg bw)[1]	Animal ID	Residue levels [2, 3, 4, 5] mg/kg (glyphosate equivalents)			
			N-acetyl glyphosate	Glyphosate	AMPA	N-acetyl AMPA
Kidney	0.0	015F	ND	<0.05	ND	ND
	1.25	001F	0.079	<0.05	ND	<0.05
		002F	0.11	<0.05	ND	<0.05
		003F	0.060	<0.05	ND	ND
		Mean =	0.082	*<0.05*	ND	*<0.05*
	3.75	004F	0.16	<0.05	ND	ND
		005F	0.24	<0.05	ND	<0.05
		006F	0.11	<0.05	ND	<0.05
		Mean =	0.17	*<0.05*	ND	*<0.05*
	12.5	007F	0.62	0.072	<0.05	<0.05
		008F	0.69	0.071	<0.05	<0.05
		009F	0.71	0.078	<0.05	<0.05
		Mean =	0.67	0.074	<0.05	*<0.05*
	37.5	010F	2.0	0.19	<0.05	0.069
		011F	3.2	0.23	0.089	0.083
		012F	3.2	0.20	<0.05	0.078
		Mean =	2.8	0.21	*0.063*	0.077
	37.5 (4-day depuration)	013F	0.087	<0.05	<0.05	ND
	37.5 (8-day depuration)	014F	<0.05	<0.05	<0.05	ND

1 Nominal dose level; mg *N*-acetylglyphosate / kg bodyweight / day
2 Results for each analyte are reported as glyphosate equivalents
3 LOQ for all 4 analytes in kidney = 0.05 mg/kg; ND = Not Detected; (LODs in kidney: *N*-acetylglyphosate: 0.014 mg/kg, glyphosate: 0.004 mg/kg, AMPA: 0.009 mg/kg, *N*-acetyl AMPA: 0.008 mg/kg).
4 Residues results for detected residues that were below the LOQ (i.e. values ≥ LOD, but <LOQ) are listed as in the table as <LOQ (i.e. <0.05), although in the study report the numerical values between LOD and LOQ were listed
5 For purposes of calculating means, values <LOQ (i.e.<0.05) were assigned a value of the LOQ (i.e.0.05) if being averaged with a result ≥ LOQ or with a ND result. ND results were assigned a value of 0. Means are displayed in italics when the value is different than that listed in the study report due to different handling of residue results ≥ LOD, but <LOQ.

Table 6.4.2-8: Residue level results in liver

Matrix	*N*-acetyl glyphosate dose level (mg/kg bw)[1]	Animal ID	Residue levels [2, 3, 4, 5] mg/kg (glyphosate equivalents)			
			N-acetyl glyphosate	Glyphosate	AMPA	N-acetyl AMPA
Liver	0.0	015F	ND	ND	ND, <0.05 [6]	ND
	1.25	001F	<0.05	ND	ND	ND
		002F	<0.05	ND	ND	ND
		003F	ND	ND	ND	ND
		Mean =	*<0.05*	ND	ND	ND
	3.75	004F	<0.05	ND	ND	ND
		005F	<0.05	ND	ND	ND
		006F	<0.05	ND	ND	ND
		Mean =	*<0.05*	ND	ND	ND
	12.5	007F	0.10	ND	ND	ND
		008F	0.10	ND	ND	ND
		009F	0.12	ND	<0.05	ND
		Mean =	0.10	ND	*<0.05*	ND

Table 6.4.2-8: Residue level results in liver

Matrix	*N*-acetyl glyphosate dose level (mg/kg bw)[1]	Animal ID	Residue levels [2, 3, 4, 5] mg/kg (glyphosate equivalents)			
			N-acetyl glyphosate	Glyphosate	AMPA	N-acetyl AMPA
	37.5	010F	0.37	<0.05	<0.05	ND
		011F	0.52	<0.05	ND	ND
		012F	0.38	<0.05	<0.05	ND
		Mean =	0.43	*<0.05*	*<0.05*	ND
	37.5 (4-day depuration)	013F	0.10	<0.05	<0.05	ND
	37.5 (8-day depuration)	014F	<0.05	<0.05	<0.05	ND

1 Nominal dose level; mg *N*-acetylglyphosate / kg bodyweight / day
2 Results for each analyte are reported as glyphosate equivalents
3 LOQ for all 4 analytes in liver = 0.05 mg/kg. ND = Not Detected; (LODs in liver: *N*-acetylglyphosate: 0.018 mg/kg, glyphosate: 0.009 mg/kg, AMPA: 0.019 mg/kg, *N*-acetyl AMPA: 0.008 mg/kg).
4 Residues results for detected residues that were below the LOQ (i.e. values ≥ LOD, but <LOQ) are listed as in the table as <LOQ (i.e. <0.05), although in the study report the numerical values between LOD and LOQ were listed
5 For purposes of calculating means, values <LOQ (i.e.<0.05) were assigned a value of the LOQ (i.e.0.05) if being averaged with a result ≥ LOQ or with a ND result. ND results were assigned a value of 0. Means are displayed in italics when the value is different than that listed in the study report due to different handling of residue results ≥ LOD, but <LOQ.
6 Apparent contamination in 1 of 2 analyses of the control sample. ND used in calculations

Table 6.4.2-9: Residue level results in fat

Matrix	*N*-acetyl glyphosate dose level (mg/kg bw)[1]	Animal ID	Residue levels [2, 3, 4, 5] mg/kg (glyphosate equivalents)			
			N-acetyl glyphosate	Glyphosate	AMPA	N-acetyl AMPA [6]
Fat	0.0	015F	ND	ND	ND	ND, <0.05
	1.25	001F	<0.05	ND	Not analysed	ND
		002F	<0.05	ND		ND
		003F	<0.05	<0.05		ND
		Mean =	*<0.05*	*<0.05*		ND
	3.75	004F	<0.05	ND	Not analysed	ND
		005F	0.17	<0.05		ND
		006F	ND	<0.05		ND
		Mean =	*0.073*	*<0.05*		ND
	12.5	007F	0.054	<0.05	Not analysed	<0.05
		008F	0.051	<0.05		<0.05
		009F	<0.05	ND		<0.05
		Mean =	*0.052*	<0.05		*<0.05*
	37.5	010F	0.22	<0.05	ND	<0.05
		011F	0.055	<0.05	ND	<0.05
		012F	0.075	ND	ND	<0.05
		Mean =	0.12	*<0.05*	ND	*<0.05*
	37.5 (4-day depuration)	013F	0.058	<0.05	Not analysed	<0.05
	37.5 (8-day depuration)	014F	0.14	<0.05	Not analysed	<0.05

Table 6.4.2-9: Residue level results in fat

1 Nominal dose level; mg *N*-acetylglyphosate / kg bodyweight / day
2 Results for each analyte are reported as glyphosate equivalents
3 LOQ for all 4 analytes in fat = 0.05 mg/kg. ND = Not Detected; (LODs in fat: *N*-acetylglyphosate: 0.015 mg/kg, glyphosate: 0.004 mg/kg, AMPA: 0.015 mg/kg, *N*-acetyl AMPA: 0.009 mg/kg).
4 Residues results for detected residues that were below the LOQ (i.e. values ≥ LOD, but <LOQ) are listed as in the table as <LOQ (i.e. <0.05), although in the study report the numerical values between LOD and LOQ were listed
5 For purposes of calculating means, values <LOQ (i.e.<0.05) were assigned a value of the LOQ (i.e.0.05) if being averaged with a result ≥ LOQ or with a ND result. ND results were assigned a value of 0. Means are displayed in italics when the value is different than that listed in the study report due to different handling of residue results ≥ LOD, but <LOQ.
6 Apparent contamination observed in control and treated samples of 12.5 mg/kg bw and 37.5 mg/kg bw. ND used in calculations for control

Table 6.4.2-10: Residue level results in muscle

Matrix	*N*-acetyl glyphosate dose level (mg/kg bw)[1]	Animal ID	Residue levels [2,3,4,5] mg/kg (glyphosate equivalents)			
			***N*-acetyl glyphosate**	**Glyphosate**	**AMPA**	**N-acetyl AMPA**
Muscle	0.0	015F	ND	ND	Not analysed	Not analysed
	1.25	001F	ND	ND	Not analysed	Not analysed
		002F	ND	ND		
		003F	ND	<0.025		
		Mean =	ND	*<0.025*		
	3.75	004F	ND	<0.025	Not analysed	Not analysed
		005F	<0.025	<0.025		
		006F	ND	<0.025		
		Mean =	*<0.025*	*<0.025*		
	12.5	007F	<0.025	ND	Not analysed	ND
		008F	<0.025	ND		ND
		009F	ND	ND		ND
		Mean =	*<0.025*	ND	ND	ND
	37.5	010F	<0.025	ND	ND	ND
		011F	<0.025	ND	ND	ND
		012F	0.053	ND	ND	ND
		Mean =	*0.034*	ND	ND	ND
	37.5 (4-day depuration)	013F	<0.025	ND	Not analysed	Not analysed
	37.5 (8-day depuration)	014F	<0.025	<0.025	Not analysed	Not analysed

1 Nominal dose level; mg *N*-acetylglyphosate / kg bodyweight / day
2 Results for each analyte are reported as glyphosate equivalents
3 LOQ for all 4 analytes in muscle = 0.025 mg/kg. ND = Not Detected; (LODs in muscle: *N*-acetylglyphosate: 0.006 mg/kg, glyphosate: 0.004 mg/kg, AMPA: 0.008 mg/kg, *N*-acetyl AMPA: 0.006 mg/kg).
4 Residues results for detected residues that were below the LOQ (i.e. values ≥ LOD, but <LOQ) are listed as in the table as <LOQ (i.e. <0.025), although in the study report the numerical values between LOD and LOQ were listed
5 For purposes of calculating means, values <LOQ (i.e.<0.025) were assigned a value of the LOQ (i.e.0.025) if being averaged with a result ≥ LOQ or with a ND result. ND results were assigned a value of 0. Means are displayed in italics when the value is different than that listed in the study report due to different handling of residue results ≥ LOD, but <LOQ.

III. Conclusion

N-Acetylglyphosate was orally administered to lactating dairy cattle for 28 consecutive days at nominal dose levels of 1.25, 3.75, 12.5, and 37.5 mg/kg bodyweight. The actual dose levels attained during the study were in good agreement with the nominal levels.

All cows used in the study were in good general health throughout the acclimation and treatment periods. No treatment-related effects on feed consumption, milk production, or bodyweight were observed during the study.

Milk and tissue samples were analysed for *N*-acetylglyphosate, glyphosate, AMPA, and *N*-acetyl AMPA. Concentrations of each analyte were expressed as glyphosate equivalents.

In whole milk, residues of *N*-acetylglyphosate and glyphosate were below the LOQ of 0.025 mg/kg in the samples analyzed from all dose levels and sampling intervals. Although the level of *N*-acetylglyphosate found did not exceed the LOQ of 0.025 mg/kg in any milk samples, it was detected in a significantly greater number of samples compared to glyphosate. Generally, there were very few detections of residues in the 1.25 mg/kg bw and 3.75 mg/kg bw dose groups. Additionally, screening of all dose group cows in Day 7 and Day 14 whole milk samples and the 12.5 mg/kg bw and 37.5 mg/kg bw dose groups in Day 21 whole milk samples showed no detectable residues of AMPA or *N*-acetyl AMPA. Although residue levels in milk were too low for the samples collected during the 7-day depuration period to provide clear results concerning a rate of residue decline, residues of *N*-acetylglyphosate were still detectable in milk samples collected at 7 days after dosing at 37.5 mg/kg bw/day was terminated. Glyphosate was not detected in milk during the depuration phase of the study.

In tissue samples obtained within 24 hours of completion of 28 consecutive days of dosing with *N*-acetylglyphosate, residue levels were highest in kidney followed generally in decreasing order by liver, fat, and muscle. In each tissue, *N*-acetylglyphosate was found in higher concentrations than concentrations of glyphosate, AMPA, or *N*-acetyl AMPA.

In kidney, *N*-Acetylglyphosate, glyphosate, and *N*-acetyl AMPA were detected in kidney in all dose groups. *N*-Acetylglyphosate was found at levels above the LOQ of 0.05mg/kg bw at all dose levels. *N*-Acetylglyphosate residues in kidney ranged from 0.060 mg/kg glyphosate equivalents in the 1.25 mg/kg bw dose group to 3.2 mg/kg in the 37.5 mg/kg bw dose group. Glyphosate was found at or above the LOQ of 0.05 mg/kg in kidney in the two highest dose groups, 12.5 mg/kg bw and 37.5 mg/kg bw. AMPA and *N*-acetyl AMPA were found at or above the LOQ of 0.05 mg/kg in kidney only in the highest dose group, 37.5 mg/kg bw.

In liver, *N*-acetylglyphosate was detected at all dose levels and exceeded the LOQ of 0.05 mg/kg in the two highest dose levels (12.5, and 37.5 mg/kg bw). *N*-Acetylglyphosate residues in liver ranged from 0.010 mg/kg at the 12.5 mg/kg bw dose level to 0.52 mg/kg bw at the 37.5 mg/kg bw dose level. AMPA was detected, but <LOQ of 0.05 mg/kg and only at the 12.5 and 37.5 mg/kg bw dose levels. Glyphosate was detected, but <LOQ of 0.05 mg/kg and only at the highest dose level, 37.5 mg/kg bw. *N*-Acetyl AMPA was not detected in liver at any of the dose levels evaluated.

In fat, concentrations of *N*-acetylglyphosate ranged from <0.05 mg/kg at the lowest dose level of 1.25 mg/kg bw to 0.22 mg/g at the 37.5 mg/kg bw dose level. Glyphosate was detected in fat samples at all four dose levels, but did not exceed the LOQ of 0.05 mg/kg. *N*-acetyl AMPA was detected in fat at only the two highest dose levels, 12.5 and 37.5 mg/kg bw/day, but did not exceed he LOQ of 0.05 mg/kg bw. AMPA residues were not detected at the highest dose level, 37.5 mg/kg bw.

In muscle, *N*-acetylglyphosate was not detected in the 1.25 mg/kg bw dose group, and concentrations ranged from <LOQ of 0.025 mg/kg in the 3.75 mg/kg bw dose group to 0.053 mg/kg in the 37.5 mg/kg bw dose group. Glyphosate residues were not detected in muscle from the two highest dose groups, 12.5 and 37.5 mg/kg bw. AMPA and *N*-acetyl AMPA were not detected in muscle samples analysed.

Following cessation of dosing, residues in tissues generally declined during the depuration period. In kidney and liver, residues of *N*-acetylglyphosate, glyphosate and AMPA were detected, but <LOQ of 0.05 mg/kg after an 8-day depuration period. *N*-Acetyl AMPA residues in kidney were not-detected (ND) after a 4-day depuration period. In liver, residues of *N*-acetylglyphosate, glyphosate, and AMPA were

detected, but <LOQ of 0.05 mg/kg after an 8-day depuration period. *N*-Acetyl AMPA residues in liver were not-detected (ND) either at the end of the 28-day dosing period or during the depuration phase. In fat, the level of *N*-acetylglyphosate appeared to decline slower than in other tissues and was found at 0.14 mg/kg after an 8-day depuration period. Residues of glyphosate and *N*-acetyl AMPA were detected, but remained below the LOQ of 0.05 mg/k at the 4-day and 8-day depuration intervals, although both compounds were found at the same levels at the end of the 28-day dosing period. In muscle, *N*-acetylglyphosate and glyphosate residues were detected after the 8-day depuration period, but were below the LOQ of 0.025 mg/kg.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the magnitude of residues of *N*-acetylglyphosate in ruminant (cattle) milk and tissues (fat, muscle, liver and kidney) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of residues of *N*-acetylglyphosate that may transfer from the livestock diet to milk and edible livestock tissues. The study was performed under GLP and. The study is considered to be scientifically valid and complies with the OECD Guideline for the Testing of Chemicals, 505, Residues in Livestock with one deviation.

For the depuration phase only 2 intervals instead of 3 intervals were analysed. Nevertheless, decline of the residues in milk and tissues can clearly be seen.

The study is considered valid as this deficit is not expected to significantly impact the quality or reliability of the study.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.2/002
Report author	[redacted]
Report year	1987
Report title	Residue determination of Glyphosate and AMPA in dairy cow tissues and milk following a 28-day feeding study
Report No	[redacted]6729
Document No	M-650790-02-1
Guidelines followed in study	US EPA: Subdivision O, Pesticide Assessment Guidelines for Residue Chemistry
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 505: • For meat other pieces than loin, flank or hind-leg collected (triceps, gracilis, and longissimus dorsi muscle) • Sample weights after slaughter not reported • Depuration phase with only 2 intervals instead of 3 intervals • Insufficient detail provided in the study report to determine the interval of sample frozen storage before extraction and analysis.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised	Yes

testing facilities	
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate (N-phosphonomethyl glycine) and AMPA (aminomethylphosphonic acid) in milk and tissues of lactating dairy cows dosed with glyphosate and AMPA for a period of 28 consecutive days, and at 7 and 28 days after dosing ended (i.e. after a withdrawal period of 7 days and 28 days).

Glyphosate and AMPA (in a 9:1 ratio) were administered to cattle through dietary intake for a period of 28 consecutive days with use of a concentrate milking ration that was fortified with glyphosate and AMPA at each of three levels (1X, 3X, and 10X treatment groups). The nominal concentration of glyphosate in the diet for the 1X, 3X, and 10X treatment groups was 36, 108 and 360 mg/kg feed, respectively. The nominal concentration of AMPA in the diet for the 1X, 3X, and 10X treatment groups was 4.0 mg/kg feed, 12 mg/kg feed, and 40 mg/kg feed, respectively. Measured levels of glyphosate and AMPA attained in the cattle diet were near nominal values. Actual levels of glyphosate in the 1X, 3X, and 10X treatments groups in feed on a dry weight basis averaged 34.6 mg/kg, 108.8 mg/kg, and 347.9 mg/kg, respectively. Expressed on a body weight basis, the average dose levels of glyphosate in the 1X, 3X, and 10X groups was 1.4 mg/kg bw/day, 4.1 mg/kg bw/day, and 12.7 mg/kg bw/day, respectively. The actual level of AMPA in the 1X, 3X, and 10X treatments groups in feed on a dry weight basis averaged 3.8 mg/kg, 12.0 mg/kg, and 38.6 mg/kg, respectively. Expressed on a body weight basis, the average dose levels of glyphosate in the 1X, 3X, and 10X groups was 0.16 mg/kg bw/day, 0.46 mg/kg bw/day, and 1.42 mg/kg bw/day, respectively.

The analytical method LOQ for glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) was 0.025 mg/kg in milk and 0.05 mg/kg in fat, muscle, liver, and kidney.

Residues of glyphosate and AMPA in all milk samples from the 10X treatment group were below the LOQ (<0.025 mg/kg); samples from lower dose levels were not analyzed. Glyphosate and AMPA residues in fat and muscle samples collected from all animals in all treatment levels (1X, 3X, and 10X treatment groups) were below the LOQ (<0.05 mg/kg) at the end of the dosing period (Study Day 28) and after the 7-day and 28-day withdrawal periods.

Residues of glyphosate and AMPA at quantifiable levels (≥ 0.05 mg/kg) were found among liver and kidney samples.

The average level of glyphosate in liver samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.06 mg/kg, 0.06 mg/kg, and 0.20 mg/kg, respectively. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA levels were below the LOQ (<0.05 mg/kg) in all liver samples from the 1X treatment group. In the 3X treatment group, AMPA was found in one sample at 0.05 mg/kg, but was below the LOQ (<0.05 mg/kg) in all other samples in that group. The average level of AMPA found in liver samples from the 10X treatment group at the end of the dosing period was 0.15 mg/kg. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

The average level of glyphosate found in kidney at the end of the 28-day dosing period for the 1X, 3X, and 10X treatments groups was 0.24 mg/kg, 0.75 mg/kg, and 3.00 mg/kg, respectively. The average level

of AMPA found in kidney samples at the end of the 28-day dosing period for the 1X, 3X and 10X treatment groups was 0.07 mg/kg, 0.19 mg/kg, and 0.85 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

Test facilities

Study directory:	Monsanto Agricultural Products, St. Louis, Missouri 63167, USA
In-Life phase:	Hazleton Laboratories America, Inc., Madison, Wisconsin 53704, USA
Analytical phase:	Monsanto Agricultural Products, St. Louis, Missouri 63167, USA

I. Materials and Methods

A. Materials

Two test materials, glyphosate and AMPA, were administered to the treated animals in this study. Further information on the test materials is listed in the tables below.

4. Test materials

Test material number 1:

Description:	Glyphosate
Batch number:	Not reported
HLA sample number:	50809703
Active ingredient(s):	Glyphosate (N-phosphonomethyl glycine)
CAS number:	1071-83-6
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	99.9 %
Formulation type:	NA
Appearance/colour:	Powdered solid
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in screw-top glass jar
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Test material number 2:

Description:	AMPA
Batch number:	Not reported
HLA sample number:	50809702
Active ingredient(s):	AMPA (aminomethylphosphonic acid)
CAS number:	1066-51-9
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	97.0 %
Formulation type:	NA

Appearance/colour:	Powdered solid
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in screw-top glass jar
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Lactating Holstein dairy cows were the test animals used in this study. Details are listed in the table below.

5. Test animals

Species:	Lactating dairy cattle; Bovine (*Bos Taurus)*
Gender:	Female
Breed:	Holstein
Source:	[redacted]
Age:	1 to 7 lactations (age in years was not specified)
Weight at dosing, (Day-1):	Ranged from 460-642 kg
Lactation:	Cows selected for use in the study were producing 15 kg or more of milk per day
Number of animals:	19 cows selected out of a group of 22: (4 in untreated control group, and 5 in each of 3 treated groups (1X, 3X, and 10X dose levels)
Animal Identification:	Collar with uniquely numbered tag
Animal health / observations:	Physical examination of each animal by staff veterinarian at the beginning of acclimation (Day -13), at the beginning of the test period (Day-1), and just before sacrifice (Days 27, 34, and 55). The animals were approved for use in the study by the staff veterinarian on 18-Nov-1985.
Acclimation period:	14 days
Diet:	The basal diet was composed of 75 % alfalfa hay (roughage) and 25 % of a concentrate milking ration [Purina Milk Generator #1000(B) 16 %]. The concentrate milking ration was fed at a rate of 1 kg of concentrate ration to 2.5 kg of milk produced, but never less than 7 kg per day. The roughage was fed *ad libitum* and the concentrate milking ration was limit fed with half given at the a.m. milking and the remainder at the p.m. milking. There were no known contaminants in the animal diet that would be expected to have had an impact on the outcome of the study.
Water:	Water was supplied *ad libitum* from automatic waterers
Housing:	The animals were confined to individual stanchion stalls during the study. The animals were released for an exercise period of approximately 1 hour each day.

The environmental conditions at the test facility during the in-life phase of the study are summarised in the table below.

6. Environmental conditions

Temperature:	Ambient; ranged from 7-24 °C
Humidity:	Ranged from 42-80 %
Air change:	Not reported
Photoperiod:	Not reported

B. Study Design and Methods

The study included 4 treatment groups, an untreated control and 3 treated groups (1X, 3X, and 10X dose levels). The 1X dose level was based on the maximum expected level of glyphosate and AMPA residues in the dairy cattle diet based on uses considered at the time the study was conducted. Exaggerated dose levels (3X and 10X) were also included in the study, consistently with guidelines for livestock feeding studies. The animals were assigned to treatment groups late in the acclimation period. Animals were randomly assigned to treatment groups based on feed consumption and milk production. Four cows were assigned to the untreated control group and 5 cows were assigned to each of the three treated groups.

The control group was fed a non-treated diet while the three treated groups were fed rations containing both glyphosate and AMPA in a 9:1 ratio. Dosing of treated animals continued for 28 consecutive days. Upon completion of dosing, 3 animals from each treatment group were sacrificed and tissue samples were collected. The remaining cows were retained for use in a withdrawal phase of the study to evaluate reduction in any residues in milk or tissues after dosing ended. One cow from each of the 3 treated groups was sacrificed 7 days after the end of the dosing period (i.e. Study Day 35), and the remaining 4 cows (1 control and 1 cow in each of the 3 treated groups) were sacrificed 28 days after the end of the dosing period (i.e. Study Day 56).

Further details on the dosing regimen, including target dose levels, are summarised in the table below.

1. Dosing regimen

Route:	Oral via dietary intake
Vehicle:	Concentrate milking ration which was fortified with glyphosate and AMPA
Timing / frequency per day:	Twice per day, with half of the daily dosage each at the a m. and p.m. milking
Duration:	28 consecutive days
Treatment groups (dose levels):	4 treatment groups; untreated control and 3 dose levels (dry feed basis): 1X: nominal at 36 mg/kg glyphosate + 4 g/kg AMPA in total diet 3X: nominal at 108 mg/kg glyphosate + 12 mg/kg AMPA in total diet 10X: nominal at 360 mg/kg glyphosate + 40 mg/kg AMPA in total diet

The concentrate milking ration which was fortified with glyphosate and AMPA for use in dosing the animals in the treated groups was prepared by addition of the powdered solid test materials to the concentrate ration and blending a series of steps including use of a large ribbon blender to achieve a uniform concentration of glyphosate and AMPA.

The concentrate milking ration was targeted at 25 % of the total feed intake in the cow's diet. Therefore, the concentrate ration was treated with glyphosate and AMPA at 4X the indicated dose levels above in order to achieve the desired level in total diet (i.e. glyphosate in the concentrate ration targeted at 144 mg/kg, 432 mg/kg, and 1440 mg/kg in the 1X, 3X and 10X dose levels, respectively; AMPA in the

concentrate ration targeted at 16 mg/kg, 48 mg/kg, and 160 mg/kg in the 1X, 3X and 10X dose levels, respectively).

Fortified feed samples were collected and analysed to confirm that the blending procedure produced a uniform concentration of the test materials throughout the treated batch. Samples were collected from the top, bottom, left and right positions of the mixer for the 3X and 10X dose levels and from the top, middle and bottom positions for the 1X dose level. Results from analysis of the samples confirmed that uniform distribution of the test materials in the feed concentrate was achieved. Additionally, stability of glyphosate and AMPA in the milking concentrate ration was evaluated. Analysis of fortified ration indicated no significant decrease in glyphosate or AMPA concentrations when stored for 14 days at 25°C. The batches of treated milking concentrate ration used to administer the test materials to the cows in this study were stored no longer than 7 days before use. Therefore, the period of demonstrated test material stability in the milking concentrate ration covers the maximum period of storage experienced in the study.

Samples (500 g each) were collected from each batch of fortified concentrate milking ration fed to the cows in this study and were analysed to determine levels of glyphosate and AMPA.

2. Daily observations and animal data collection

All animals were observed daily for general condition and behaviour. Feed consumption for all animals was determined daily for each animal individually based on weight of roughage and concentrate milking ration offered and refused. Moisture content of feed commodities was determined and feed consumption is expressed on a dry weight basis. Body weight was recorded at the beginning, midpoint, and end of the acclimation period, and weekly thereafter through the end of the dosing period and then during the withdrawal period for those animals retained for use in that phase of the study. All cows were milked twice daily and the weight of the milk produced by individual animal was recorded.

3. Milk and tissue sample collection

Samples of milk and tissues were collected for residue analysis. Milk samples were collected at specified intervals during the 28-day dosing phase of the study (Study Days 0–28) and after completion of dosing during the withdrawal phase of the study (Study Days 29–56). Milk samples were collected from individual animals and a given sample was produced by composting approximately 400 mL of milk from the evening milking and 600 mL of milk from the following morning milking. The sample was identified by the day of the morning milking. The composite sample was thoroughly mixed and four subsamples (~200 mL each) were removed and stored in polyethylene containers. Each subsample is of sufficient size for one analysis and avoided thawing / refreezing the larger container, should repeat analyses be needed.

At the time of tissue sample collection, specified animals were euthanised (stunning gun followed by exsanguination). Samples of fat (composite of equal amounts of omental and subcutaneous back fat), muscle (composite of equal amounts of triceps, gracilis, and longissimus dorsi muscle), liver, and kidney were collected from animals individually upon completion of the 28-day dosing period (within 1 day of administration of the final dose) or during the withdrawal phase of the study at 7 days or 28 days after the end of the dosing period (Study Days 35, and 56, respectively). Gross necropsy was performed on sacrificed animals. Tissue samples were stored frozen in polyethylene containers.

Milk and tissue samples were stored frozen (<-20 °C) initially at the In-life facility, Hazleton Laboratories, and then shipped to the Analytical Phase facility (Monsanto, St Louis, Missouri, USA) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table 6.4.2-11: Milk and tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Milk	Dosing phase: -1, 1, 2, 4, 7, 14, 21, 28; Withdrawal phase: 29, 30, 32, 35, 42, 49, 56	Composite of ~ 400 mL evening milk and 600 mL of following morning milk. 4 x ~200 mL subsamples collected from composite milk sample from each animal at each sampling interval
Muscle[1]	End of dosing: Study Day 28 Withdrawal phase: Study days 35 and 56	~ 500 g/animal[3]
Fat[2]		~ 500 g/animal[3]
Liver		~ 500 g/animal[3]
Kidney		~ 500 g/animal[3]

1 Composite of equal amounts of triceps, gracilis, and longissimus dorsi muscle.
2 Composite of equal amounts of omental and subcutaneous back fat.
3 Duplicate samples were collected; one shipped for analysis and one held as a reserve sample.

4. Analytical phase

Analysis of feed samples as well as milk and tissue samples was conducted at the Analytical Phase facility, Monsanto, St. Louis, Missouri, USA.

An analytical methodology was developed and validated for the determination of glyphosate and AMPA in the feed diet. The procedure consists of extracting the feed diets with an aqueous/organic partition extraction (2:1 deionised water and chloroform) on a shaker, centrifuging, and ion exchange resin clean up. Quantitation was achieved by using a liquid chromatograph equipped with an Aminex A-9 analytical column, an o-phthalaldehyde post-column reactor and a fluorescence detector. The limit of validation/quantitation (LOQ) of the method was 4 mg/kg. Each feed diet was analysed in duplicate.

Recovery results with concentrate milking ration (feed) fortified with glyphosate and AMPA demonstrate that the intended dose concentration was achieved and are summarised in the table below.

Table 6.4.2-12: Recovery results: glyphosate and AMPA in feed (concentrate milking ration)

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)[1]	Relative standard deviation (%)[1]	Number analyses (n)
Feed (concentrate milking ration)	Glyphosate	144 (1X)	105, 102, 101, 101, 101, 95.4, 85.4, 79.4, 103, 101, 103, 100	97.9	*7.9*	*8.0*	12
		432 (3X)	95.7, 95.7, 102, 104, 97.9, 99.2, 102, 106	100	*3.8*	*3.8*	8
		1440 (10X)	92.9, 91.1, 97.3, 90.4, 96.7, 96.8, 96.6, 91.6, 95.1, 100	94.9	*3.2*	*3.4*	10
		Overall	79.4–106	97.6	5.9	*6.0*	30
	AMPA	16.0 (1X)	97.4, 98.0, 98.9, 102, 101, 93.3, 87.5, 89.9, 96.9, 97.6, 102, 99.6	96.9	*4.6*	*4.7*	12
		48.0 (3X)	93.6, 94.9, 105, 106, 97.6, 97.6, 102, 95.8	99.0	*4.7*	*4.7*	8
		160 (10X)	86.4, 87.0, 90.7, 94.9, 90.9, 96.4, 96.8, 93.3, 97.9, 97.8	93.1	*4.3*	*4.6*	10
		Overall	86.4–106	96.3	5.0	*5.2*	30

Table 6.4.2-12: Recovery results: glyphosate and AMPA in feed (concentrate milking ration)

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)[1]	Relative standard deviation (%)[1]	Number analyses (n)

1 Standard deviation and relative standard deviation values in italics were not included in the study report, but were calculated separately for this study summary.

Another analytical methodology was developed and validated for the determination of glyphosate and AMPA in cow milk, as well as fat, muscle, liver, and kidney tissues. All samples were analysed using the analytical method based on the well-established method DFG 405 (refer to CA 4.1.2). The procedure used an aqueous/organic partition extraction (2:1 deionised water and chloroform). Glyphosate and AMPA were isolated from cow fat, muscle, liver, kidney and milk extracts by elution through Chelex 100 resin in the Fe(III) form. Glyphosate and AMPA are eluted from the resin with hydrochloric acid and the iron is removed using an ion exchange resin. After concentration to dryness to remove the hydrochloric acid, samples are analysed using a two column switching high pressure liquid chromatograph equipped with an OPA post-column reactor and a fluorescence detector.

The limit of validation/quantitation (LOQ) is 0.05 mg/kg each for glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) in a fat, muscle, liver and kidney, and is 0.025 mg/kg each for glyphosate and AMPA in milk. Each tissue and milk sample was analysed in duplicate with a typical analytical set consisting of 2 control samples, 2 fortified controls and 8 treated samples. Recovery results with samples of milk, fat, muscle, liver, and kidney fortified with glyphosate and AMPA are summarised in the table below.

Table 6.4.2-13: Recovery results: glyphosate and AMPA in milk and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Glyphosate	Milk	0.025	83.6, 89.8, 98.7, 91.6, 107, 96.1, 84.3, 95.4, 95.9, 91.2, 91.7, 105, 96.1, 93.8, 95.6, 98.3, 95.2, 94.6, 101, 99.9, 99.3, 96.1	95.5	5.6	5.9	22
	Fat	0.05	94.0, 97.1, 97.5, 98.1, 96.6, 96.1, 94.2, 95.3	96.1	1.5	1.6	8
	Muscle	0.05	89.0, 92.3, 92.9, 98.7, 102, 100, 89.6, 90.6	94.4	5.1	5.4	8
	Liver	0.05	80.3, 85.6, 104, 76.9	*86.7*	*12.1*	*13.9*	4
		0.1	70.3, 72.9	*71.6*	-	-	2
		1.0	89.3, 87.0	*88.2*	-	-	2
		Overall	70.3–104	83.3	10.8	13.0	8
	Kidney	0.05	94.5, 94.2	*94.4*	-	-	2
		0.5	96.4, 93.8, 94.3, 91.9	*94.1*	*1.8*	*2.0*	4
		5.0	98.0, 96.1	*97.1*	-	-	2
		Overall	91.9–98.0	94.9	1.9	2.0	8

Table 6.4.2-13: Recovery results: glyphosate and AMPA in milk and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
AMPA	Milk	0.025	93.6, 91.8, 93.1, 92.8, 104, 101, 90.2, 99.0, 109, 107, 107, 107, 95.8, 95.4, 96.4, 99.2, 95.6, 98.4, 103, 103, 102, 103	99.4	5.6	5.6	22
	Fat	0.05	88.5, 89.4, 77.6, 79.2, 97.3, 102, 86.5, 91.1	89.0	8.2	9.2	8
	Muscle	0.05	85.0, 89.1, 92.6, 106, 96.3, 94.0, 95.3, 92.0	93.8	6.1	6.5	8
	Liver	0.05	99.0, 99.0, 80.6, 84.3	*90.7*	*9.7*	*10.7*	4
		0.1	91.2, 91.4	*91.3*	-	-	2
		1.0	88.7, 87.5	*88.1*	-	-	2
		Overall	80.6–99.0	90.2	6.5	7.2	8
	Kidney	0.05	95.6, 98.0	*96.8*	-	-	2
		0.5	89.8, 86.0, 90.2, 87.2	*88.3*	*2.0*	*2.3*	4
		5.0	89.8, 87.3	*88.6*	-	-	2
		Overall	86.0–98.0	90.5	4.2	4.6	8

Means, standard deviations, and relative standard deviations for individual fortification levels which were not included in the study report were calculated separately for this study summary and are presented here in italics.

II. Results and Discussion

A. Dose levels

As indicated previously, concentrate milking ration was fortified with glyphosate and AMPA at specified levels as the vehicle to administer the test materials through dietary intake to cows in the treated dose group. The concentrate milking ration was limit fed and was targeted to compose 25 % of total dry feed intake. The remainder of the diet was composed of roughage (alfalfa hay), which was fed *ad libitum*. Since the fortified ration only composed 25 % of the targeted total of amount of dry feed consumption (concentrate milking ration + alfalfa hay), the fortification levels targeted for the concentrate milking ration were 4x the desired level in the total diet. Therefore, for targeted dietary levels of glyphosate at 36 mg/kg (1X), 108 mg/kg (3X) and 360 mg/kg (10X), the intended fortification levels of glyphosate in the concentrate milking ration were 144 mg/kg, 432 mg/kg, and 1440 mg/kg, respectively. Similarly, for targeted dietary levels of AMPA at 4 mg/kg (1X), 12 mg/kg (3X) and 40 mg/kg (10X), the intended fortification levels of AMPA in the concentrate milking ration were 16 mg/kg, 48 mg/kg, and 160 mg/kg, respectively.

Analysis of samples of fortified concentrate milking ration collected during the dosing phase of the study confirmed that actual dose levels were close the nominal / targeted dose levels. A summary results of analysis of fortified concentrate milking ration to determine actual dose levels of glyphosate and AMPA is shown in the table below.

Table 6.4.2-14: Actual dose levels of glyphosate and AMPA in concentrate milking ration

Nominal dose level	Week number	Average Glyphosate (mg/kg)	Average AMPA (mg/kg)

1X Glyphosate: 144 mg/kg AMPA: 16 mg/kg	1	133	15.0
	2	143	15.6
	3	143	15.6
	4	144	15.1
	Overall average:	141 ± 5	15.3 ± 0.3
3X Glyphosate: 432 mg/kg AMPA: 48 mg/kg	1	407	47.1
	2	416	48.2
	3	430	47.9
	4	405	44.7
	Overall average:	415 ± 11	46.8 ± 1.5
10X Glyphosate: 1440 mg/kg AMPA: 160 mg/kg	1	1357	155
	2	1372	162
	3	1403	172
	4	1335	148
	Overall average:	1367 ± 29	159 ± 10

Based on the quantity of milking concentrate ration and the associated nominal levels of glyphosate and AMPA along with total dry feed consumption, the concentration of glyphosate and AMPA in the total diet was calculated for each treated animal during the dosing phase of the study. Results showed that actual levels of glyphosate and AMPA in each of the 3 dose levels were close to nominal / target levels. The overall average for glyphosate in the total diet on a dry feed basis for the 1X, 3X, and 10X treated groups was 34.6 mg/kg, 108.8 mg/kg, and 347.9 mg/kg, respectively. The overall average for AMPA in the total diet on a dry feed basis for the 1X, 3X, and 10X treated groups was 3.8 mg/kg, 12.0 mg/kg, and 38.6 mg/kg, respectively. These results are summarised in the table below.

Additionally, in a second table below, dosage was calculated for this summary and expressed on the basis of individual animal body weight (i.e. mg test material / kg bw/day). These results were calculated using average daily intake of glyphosate and AMPA and average body weight of individual animals during the dosing phase of the study. The overall average for glyphosate dosage on a body weight basis in the 1X, 3X, and 10X treated groups was 1.4 mg/kg bw/day, 4.1 mg/kg bw/day, and 12.7 mg/kg bw/day, respectively. The overall average for AMPA dosage on a body weight basis in the 1X, 3X, and 10X treated groups was 0.16 mg/kg bw/day, 0.46 mg/kg bw/day, and 1.42 mg/kg bw/day, respectively.

Table 6.4.2-15: Actual dose levels of glyphosate and AMPA administered to lactating dairy cows for 28 days expressed on basis of basis of body weight (bw) and concentration in total diet (dry feed)

Nominal dose level	Animal number	Average milking concentrate ration per day (kg)[1,2]	Average body weight during dosing (kg)[2]	Average daily dry feed consumption (kg)[2]	Glyphosate dose / day[2]			AMPA dose / day[2]		
					mg/ animal	mg/kg bw	mg/kg dry feed	mg/ animal	mg/kg bw	mg/kg dry feed
1X [36 mg/kg glyphosate + 4 mg/kg AMPA in dry feed (total diet)]	079	*4.9*	*562*	*20.4*	*709.2*	*1.3*	*34.9*	*78.8*	*0.14*	*3.9*
	055	*6.8*	*617*	*28.4*	*972.0*	*1.6*	*34.3*	*108.0*	*0.17*	*3.8*
	097	*5.5*	*613*	*24.5*	*795.6*	*1.3*	*32.5*	*88.4*	*0.14*	*3.6*
	053	*4.8*	*487*	*20.4*	*684.0*	*1.4*	*33.5*	*76.0*	*0.16*	*3.7*
	086	*5.3*	*485*	*20.2*	*766.8*	*1.6*	*38.1*	*85.2*	*0.18*	*4.2*
	Average:	*5.5*	*553*	*22.7*	*785.5*	***1.4***	***34.6***	*87.3*	***0.16***	***3.8***
3X [108 mg/kg glyphosate + 12 mg/kg AMPA in dry feed	059	*5.5*	*571*	*21.1*	*2365*	*4.1*	*112.0*	*263*	*0.46*	*12.4*
	105	*6.4*	*609*	*26.8*	*2743*	*4.5*	*102.4*	*305*	*0.50*	*11.4*
	102	*5.9*	*634*	*22.5*	*2560*	*4.0*	*113.8*	*284*	*0.45*	*12.6*
	080	*4.6*	*511*	*17.5*	*1987*	*3.9*	*113.6*	*221*	*0.43*	*12.6*
	106	*5.6*	*589*	*23.8*	*2430*	*4.1*	*102.2*	*270*	*0.46*	*11.4*

Table 6.4.2-15: Actual dose levels of glyphosate and AMPA administered to lactating dairy cows for 28 days expressed on basis of basis of body weight (bw) and concentration in total diet (dry feed)

Nominal dose level	Animal number	Average milking concentrate ration per day (kg)[1,2]	Average body weight during dosing (kg)[2]	Average daily dry feed consumption (kg)[2]	Glyphosate dose / day[2]			AMPA dose / day[2]		
					mg/ animal	mg/kg bw	mg/kg dry feed	mg/ animal	mg/kg bw	mg/kg dry feed
(total diet)]	**Average:**	*5.6*	*583*	*22.3*	*2417*	***4.1***	***108.8***	*269*	***0.46***	***12.0***
10X [360 mg/kg glyphosate + 40 mg/kg AMPA in dry feed (total diet)]	100	*6.0*	*632*	*25.4*	*8676*	*13.7*	*342.2*	*964*	*1.53*	*38.0*
	090	*4.8*	*554*	*20.6*	*6840*	*12.4*	*331.6*	*760*	*1.37*	*36.8*
	098	*5.6*	*637*	*21.8*	*8100*	*12.7*	*371.1*	*900*	*1.41*	*41.2*
	107	*6.0*	*629*	*24.9*	*8568*	*13.6*	*343.8*	*952*	*1.51*	*38.2*
	093	*4.1*	*540*	*16.9*	*5940*	*11.0*	*351.1*	*660*	*1.22*	*39.0*
	Average:	*5.3*	*598*	*21.9*	*7625*	***12.9***	***347.9***	*847*	***1.42***	***38.6***

1 The milking concentrate ration was fortified with glyphosate and AMPA at 4X the target level in the total diet on a dry feed basis. The nominal concentration of glyphosate in the milking concentrate ration in the 1X, 3X, and 10X dose level was 144 mg/kg, 432 mg/kg, and 1440 mg/kg, respectively. The nominal concentration of AMPA in the milking concentrate ration in the 1X, 3X, and 10X dose level was 16 mg/kg, 48 mg/kg, and 160 mg/kg, respectively.

2 All values were calculated for this summary and are thus shown in italics.

B. Animal health and daily observations

There were no findings concerning animal health or behavior that were considered to be test related. Feed consumption for all animals in each test group remained essentially stable during the test period. Body weight fluctuations seen during the study were considered normal for adult animals. Following animal sacrifice, necropsy / pathology evaluation indicated no macroscopic or microscopic observations that appear treatment related.

C. Residue levels in milk and tissues

Residues of glyphosate and AMPA in milk and tissues (fat, muscle, liver, and kidney) collected from untreated control animals were below the LOQ (<0.05 mg/kg).

Frozen storage stability of glyphosate and AMPA in cattle matrices (milk, fat, muscle, liver and kidney) was evaluated in a separate study completed subsequent to this feeding study. No significant degradation of glyphosate or AMPA in cattle fat, muscle, liver, or kidney was observed for 24 months, which was the maximum period of frozen storage evaluated. No significant degradation of cattle milk was observed for a period of 16 months, which was the maximum period of frozen storage evaluated.

Residues of glyphosate and AMPA in all milk samples from the 10X treatment group were below the LOQ (<0.025 mg/kg). Since residues were <0.025 mg/kg in the highest dose group, milk samples from the lower dose levels (1X and 3X treatment levels) were not analysed. Since there were no quantifiable residues in milk from the samples analyzed from treated animals, the results were not included in a table in this summary.

Glyphosate and AMPA residues in fat and muscle samples collected from all animals in all treatment levels (1X, 3X, and 10X treatment groups) were below the LOQ (<0.05 mg/kg) at the end of the dosing period (Study Day 28) and after the 7-day and 28-day withdrawal periods. Since there were no measureable residues of glyphosate or AMPA in these samples, results for these matrices were not included in tables in this summary.

A summary of residue results for glyphosate and AMPA in liver is shown in the table below, and a summary for glyphosate and AMPA in kidney is shown a second table below. The residue values presented in the summary in the study report had been corrected for recovery. The residue values for liver and kidney in the two tables below were not corrected for recovery.

Glyphosate was found at levels at or above the LOQ (≥ 0.05 mg/kg) in one or more liver samples for each dose level (1X, 3X and 10X) at the end of the 28-day doing period (Study Day 28). The average level of glyphosate in liver samples at the end of the 28-day dosing period in the 1X, 3X and 10X treatment groups was 0.06 mg/kg, 0.06 mg/kg, and 0.20 mg/kg, respectively. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and were 0.11 mg/kg for the 10X treatment group. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period in the 10X treatment group. AMPA levels were below the LOQ (<0.05 mg/kg) in all liver samples from the 1X treatment group. In the 3X treatment group, AMPA was found in one sample at 0.05 mg/kg, but was below the LOQ (<0.05 mg/kg) in all other samples in that group. The average level of AMPA found in liver samples from the 10X treatment group at the end of the dosing period was 0.15 mg/kg. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and were 0.08 mg/kg for the 10X treatment group. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period in the 10X treatment groups.

The average level of glyphosate found in kidney at the end of the 28-day dosing period for the 1X, 3X, and 10X treatments groups was 0.24 mg/kg, 0.75 mg/kg, and 3.00 mg/kg, respectively. The average level of AMPA found in kidney samples at the end of the 28-day dosing period for the 1X, 3X, and 10X treatment groups was 0.07 mg/kg, 0.19 mg/kg, and 0.85 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and were 0.06 mg/kg for the 10X treatment group. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period in the 10X treatment group. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and were 0.07 mg/kg for the 10X treatment group. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period in the 10X treatment group.

Table 6.4.2-16: Residues of glyphosate and AMPA in liver

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
1X Glyphosate (average): 34.6 mg/kg in feed; 1.4 mg/kg bw AMPA (average): 3.8 mg/kg in feed; 0.16 mg/kg bw	055	28	1	<0.05	*0.05*	<0.05	*<0.05*
			2	0.05		<0.05	
	079	28	1	0.09	*0.07*	<0.05	*<0.05*
			2	<0.05		<0.05	
	097	28	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
	Study Day 28, 1X treatment group average:				*0.06*		*<0.05*
	053	35	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
	086	56	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
3X Glyphosate (average):	059	28	1	0.08	*0.08*	<0.05	*<0.05*
			2	0.07		<0.05	
	102	28	1	<0.05	*0.05*	0.05	*0.05*
			2	0.05		0.05	

Table 6.4.2-16: Residues of glyphosate and AMPA in liver

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
108.8 mg/kg in feed; 4.1 mg/kg bw AMPA (average): 12.0 mg/kg in feed; 0.46 mg/kg bw	105	28	1	0.06	*0.06*	<0.05	*<0.05*
			2	0.06		<0.05	
	Study Day 28, 3X treatment group average:				*0.06*		*0.05*
	080	35	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
	106	56	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
10X Glyphosate (average): 347.9 mg/kg in feed; 12.7 mg/kg bw AMPA (average): 38.6 mg/kg in feed; 1.42 mg/kg bw	090	28	1	0.22	*0.21*	0.12	*0.12*
			2	0.19		0.12	
	100	28	1	0.20	*0.20*	0.18	*0.18*
			2	0.20		0.18	
	098[5]	28	1	-	-	-	-
			2	-		-	
	Study Day 28, 10X treatment group average:				*0.20*		*0.15*
	107	35	1	0.10	*0.11*	0.08	*0.08*
			2	0.11		0.08	
	093	56	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	

1 Study Day 28 is at the end of the 28-day dosing period; Study Days 35 and 56 are during the withdrawal period, 7 days and 28 days after the end of dosing, respectively.
2 LOQ (limit of quantitation): 0.05 mg/kg.
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater. Averages of uncorrected residues were calculated for this summary and thus are shown in italics.
5 Samples from animal 098 were not analyzed due to health issues with the animal, which were not treatment-related.

Table 6.4.2-17: Residues of glyphosate and AMPA in kidney

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
1X Glyphosate (average): 34.6 mg/kg in feed; 1.4 mg/kg bw AMPA (average): 3.8 mg/kg in feed; 0.16 mg/kg bw	055	28	1	0.28	*0.33*	0.06	*0.07*
			2	0.37		0.08	
	079	28	1	0.24	*0.24*	0.08	*0.08*
			2	0.24		0.07	
	097	28	1	0.16	*0.16*	<0.05	*<0.05*
			2	0.16		<0.05	
	Study Day 28, 1X treatment group average:				*0.24*		*0.07*
	053	35	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
	086	56	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
3X Glyphosate (average): 108.8 mg/kg in feed;	059	28	1	0.75	*0.74*	0.13	*0.13*
			2	0.72		0.13	
	102	28	1	0.81	*0.82*	0.24	*0.25*
			2	0.83		0.25	

Table 6.4.2-17: Residues of glyphosate and AMPA in kidney

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
4.1 mg/kg bw AMPA (average): 12.0 mg/kg in feed; 0.46 mg/kg bw	105	28	1	0.69	*0.69*	0.19	*0.20*
			2	0.69		0.20	
	Study Day 28, 3X treatment group average:				*0.75*		*0.19*
	080	35	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
	106	56	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	
10X Glyphosate (average): 347.9 mg/kg in feed; 12.7 mg/kg bw AMPA (average): 38.6 mg/kg in feed; 1.42 mg/kg bw	090	28	1	2.70	*2.73*	0.91	*0.91*
			2	2.76		0.91	
	100	28	1	3.25	*3.27*	0.79	*0.79*
			2	3.28		0.78	
	098[5]	28	1	-	-	-	-
			2	-		-	
	Study Day 28, 10X treatment group average:				*3.00*		*0.85*
	107	35	1	0.05	*0.06*	0.07	*0.07*
			2	0.07		0.07	
	093	56	1	<0.05	*<0.05*	<0.05	*<0.05*
			2	<0.05		<0.05	

1 Study Day 28 is at the end of the 28-day dosing period; Study Days 35 and 56 are during the withdrawal period, 7 days and 28 days after the end of dosing, respectively.
2 LOQ (limit of quantitation):0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater. Averages of uncorrected residues were calculated for this summary and thus are shown in italics.
5 Samples from animal 098 were not analyzed due to health issues with the animal, which were not treatment-related.

III. Conclusion

Residues of glyphosate and AMPA in all milk samples from the 10X treatment group were below the LOQ (<0.025 mg/kg); samples from lower dose levels were not analyzed. Glyphosate and AMPA residues in fat and muscle samples collected from all animals in all treatment levels (1X, 3X, and 10X treatment groups) were below the LOQ <0.05 mg/kg) at the end of the dosing period (Study Day 28) and after the 7-day and 28 day withdrawal periods.

Residues of glyphosate and AMPA at quantifiable levels (≥ 0.05 mg/kg) were found among liver and kidney samples.

The average level of glyphosate in liver samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups was 0.06 mg/kg, 0.06 mg/kg, and 0.20 mg/kg, respectively. Glyphosate residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA levels were below the LOQ (<0.05 mg/kg) in all liver samples from the 1X treatment group. In the 3X treatment group, AMPA was found in one sample at 0.05 mg/kg, but was below the LOQ (<0.05 mg/kg) in all other samples in that group. The average level of AMPA found in liver samples from the 10X treatment group at the end of the dosing period was 0.15 mg/kg. AMPA residues in liver were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

The average level of glyphosate found in kidney at the end of the 28-day dosing period for the 1X, 3X, and 10X treatments groups was 0.24 mg/kg, 0.75 mg/kg, and 3.00 mg/kg, respectively. The average level of AMPA found in kidney samples at the end of the 28-day dosing period for the 1X, 3X and 10X treatment groups was 0.07 mg/kg, 0.19 mg/kg, and 0.85 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 7-day withdrawal period for the 1X and 3X treatment groups, and after a 28-day withdrawal period in the 10X treatment group.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the residues of glyphosate and AMPA in ruminant (cattle) milk and tissues (fat, muscle, liver and kidney) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of glyphosate and AMPA residues that may transfer from the livestock diet to milk and edible livestock tissues. It was performed under GLP, although the report lacks a GLP compliance page for the analytical portion of the study, but it is considered to be scientifically valid. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 505 and OECD Guidance Document on Residues in Livestock (Series on Pesticides No. 73) with a few deviations.

For meat other pieces than loin, flank or hind-leg were collected (triceps, gracilis, and longissimus dorsi muscle). The sample weights after slaughter were not reported. For the depuration phase only 2 intervals instead of 3 intervals were analysed. The period for which samples were stored frozen before extraction/analysis is not provided, as the date of analysis is not stated within the report. The dates of sacrifice are 17.12.1985, 24.12.1985 and 14.01.1986 for the first, second and the final sacrifice, respectively. The final report was issued in September, 1987. Thus, the maximum storage period from the first sacrifice to the final report could be estimated as 652 days (22 months). The storage stability of glyphosate and AMPA upon frozen storage was demonstrated in cow milk, kidney, liver, fat, and muscle for a minimum of 671 days (22 months) in a feeding study resented within this chapter (CA.6.4.2/003 [redacted] 1987). Thus, the storage stability is covered.

The study is considered valid as these deficits are not expected to significantly impact the quality or reliability of the study.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.2/003
Report author	[redacted]
Report year	1987
Report title	Magnitude of SC-0224 Residues in Meat and Milk
Report No	[redacted] 87-44
Document No	Not available
Guidelines followed in study	U.S. Environmental Protection Agency Publication EPA 540/9-82-023, Subdivision O, Residue Chemistry Section of Pesticide Assessment Guidelines, October, 1982
Deviations from current test	A review of this study indicates the following deviations from OECD

guideline	Guideline for the Testing of Chemicals, 505: • The report did not confirm that the weight of feed commodities was on a dry weight basis (i.e. corrected for moisture content). • Although the study included 3 animals per dose level, as specified in guideline 505, tissues were sampled from only 2 animals at the end of the dosing period since 1 of the animals was retained for use in a withdrawal / depuration phase of the study. The fat sample was a composite of omental and renal fat. Test guideline 505 indicates that the fat sample should also include subcutaneous fat. • For meat other pieces than loin, flank or hind-leg collected (triceps, gracilis, and longissimus dorsi muscle) • Depuration phase with only 1 interval instead of 3 intervals • GLP assay / certificate of analysis for test materials was not provided
Previous evaluation	Yes, evaluated and accepted *in the RAR (2015)*
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of this study was to determine the magnitude of the residues in milk and tissues of lactating dairy cattle dosed with of glyphosate-trimesium (SC-0224), the trimethylsulfonium salt of glyphosate, for a period of 28 consecutive days, and 7 days after dosing ended (i.e. after a withdrawal period of 7 days). Residue analysis was conducted for the N-phosphonomethyl glycine anion (PMG) (also known as carboxymethylaminomethyl phosphonate (CMP)), the trimethylsulfonium cation (TMS), and AMPA (aminomethylphosphonic acid). TMS is not a relevant analyte in this dossier, therefore data with respect to this analyte is not presented in the following summary.

The study included 6 treatment groups, an untreated control group and 5 treated groups (T1, T2, T3, T4, and T5 with nominal dose levels of glyphosate-trimesium in feed at 0.5, 5, 50, 300, and 1000 mg glyphosate trimesium/kg feed or 0.345, 3.45, 34.5, 207 and 690 mg glyphosate equivalents/kg feed, respectively). There were 3 cows assigned to each treatment group. Two animals in each treated group were sacrificed within 24 hours after their 28th daily dose and tissue samples were collected. The remaining animals were sacrificed 7 days after dosing had been discontinued (i.e. after a 7-day withdrawal period). Milk samples were collected during the 28-day doing period and in the withdrawal phase of the study.

The actual dose levels attained were relatively close to nominal levels, except for the highest dose level (T5) where the dosage when expressed on the basis of concentration in the diet was higher than the nominal level. The average dose levels of glyphosate-trimesium expressed as concentration in the diet (mg/kg feed) for treatment groups T1, T2, T3, T4, and T5 were 0.51, 4.45, 43.0, 299, and 1383 mg/kg feed, respectively. Additionally, the dose levels expressed on the basis of animal bodyweight were calculated with use of data provided in the study report. The average dose levels of glyphosate-trimesium expressed on the basis of bodyweight for treatment groups T1, T2, T3, T4, and T5 were 0.018, 0.173, 1.81, 10.7, and 36.6 mg/kg bw/day, respectively. If expressed as glyphosate equivalents, based on a conversion factor of 0.69 derived from the molecular weight of glyphosate and glyphosate-trimesium, the average dose levels of glyphosate equivalents based on concentration in the diet for treatment groups

T1, T2, T3, T4, and T5 were 0.41, 3.07, 29.70, 206.19, and 954 mg/kg feed, respectively. The average dose levels of glyphosate equivalents expressed on the basis of bodyweight for treatment groups T1, T2, T3, T4, and T5 were 0.012, 0.119, 1.25, 7.39, and 25.25 mg/kg bw/day, respectively.

No treatment-related effects on feed consumption, body weight or milk production were noted at levels up to the 300 mg/kg feed (T4) nominal dosage level. At the 1000 mg/kg feed (T5) nominal dosage level, feed consumption, body weight and milk production were initially adversely impacted, but returned to near pretreatment levels when dosage levels were adjusted to 1000 mg/kg in feed based on daily feed consumption, which was reduced from pre-treatment levels. All animals were considered healthy at the pre-sacrifice examination.

Calculated background concentrations of the analytes were below the detection limit of the methods for most control samples; therefore, residue concentrations are listed as less than the detection limit. The analytical method LOD for both CMP and AMPA in muscle, fat, and kidney was 0.05 mg/kg. The LOD for both CMP and AMPA in milk was 0.02 mg/kg and was 0.2 mg/kg in liver. The residues as well as LODs are expressed as glyphosate for glyphosate and as AMPA for AMPA.

All samples were analyzed within 69 days of collection. The study report includes storage stability data showing that CMP and AMPA are stable in milk, kidney, liver, fat, and muscle upon frozen storage for a minimum of 671 days. These data adequately cover the maximum periods of frozen storage encountered in this study.

In samples of milk and tissues collected from untreated control animals, the residues of CMP and AMPA were generally below the analytical method LOD. However, there were occasionally control samples in which residue results were at or slightly above the analytical LOD. CMP was observed at 0.05 mg/kg in some of the untreated control fat samples. The study report indicated that residues observed in untreated control samples should be considered as potential background levels of residue and should be used for comparison when evaluating residue results in samples from treated animals.

In general, the study results indicate a direct relationship between the dose level of glyphosate-trimesium and the concentrations of CMP and AMPA residues in the cow milk and tissues. The highest residue concentrations were present in milk and tissues from the highest dosage levels.

In milk, CMP residues in the highest dose level, T5, ranged from <0.02 mg/kg to 0.04 mg/kg during the dosing period. In the T4 dose level CMP residues in milk were typically below the LOD (<0.02 mg/kg), but were observed at 0.02 mg/kg in a few samples. In the lower dose levels (T1 – T3), CMP residues in milk remained below the LOD. Residues of CMP in tissues (kidney, liver, fat, and muscle) remained below the LOD in the two lowest dose levels, T1 and T2. Among the four tissues, residues of CMP were highest in kidney in treatment groups T3–T5. The average level of CMP in kidney at the end of the doing period in Treatments Groups T3, T4, and T5 was 0.385 mg/kg, 2.2 mg/kg, and 5.85 mg/kg, respectively. CMP residues in fat were less responsive to increased dosage level and averaged 0.055 mg/kg, 0.06 mg/kg, and 0.08 mg/kg in treatment groups T3, T4, and T5, respectively. Residue of CMP in liver and muscle remained below the LOD, except in the highest dose group (T5) where average residue levels were 0.365 mg/kg and 0.08 mg/kg, respectively.

AMPA residues were below the LOD in milk, liver, fat, and muscle at the end of the dosing period in all dosage levels evaluated (T1-T5). In kidney, AMPA residues were below the LOD in the two lowest dose levels, (T1 and T2). However, the average level of AMPA found in kidney at the end of the dosing period in treatment groups T3, T4, and T5 was 0.07 mg/kg, 0.525 mg/kg, and 1.65 mg/kg, respectively.

Residues of CMP and AMPA, when found in milk and tissues at the end of the dosing period, decreased significantly during the 7-day withdrawal period when dosing was discontinued, indicating that these residues do not accumulate irreversibly under the conditions tested.

Test facilities

Study directory: [redacted]

In-Life phase: [redacted]

Analytical phase: [redacted]

I. Materials and Methods

A. Materials

The test material, SC-0224, which contains the active substance trimethylsulfonium carboxymethylaminomethylphosphonate (glyphosate-trimesium), was administered to the treated animals in this study. Further information on the test material is listed in the table below.

1. Test material:

Description:	SC-0224
Lot number:	8289-35-1
HLA sample number:	789030
Active ingredient(s):	Glyphosate-trimesium
CAS number:	81591-81-3
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	56.29 wt%
Formulation type:	NA; technical grade active substance
Appearance/colour:	Aqueous solution, colour not reported
Analysis date:	06/06/1983
Expiry date:	06/23/1986
Storage conditions:	Stored at room temperature in original glass shipping containers
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Lactating Holstein dairy cows were the test animals used in this study. Details are listed in the table below.

2. Test animals

Species:	Lactating dairy cattle; Bovine (*Bos Taurus)*
Gender:	Female
Breed:	Holstein
Source:	[redacted]
Age:	1 year 10 months to 8 years 0 months (1 to 5 lactations)
Weight at dosing, (Day-1):	Ranged from 484-737 kg at the end of acclimation (Study Day -1)
Milk production:	Cows selected for use in the study were producing 13.2 kg or more of milk per day. In the last week of acclimation before dosing began (Study Days -7 to -1) average daily milk production per animal ranged from 13.2 kg to 27.5 kg.

Number of animals:	18 cows selected out of a group of 22: (3 cows in each of 6 treatment groups (untreated control and 5 treated dose levels)
Animal Identification:	Uniquely numbered neck chain
Animal health / observations:	A staff veterinarian examined each animal prior to purchase, at the end of acclimation, and just before sacrifice. The initial examination included hematology and blood chemistry tests, and fecal evaluation for intestinal parasites. The animals were not used for the study until released/approved by the veterinarian. All animals used in the study were considered healthy at the pre-test evaluation.
Acclimation period:	14 days, except for two animals: Animal Nos. L00014 and L00016, which were acclimated 6 days and 8 days, respectively. However, the two animals that were acclimated less than 14 days appeared to be adjusted to the diet and management as they entered the study.
Diet:	The animals were fed alfalfa hay *ad libitum.* A concentrate diet, Purina Milk Generator #1286 (Lot No. 16722), was limit-fed during milking periods. There were no known contaminants in the dietary components that would interfere with this study.
Water:	Tap water was supplied *ad libitum* from automatic waterers.
Housing:	The animals were confined to individual stanchion stalls during the study. The animals were released for an exercise period of approximately 1 hour each day. Care was taken to avoid contamination of water or feed from one animal to another.

3. Environmental conditions

The environmental conditions at the test facility during the in-life phase of the study are summarised in the table below:

Temperature:	Ambient; ranged from 10-36 °C
Humidity:	Ranged from 57-100 %
Air change:	Not reported
Photoperiod:	Not reported

B. Study Design and Methods

The study included 6 treatment groups, an untreated control and 5 treated groups (T1, T2, T3, T4, and T5 with nominal dose levels of glyphosate-trimesium in dry feed at 0.5, 5, 50, 300, and 1,000 mg/kg, respectively). At the time this feeding study was designed, there were limited residue measurements in livestock feed commodities available to provide a basis for estimation of potential residue dietary burden for livestock. For this reason, five test concentrations were chosen for evaluation that were expected to span the range of residue dietary burden levels thought to be possible. Testing of a range of dose levels, including at least 3 dose levels which include exaggerated dose levels is consistent with current OECD guidance for conduct of livestock feeding studies.

The animals were assigned to treatment groups based upon a stratified randomisation procedure. Feed consumption was the primary criterion for selection, with milk production a secondary consideration. Three animals were assigned to each of the 6 treatment groups.

Empty gelatine capsules (i.e. containing no SC-0224 / glyphosate-trimesium) were administered to the animals in the untreated control group. Dosing of treated animals continued for 28 consecutive days

(Study Days 0 to 27). Upon completion of dosing, 2 animals from each treatment group were sacrificed and tissue samples were collected. The remaining one cow in each treatment group was retained for use in a withdrawal phase of the study to evaluate reduction in any residues in milk or tissues seven days after the end of the dosing period (i.e. by Study Day 35).

Further details on the dosing regimen, including target dose levels, are summarised in the table below.

1. Dosing regimen

Route:	Oral via gavage (gelatine capsule)
Vehicle:	Gelatine capsules administered orally with use of a balling gun
Timing / frequency per day:	Once per day at approximately 11:00 a.m.
Duration:	28 consecutive days
Treatment groups (dose levels):	6 treatment groups; untreated control and glyphosate-trimesium at 5 dose levels (dry feed basis):

Treatment Group	Nominal dose level in diet, dry feed basis (mg/kg)	
	Glyphosate-trimesium	Glyphosate equivalent [1]
Untreated control	0	0
T1	0.5	0.345
T2	5	3.45
T3	50	34.5
T4	300	207
T5	1000	690

1 Based on molecular weight of glyphosate-trimesium and glyphosate, multiplication of the glyphosate-trimesium dose level by a factor of 0.6896 results in the expression of the dose level in glyphosate equivalents.

Daily doses were based first upon average daily feed consumption data collected during a 5-day period in late acclimation. These doses were maintained unless the feed consumption in subsequent weeks increased by more than 25 % over the base-line value. Reductions in dose, in response to reduced feed consumption, were made only in T-5 (1,000 mg/kg) animals because feed consumption was severely depressed and the animals were showing other negative effects from the dosing (i.e. reduced milk production and body weight). Therefore, during the second week of the study, their doses were adjusted daily based upon the previous day's feed consumption.

The test material was diluted in deionised water to provide dosing solutions for the T-l and T-2 treatment groups. The test material was used undiluted for the T-3, T-4, and T-5 treatment groups. In all cases, the doses were prepared based upon the active substance (glyphosate-trimesium) content of the test material (56.29 %) and a specific gravity of l.25 g/mL for the undiluted SC-0224.

The test material solutions or test material (undiluted) were measured by volume to gelatine capsules. The capsules were sealed and administered by balling gun (control animals received empty capsules). The animals were dosed each day at approximately 11:00 a rn. Each week a sample capsule was prepared for each treatment level and placed in a glass container. The dosing solutions used were also sampled weekly. The samples were frozen and sent to the analytical phase facility. Analysis of the capsule contents and the dosing solution (undiluted technical test material for treatment groups T3–T5, and dilution in water for treatment groups T1 and T2) indicated that concentrations of test material in dosing solutions and capsules agreed with nominal (calculated) values, except in the case of the T1 capsule contents which at an average of 2.09 mg/mL was somewhat higher than the nominal value of 1.5 mg/mL.

Three consecutive starting dates for dosing were used to reduce the number of animals that would be sacrificed each day at the end of the test period. On a Tuesday, Wednesday, and Thursday, one animal from each treatment group entered the test period. Twenty-eight days later, the Tuesday and Thursday

animals were sacrificed. The Wednesday animals were removed from treatment (dosing) on Day 28, and 7 days later, they also were sacrificed.

Animals had access to roughage at all times except during the milking period when the concentrate diet was fed. The total daily concentrate (milking) ration was divided into a m. and p m. feedings. The daily feeding of concentrate diet was gradually brought to 7.0 kg during acclimation and was limit fed at this rate throughout the remainder of the study.

2. Daily observations and animal data collection

All animals were observed daily for general condition and behaviour. Feed consumption for all animals was determined daily for each animal individually based on weight of roughage and concentrate milking ration offered and refused. Body weight was recorded at the beginning and end of the acclimation period, and weekly thereafter. All cows were milked twice daily and the weight of the milk produced by individual animal at each milking was recorded.

3. Milk and tissue sample collection

Samples of milk and tissues were collected for residue analysis.
Milk samples were collected on Study Days -1, 1, 2, 4, 7, 14, 21, 28, 31, and 35. In addition, samples were collected daily from the T1 (0.5 mg/kg) animals following the dosing error which occurred on Day 21. (This daily collection continued through Day 27). Each sample was a composite of equal amounts (at least 600 mL) of evening and morning milk identified by the day of the morning milking. At each milking, the milk in the milking machine bucket was poured back and forth into an additional clean pail at least four times to ensure adequate mixing. The two subsamples (evening and morning) were composited and thoroughly mixed. Six individual 200 mL samples were drawn from the composite. All samples were stored frozen in polyethylene containers. Milk produced on days when samples were not scheduled for collection was discarded.

Two animals in each treatment were sacrificed within 24 hours after their 28th daily dose. The remaining animals were withdrawn from treatment and were sacrificed after a 7-day withdrawal (i.e. at Study Day 35). The animals were sacrificed using a stunning gun followed by exsanguination.

A macroscopic examination was conducted at sacrifice under the supervision of a staff veterinary pathologist.

Duplicate samples (approximately 500 g each) of liver, kidney, fat (a composite of equal amounts of omental and renal fat), skeletal muscle (a composite of triceps, gracilis, and longissimus dorsi muscle) were collected from each animal. All surgical instruments were cleaned and rinsed with an appropriate solvent after each sample had been collected. The remaining carcass and its contents were discarded. Each tissue sample was chilled and then thoroughly ground and mixed. The samples were then divided and frozen in polyethylene bags.

A summary of the sampling information is shown in the table below.

Table 6.4.2-18: Milk and tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Milk	Dosing phase: -1, 1, 2, 4, 7, 14, 21, 28; Withdrawal phase: 31, 35	Composite of equal amounts (≥ 600 mL) of milk each from the evening and morning milking (identified by the day of the morning milking).Six individual 200 mL subsamples were then collected from the composite milk sample from each animal at each sampling interval
Muscle[1]	End of dosing: Study Day 28 Withdrawal phase: Study day 35 (7 days after dosing ended)	~ 500 g [3]
Fat[2]		~ 500 g [3]
Liver		~ 500 g [3]
Kidney		~ 500 g [3]

1 Composite of equal amounts of triceps, gracilis, and longissimus dorsi muscle.
2 Composite of equal amounts of omental and renal fat.
3 Duplicate samples were collected; one shipped for analysis and one held as a reserve sample.

All samples were frozen at the In-life test facility and were shipped by air to the Analytical test facility (Stauffer Chemical Company, Richmond, California USA) frozen on dry ice in insulated containers. Samples were received at the analytical facility frozen, in good condition, with dry ice remaining in the shipping containers. Samples were stored frozen at the analytical phase facility at -29 °C (-20 °F).

4. Analytical phase

Analysis of milk and tissue samples was conducted at the Analytical Phase facility, Stauffer Chemical Company, Richmond, California, USA.

Residues of CMP and AMPA in milk and tissues were determined using analytical Method RRC 87-41. In general, with this method, sample analysis was carried out by extraction in an aqueous medium. The resulting extract was cleaned up by passage through a cation exchange resin column and the analytes were collected in separate fractions of the eluate. The extracts were cleaned, then derivatised with 9-fluorenylmethyl chloroformate; and the derivatives were quantitated with the use of a liquid chromatographic (HPLC) system with an anion column and a UV detector. For mi1k, during extraction, glacial acetic acid (6:94 (v/v) acetic acid:water) was used to precipitate the proteins. The supernatant was used for subsequent clean-up and analysis. The buffer system used for the HPLC ana1ysis consisted of 30 % methanol/20 % pH 3.3 buffer in deionised water. For tissues, extraction was carried out with water along with addition of an acidic modifier solution (KH_2PO_4, methanol, and HCl), followed by cation exchange resin clean-up and then derivatised with 9-fluorenylmethyl chloroformate. Analyses of kidneys, fat, muscle, and 1iver were conducted with a HPLC solvent system that consisted of 22 % methanol/20 % pH 3.3 buffer in de1on1zed water. Calculated background concentrations of the analytes were below the detection limit of the methods for most control samples; therefore, residue concentrations are listed as less than the detection limit. The lower limit of detection (LOD) of this method for both CMP and AMPA in muscle, fat, and kidney was 0.05 mg/kg. The lower limit of detection (LOD) of this method for both CMP and AMPA in milk and liver was 0.02 mg/kg and 0.2 mg/kg, respectively. The lowest fortification level for glyphosate in milk, kidney, liver, fat and muscle were 0.02, 0.5, 0.05, 0.2 and 0.2 mg/kg. For AMPA the lowest fortification level was the same, except for liver at 0.5 mg/kg. The fortification levels, LODs and residues of glyphosate is expressed as glyphosate and AMPA is expressed as AMPA.

Recovery results with samples of milk, kidney, liver, fat and muscle fortified with CMP and AMPA are summarised in the table below.

Table 6.4.2-19: Recovery results: CMP and AMPA in milk and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery [1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
CMP	Milk	0.02	99, 107	*103*	-	-	2
		0.05	83, 98, 88, 88, 100	*91.4*	*7.3*	*8.0*	5
		0.1	83, 91	*87*	-	-	2
		0.2	105	-	-	-	1
		0.5	107	-	-	-	1
		Overall	83–107	*95.4*	*9.2*	*9.6*	11
	Kidney	0.5	84	-	-	-	1
		1.0	87	-	-	-	1
		2.0	81	-	-	-	1
		Overall	81–87	*84.0*	*3.0*	*3.6*	3
	Liver	0.05	75	-	-	-	1
		1.0	67, 73	*70*	-	-	2
		Overall	67–75	*71.7*	*4.2*	*5.8*	3
	Fat	0.2	84	-	-	-	1
		0.5	84, 76, 65	*75.0*	*9.5*	*12.7*	3
		Overall	65–84	*77.3*	*9.0*	*11.6*	4
	Muscle	0.2	96	-	-	-	1
		0.5	73, 69	*71*	-	-	2
		Overall	69–96	*79.3*	*14.6*	*18.4*	3
AMPA	Milk	0.02	73, 89	*81.0*	-	-	2
		0.05	94, 121, 82, 84, 106	*97.4*	*16.3*	*16.7*	5
		0.1	98, 95	*96.5*	-	-	2
		0.2	95	-	-	-	1
		0.5	95	-	-	-	1
		Overall	73–121	*93.8*	*12.7*	*13.5*	11
	Kidney	0.5	91, 69	*80.0*	-	-	2
		1.0	64	-	-	-	1
		2.0	69	-	-	-	1
		Overall	64–91	*73.3*	*12.1*	*16.5*	4
	Liver	0.5	46	-	-	-	1
		1.0	66, 58	*62.0*	-	-	2
		Overall	46 - 66	*56.7*	*10.1*	*17.8*	3
	Fat	0.2	100				
		0.5	83, 84	*83.5*	-	-	2
		Overall	83–100	*89.0*	*9.5*	*10.7*	3
	Muscle	0.2	86	-	-	-	1
		0.5	83, 73	*78.0*	-	-	2
		Overall	73–86	*80.7*	*6.8*	*8.4*	3

Table 6.4.2-19: Recovery results: CMP and AMPA in milk and tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery [1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)

[1] Mean, standard deviation, and relative standard deviation values were not included in the study report. Values listed in this table were calculated based on reported recovery results and are shown in italics.

II. Results and Discussion

A. Dose levels

As indicated previously, glyphosate-trimesium was administered orally once per day in gelatine capsules for 28 consecutive days. There were 6 treatment groups, which included an untreated control group as well as 5 dose levels (T1–T5). The dose level of glyphosate-trimesium was based on concentration in the diet with nominal dose levels for T1, T2, T3, T4 and T5 at 0.5, 5, 50, 300, and 1000 mg/kg feed, respectively.

Initial daily doses for all animals were incorrectly based on milk production rather than feed consumption data. However, review of the data indicated this error had little impact on the target level of test material administered. Additionally, on Study Day 21 there was an error in dosing solution preparation for the T1 treatment group that resulted in the animals in that group receiving approximately two times the nominal level of 0.5 mg/kg in the diet on that day.

As discussed previously, the animals in the T5 treatment group initially demonstrated depressed feed consumption in response to treatment. The initial fixed dose for these animals was discontinued on Day 10 of the study and subsequent doses were based, each day, upon the previous day's feed consumption. Reducing the total amount of test material administered allowed the animals to recover from negative treatment effects and allowed better alignment of dose level with the target / nominal dose level, which was expressed on the basis of concentration in the diet.

A summary of the actual dose levels attained for the 5 treatment groups is shown in the table below. Results are presented for individual animals as well as an average for each treatment group. The average dose levels of glyphosate-trimesium expressed as concentration in the diet (mg/kg feed) for treatment groups T1, T2, T3, T4, and T5 were 0.51, 4.45, 43.0, 299, and 1383 mg/kg feed, respectively. If expressed as glyphosate equivalents, based on a conversion factor of 0.69 derived from the molecular weight of glyphosate and glyphosate-trimesium, the average dose levels of glyphosate expressed as concentration in the diet (mg/kg feed) for treatment groups T1, T2, T3, T4, and T5 were 0.41, 3.07, 29.70, 206.19, and 954 mg/kg feed, respectively. Additionally, the dose levels expressed on the basis of animal bodyweight were calculated with use of data provided in the study report. The actual dose levels expressed as concentration in the diet along with quantity of feed consumed and body weight were used to calculate dose levels on the basis of bodyweight (i.e. mg/kg bw/day). The average dose levels of glyphosate-trimesium expressed on the basis of bodyweight for treatment groups T1, T2, T3, T4, and T5 were 0.018, 0.173, 1.81, 10.7, and 36.6 mg/kg bw/day, respectively. If expressed as glyphosate equivalents, based on a conversion factor of 0.69 derived from the molecular weight of glyphosate and glyphosate-trimesium, the average dose levels of glyphosate expressed on the basis of bodyweight for treatment groups T1, T2, T3, T4, and T5 were 0.012, 0.119, 1.25, 7.39, and 25.25, respectively.

Table 6.4.2-20: Actual dose levels of glyphosate-trimesium administered to lactating dairy cows for 28 days expressed on basis of basis of concentration in total diet (dry feed) or body weight (bw)

Treatment Group / Nominal dose level	Animal number	Average daily dry feed consumption (kg)	Average body weight during dosing (kg)	Actual glyphosate-trimesium dose		
				mg/ animal / day [1]	mg /kg dry feed	mg/kg bw [2]
T1 / 0.5 mg/kg glyphosate-trimesium in dry feed (total diet)	L00001	15.55	509	7.1	0.46	0.014
	L00018	21.78	601	11.5	0.53	0.019
	L00024	24.93	620	13.3	0.54	0.022
	Average:	20.75	577	10.6	0.51	**0.018**
T2 / 5.0 mg/kg glyphosate-trimesium in dry feed (total diet)	L00011	21.28	764	91.5	4.30	0.120
	L00020	21.60	561	91.5	4.24	0.163
	L00023	30.75	628	148	4.81	0.235
	Average:	24.54	651	110	4.45	**0.173**
T3 / 50 mg/kg glyphosate-trimesium in dry feed (total diet)	L00015	22.00	555	932	42.3	1.68
	L00019	21.28	591	897	42.1	1.52
	L00026	29.93	601	1337	44.7	2.23
	Average:	24.40	582	1055	43.0	**1.81**
T4 / 300 mg/kg glyphosate-trimesium in dry feed (total diet)	L00006	19.80	517	5557	281	10.8
	L00028	20.78	561	6211	300	11.1
	L00022	20.95	643	6610	316	10.3
	Average:	20.51	574	6126	299	**10.7**
T5 / 1000 mg/kg glyphosate-trimesium in dry feed (total diet)	L00013	15.63	558	18131	1198	32.5
	L00016	14.05	583	18992	1495	32.6
	L00025	16.35	495	22015	1456	44.6
	Average:	15.34	545	19713	1383	**36.6**

1 Value was not included in the study report, but was calculated based on reported concentration of test material in feed (mg/kg dry feed) and daily dry feed consumption (kg).

2 Value was not included in the study report, but was calculated based on reported animal body weight along with calculated value for quantity of test material administered per day (mg / animal / day).

B. Animal health and daily observations

There were no apparent treatment or dose-related effects on the animal's general condition or behavior, body weight, feed consumption, or milk production at dose levels of up to 300 mg/kg in the diet (i.e. for Treatment Groups T1 – T4). However, in the T5 group (nominal dose of 1000 mg/kg in the diet) there were treatment-related effects including lethargy with reduced feed consumption, milk production, and body weight. When the level of glyphosate-trimesium was reduced from a fixed dose to a 1,000 mg/kg dose based on the reduced daily feed consumption (after Study Day 10), the affected animals gradually improved. By week 4 (the final week) of the dosing period, feed consumption and bodyweight of the T5 animals had nearly returned to pre-treatment values. In general, milk production improved, but had not returned to pretreatment levels by the end of the dosing period.

All animals were considered healthy at the pre-sacrifice examination.

C. Residue levels in milk and tissues

All samples in this study were analyzed within 69 days of collection. The study report includes storage stability data showing that CMP and AMPA are stable in milk, kidney, liver, fat, and muscle upon frozen storage for a minimum of 671 days. These data adequately cover the maximum periods of frozen storage encountered in this study.

In samples of milk and tissues collected from untreated control animals in this study, residues of CMP and AMPA were generally below the analytical method LOD. However, there were occasionally control samples in which residue results were at or slightly above the analytical LOD. CMP was observed at 0.05 mg/kg in some of the untreated control fat samples. The study report indicated that residues observed in untreated control samples should be considered as potential background levels of residue and should be used for comparison when evaluating residue results in samples from treated animals.

Residues of CMP in milk were below the LOD in samples collected from treatment groups 1–3. The table below provides a summary of residue results for CMP in milk for samples from the two groups with the highest dose levels, T4 and T5. In the T4 group, CMP residues in milk were typically below the LOD (<0.02 mg/kg). However, in a few samples CMP residues were observed at a 0.02 mg/kg, which is at the analytical method LOD. In the T5 group, CMP residues in milk ranged from <0.02 mg/kg to 0.04 mg/kg.

AMPA residues in milk in all treatment groups were below the LOD (<0.02 mg/kg). Therefore, results for this compound were not summarised in a table.

Table 6.4.2-21: Residues of CMP in milk

Treatment Group [1]	Animal No.	CMP residue (mg/kg) [2, 3, 4]									
		Study Day									
		1	**2**	**4**	**7**	**14**	**21**	**28**	***Avg. (2-28)*** [5, 6]	**31** [7]	**35** [7]
T4, Glyphosate - trimesium *299 mg/kg in feed; 10.7 mg/kg bw*	L00006	0.02	<0.02	<0.02	<0.02	*	*	<0.02	*0.02*	-	-
	L00028	<0.02	<0.02	<0.02	<0.02	*	*	<0.02	*<0.02*	-	-
	L00022	<0.02	<0.02	0.02	<0.02	*	*	<0.02	*0.02*	<0.02	<0.02
	Avg.:	*0.02*	*<0.02*	*0.02*	*<0.02*	-	-	*<0.02*	*0.02*	-	-
T5, Glyphosate-trimesium *1383 mg/kg in feed; 36.6 mg/kg bw*	L00013	<0.02	<0.02	0.03	0.03	0.02	<0.02	0.03	*0.025*	-	-
	L00016	<0.02	0.02	0.03	0.04	0.02	<0.02	<0.02	*0.025*	-	-
	L00025	<0.02	0.02	0.02	0.03	0.02	0.02	<0.02	*0.022*	<0.02	<0.02
	Avg.:	*<0.02*	*0.02*	*0.03*	*0.03*	*0.02*	*0.02*	*0.02*	*0.024*	-	-

1 CMP residues in milk in the lower dose levels (Treatment Groups T1- T3) were below the LOD (0.02 mg/kg). To simplify reporting, results from only the two highest dose levels (T4 and T5) are presented in the table above.
2 CMP residue values were taken from table 4 of the study report. Please note that there are some inconsistencies with the values provided in Appendix E of the study report.
LOD (limit of detection): 0.02 mg/kg
3 Replicate analysis was conducted on some of the study samples. Where replicate analytical results were provided, the value listed in the table above is an average of the replicate analytical values.
4 Residue values from Study Days 2 – 28 were averaged since residue levels during Day 1 may not have reached a plateau level.
5 **-** = sample value not taken or nor applicable; * = sample not analysed
6 For purposes of calculating an average, residue values of <LOD of 0.02 mg/kg were assigned a value of 0.02 mg/kg if being averaged with a value equal to greater than the LOD of 0.02 mg/kg.
7 The last day of test material dosing was Study Day 28. Residue values reported for Study Days 31 and 35 were during the withdrawal / depuration phase of the study with Study Days 31 and 35 being 3 and 7 days after last dose administration, respectively (see Appendix E in study report).

The table below provides a summary of CMP residues in tissues in treatment groups T1–T5.

Residues of CMP in tissues (kidney, liver, fat, and muscle) remained below the LOD in the two lowest dose level treatment groups, T1 and T2. Among the four tissues, residues of CMP were highest in kidney in treatment groups T3–T5. Residues of CMP in kidney increased in proportion to the increased dose level of glyphosate-trimesium. The average level of CMP in kidneys in samples collected at the end of the

28-day doing period in treatments groups T3, T4, and T5 was 0.385 mg/kg, 2.2 mg/kg, and 5.85 mg/kg, respectively. CMP Residues in fat were less responsive to increased dosage level and averaged 0.055 mg/kg, 0.06 mg/kg, and 0.08 mg/kg in treatment groups T3, T4, and T5, respectively. Residue of CMP in liver and muscle remained below the LOD in treatment groups T3 and T4, but were above the LOD in the highest dose treatment group, T5. The average level of CMP residue in liver and muscle in the T5 treatment group at the end of the 28-day doing period was 0.365 mg/kg and 0.08 mg/kg, respectively. At 7 days after the end of the dosing period, Study Day 35, residues of CMP in kidney had decreased significantly, although they were still above the LOD in treatment groups T4 and T5. Residues of CMP in fat did not appear to change significantly during the 7-day withdrawal period, remaining above the LOD in treatment groups T4 and T5. Residues of CMP in liver and muscle were below the LOD in all treatment groups following the 7-day withdrawal period.

Table 6.4.2-22: : Residues of CMP in tissues

Treatment Group[1]	Animal No.	Study Day[2]	CMP residue found[3, 4, 5, 6] (mg/kg)			
			Kidney	Liver	Fat	Muscle
T1, Glyphosate-trimesium *0.51 mg/kg in feed; 0.018 mg/kg bw*	L00001	28	<0.05	*	*	*
	L00018	28	*	<0.2	<0.05	<0.05
	Study Day 28, T1 treatment group average		-	-	-	-
	L00024	35	<0.05	<0.2	<0.05	<0.05
T2, Glyphosate-trimesium *4.45 mg/kg in feed; 0.173 mg/kg bw*	L00011	28	*	<0.2	<0.05	<0.05
	L00020	28	<0.05	*	<0.05	*
	Study Day 28, T2 treatment group average		-	-	*<0.05*	-
	L00023	35	<0.05	<0.2	*	*
T3, Glyphosate-trimesium *43.0 mg/kg in feed; 1.81 mg/kg bw*	L00015	28	0.44	<0.2	<0.05	<0.05
	L00019	28	0.33	*	0.06	<0.05
	Study Day 28, T3 treatment group average		*0.385*	-	*0.055*	*<0.05*
	L00026	35	<0.05	<0.2	<0.05	<0.05
T4, Glyphosate-trimesium *299 mg/kg in feed; 10.7 mg/kg bw*	L00006	28	1.8	<0.2	0.06	<0.05
	L00028	28	2.6	<0.2	0.06	<0.05
	Study Day 28, T4 treatment group average		*2.2*	*<0.2*	*0.06*	*<0.05*
	L00022	35	0.12	<0.2	0.06	<0.05
T5, Glyphosate-trimesium *1383 mg/kg in feed; 36.6 mg/kg bw*	L00013	28	7.6	0.51	0.10	0.08
	L00016	28	4.1	0.22	0.06	0.08
	Study Day 28, T5 treatment group average		*5.85*	*0.365*	*0.08*	*0.08*
	L00025	35	0.18	<0.2	0.08	<0.05

1 The nominal dose of glyphosate-trimesium expressed as concentration in feed for Treatments Groups T1, T2, T3, T4, and T5 were 0.5 mg/kg, 5.0 mg/kg, 50 mg/kg, 300 mg/kg, and 1000 mg/kg, respectively. The measured dose levels achieved in the study expressed as concentration in feed (mg/kg feed) as well as per unit of animal bodyweight (mg / kg bw /day) are listed in the Table above for each Treatment Group. The corresponding dose level expressed as glyphosate equivalents in feed or per unit of animal bodyweight can be obtained by adjusting the indicated levels of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate-trimesium of 169.1 and 245.2, respectively.

2 Study Day 28 is at the end of the 28-day dosing period; Study Day 35 is at a period of 7 days after the end of dosing (i.e. 7 day withdrawal or depuration period).

3 * = sample not analysed; **-** = sample not taken or not applicable

4 LOD (limit of detection) for CMP was 0.2 mg/kg in liver and was 0.05 mg/kg in kidney, fat, and muscle.

5 For purposes of calculating averages, residue values of < LOD were assigned a value of LOD if being averaged with a value equal to greater than the LOD.

6 Replicate analysis was conducted on some of the study samples. Where replicate analytical results were available, the value listed in the table above is an average of the replicate analytical values.

The table below provides a summary of AMPA residues in tissues in treatment groups T1–T5.

Residues of AMPA remained below the LOD in liver, fat, and muscle in all dose levels evaluated (treatment groups T1–T5). In kidney, AMPA residues were below the LOD in the two lowest dose levels, treatment groups T1 and T2. However, the average level of AMPA found in kidney at the end of the dosing period in treatment groups T3, T4, and T5 was 0.07 mg/kg, 0.525 mg/kg, and 1.65 mg/kg, respectively. The increase in the level of AMPA residue in kidney was roughly proportional to increasing dose level of glyphosate-trimesium. After a 3-day withdrawal period, residues of AMPA in kidney were below the LOD in treatment groups T3 and T4, and had decreased to 0.24 mg/kg in the T5 Treatment Group.

Table 6.4.2-23: : Residues of AMPA in tissues

Treatment Group[1]	Animal No.	Study Day[2]	AMPA residue found[3, 4, 5, 6] (mg/kg)			
			Kidney	Liver	Fat	Muscle
T1, Glyphosate-trimesium *0.51 mg/kg in feed; 0.018 mg/kg bw*	L00001	28	<0.05	*	*	*
	L00018	28	*	<0.2	<0.05	<0.05
	Study Day 28, T1 treatment group average		-	-	-	-
	L00024	35	<0.05	<0.2	<0.05	<0.05
T2, Glyphosate-trimesium *4.45 mg/kg in feed; 0.173 mg/kg bw*	L00011	28	*	<0.2	<0.05	<0.05
	L00020	28	<0.05	*	<0.05	*
	Study Day 28, T2 treatment group average		-	-	*<0.05*	-
	L00023	35	<0.05	<0.2	*	*
T3, Glyphosate-trimesium *43.0 mg/kg in feed; 1.81 mg/kg bw*	L00015	28	0.06	<0.2	<0.05	<0.05
	L00019	28	0.08	*	<0.05	<0.05
	Study Day 28, T3 treatment group average		*0.07*	-	*< 0.05*	*<0.05*
	L00026	35	<0.05	<0.2	<0.05	<0.05
T4, Glyphosate-trimesium *299 mg/kg in feed; 10.7 mg/kg bw*	L00006	28	0.47	<0.2	<0.05	<0.05
	L00028	28	0.58	<0.2	<0.05	<0.05
	Study Day 28, T4 treatment group average		*0.525*	*<0.2*	*<0.05*	*<0.05*
	L00022	35	<0.05	<0.2	<0.05	<0.05
T5, Glyphosate-trimesium *1383 mg/kg in feed; 36.6 mg/kg bw*	L00013	28	1.7	<0.2	<0.05	<0.05
	L00016	28	1.6	<0.2	<0.05	<0.05
	Study Day 28, T5 treatment group average		*1.65*	*<0.2*	*<0.05*	*<0.05*
	L00025	35	0.24	<0.2	<0.05	<0.05

1 The nominal dose of glyphosate-trimesium expressed as concentration in feed for Treatments Groups T1, T2, T3, T4, and T5 were 0.5 mg/kg, 5.0 mg/kg, 50 mg/kg, 300 mg/kg, and 1000 mg/kg, respectively. The measured dose levels achieved in the study expressed as concentration in feed (mg/kg feed) as well as per unit of animal bodyweight (mg / kg bw /day) are listed in the Table above for each Treatment Group. The corresponding dose level expressed as glyphosate equivalents in feed or per unit of animal bodyweight can be obtained by adjusting the indicated levels of glyphosate-trimesium by a factor of 0.69 based on molecular weights for glyphosate and glyphosate trimesium of 169.1 and 245.2, respectively.

2 Study Day 28 is at the end of the 28-day dosing period; Study Day 35 is at a period of 7 days after the end of dosing (i.e. 7 day withdrawal or depuration period).

3 * = sample not analysed; **-** = sample not taken or not applicable

4 LOD (limit of detection) for AMPA was 0.2 mg/kg in liver and was 0.05 mg/kg in kidney, fat, and muscle.

5 For purposes of calculating averages, residue values of < LOD were assigned a value of LOD if being averaged with a value equal to greater than the LOD.

6 Replicate analysis was conducted on some of the study samples. Where replicate analytical results were available, the value listed in the table above is an average of the replicate analytical values.

III. Conclusion

Glyphosate-trimesium was orally administered to lactating dairy cattle for 28 consecutive days at nominal dose levels of 0.5, 5, 50, 300, and 1000 mg/kg in the diet (Treatment Groups T1–T5). The actual average dose levels of glyphosate-trimesium attained during the study, expressed as concentration in the diet (mg/kg feed), for treatment groups T1, T2, T3, T4, and T5 were 0.51, 4.45, 43.0, 299, and 1383 mg/kg feed, respectively. The average dose levels of glyphosate-trimesium expressed on the basis of bodyweight for treatment groups T1, T2, T3, T4, and T5 were 0.018, 0.173, 1.81, 10.7, and 36.6 mg/kg bw/day, respectively.

No treatment-related effects on feed consumption, body weight or milk production were noted at feeding levels up to 300 mg/kg (T4). At the 1000 mg/kg (T5) nominal dosage level, feed consumption, body weight and milk production were initially adversely impacted, but returned to near pretreatment levels when dosage levels were adjusted to 1000 mg/kg in feed based on daily feed consumption, which was reduced from pre-treatment levels.

In general, the study results indicate a direct relationship between the dose level of glyphosate-trimesium and the concentrations of CMP and AMPA residues in the animal's milk and tissues. The highest residue concentrations were present in milk and tissues from the highest dosage levels.

In milk, CMP residues in the highest dose level, T5, ranged from <0.02 mg/kg to 0.04 mg/kg during the dosing period. In the T4 dose level CMP residues in milk were typically below the LOD (<0.02 mg/kg), but were observed at 0.02 mg/kg in a few samples. In the lower dose levels (T1 – T3), CMP residues in milk remained below the LOD.

Residues of CMP in tissues (kidney, liver, fat, and muscle) remained below the LOD in the two lowest dose levels, T1 and T2. Among the four tissues, residues of CMP were highest in kidney in treatment groups T3–T5. The average level of CMP in kidney at the end of the doing period in treatments groups T3, T4, and T5 was 0.385 mg/kg, 2.2 mg/kg, and 5.85 mg/kg, respectively. CMP residues in fat were less responsive to increased dosage level and averaged 0.055 mg/kg, 0.06 mg/kg, and 0.08 mg/kg in treatment groups T3, T4, and T5, respectively. Residue of CMP in liver and muscle remained below the LOD, except in the highest dose group (T5) where average residue levels were 0.365 mg/kg and 0.08 mg/kg, respectively.

AMPA residues were below the LOD in milk, liver, fat, and muscle at the end of the dosing period in all dose levels evaluated (T1-T5). In kidney, AMPA residues were below the LOD in the two lowest dose levels, (T1 and T2). However, the average level of AMPA found in kidney at the end of the dosing period in treatment groups T3, T4, and T5 was 0.07 mg/kg, 0.525 mg/kg, and 1.65 mg/kg, respectively.

Residues of CMP and AMPA, when found in milk and tissues at the end of the dosing period decreased significantly during the 7-day withdrawal period when dosing was discontinued, indicating that these residues do not accumulate irreversibly under the conditions tested.

3. Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:
The study assessing the magnitude of residues of glyphosate-trimesium in ruminant (cattle) milk and tissues (fat, muscle, liver and kidney) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of residues of glyphosate-trimesium that may transfer from the livestock diet to milk and edible livestock tissues. The study was not strictly performed under GLP. However, the study is considered to be scientifically valid and largely complies with the OECD Guideline for the Testing of Chemicals, 505, Residues in Livestock) with a few deviations.

It is unclear if dose expressed on basis of feed consumption was based on dry weight of the feed commodities, but data available in the study report allowed calculation and expression of the dose on a basis of mg/kg bodyweight. Additionally, the diet was composed of low moisture feed items and the potential impact of moisture level would likely be low.

The tissue samples at the end of the dosing period were collected from 2 animals rather than from

3 animals. Fat samples did not include subcutaneous fat in addition to omental and renal fat. Meat samples were composed of triceps, gracilis, and longissimus dorsi muscle instead of loin, flank or hind-leg. For the depuration phase only 1 interval instead of 3 intervals were analysed. Nevertheless, decline of the residues in milk and tissues in the highest dose groups where residues were found can clearly be seen.

Residue concentrations are listed as less than the detection limit and not less than the quantification limit, as calculated background concentrations of the analytes were below the detection limit of the methods for most control samples. The lower limit of detection (LOD) of this method for both CMP and AMPA in muscle, fat, and kidney was 0.05 mg/kg. The lower limit of detection (LOD) of this method for both CMP and AMPA in milk and liver was 0.02 mg/kg and 0.2 mg/kg, respectively. The lowest fortification level for glyphosate in milk, kidney, liver, fat and muscle were 0.02, 0.5, 0.05, 0.2 and 0.2 mg/kg. For AMPA the lowest fortification level was the same, except for liver at 0.5 mg/kg.

The study is considered valid as these deficits are not expected to significantly impact the quality or reliability of the study.

Assessment and conclusion by RMS:

Relevant published articles from Literature Search Report

1. Information on the study

Data point	CA 6.4.2/004
Report author	Shelver, W.L. *et al*
Report year	2018
Report title	Distribution of chemical residues among fat, skim, curd, whey, and protein fractions in fortified, pasteurised milk
Document No.	DOI 10.1021/acsomega.8b00762 ISSN 2470-1343
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

The distribution of 12 environmental contaminants or metabolites with diverse polarities (2,2′,4,4′,5-pentabromodi-phenyl ether; bisphenol A; estrone; glyphosate; β-hexabromocyclo-dodecane; imidacloprid; 2,3′,4,4′,5-pentachlorobiphenyl; 3′-methylsulfone 2,2′,4,5,5′-pentachlorobiphenyl; 1,2,7,8-tetrachlorodibenzo-*p*-dioxin; 2-hydroxy-1,3,7,8-tetrachlorodibenzo-*p*-dioxin; tetrabromo-bisphenol A; and triclocarban) among skim milk, fat, curd, whey, whey retentate, and whey permeate was characterised. Analysis of these compounds along with 15 drugs previously studied provided a robust linear model predicting the distribution between skim and fat and the chemical's lipophilicity (log P, r^2 = 0.71; log D, r^2 = 0.79). Similarly, distribution between curd and whey was correlated with lipophilicity (log P, r^2 = 0.63; log D, r^2 = 0.73). Phenolic compounds had less predictable distribution patterns based on their lipophilicities. Within the whey fraction, chemicals with greater lipophilicity are associated with whey proteins more than hydrophilic chemicals. The resultant model could help predict the potential distribution of chemical contaminants among milk products in cow milk, if present.

Materials and Methods

Selection of drugs and concentrations
Chemicals selected for study had to be potential environmental contaminants, encompass a wide range of lipophilicities, and be available with radiolabel (^{3}H or ^{14}C) incorporation. The chemicals selected had a log *P* range of − 3.3 to 7.3. Chemical structures, site of radiolabel, specific activity (SA), and physio-chemical properties are provided in **Table 1**.

To detect potential concentration-dependent distribution, chemical concentrations spanning 3 orders of magnitude (*i.e.*, 20 − 2000 nM) were generally used. The lowest concentration (usually 20 nM) was typically relevant to possible contamination scenarios with sufficient activity to allow radiochemical detection. Higher concentrations were used to determine whether concentration influenced overall xenobiotic distribution. In some instances, concentrations were adjusted because of limited solubility or if the SA of the radiolabeled compound was inadequate for the sensitivity of the analysis (**Table 1**). As a result of adding unlabeled chemical (typically 9:1 parts) for the highest dose, SA was lowered, relative to low concentration.

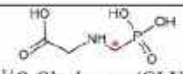
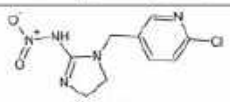
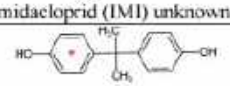
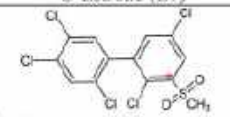
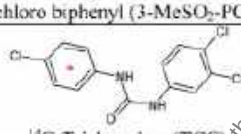
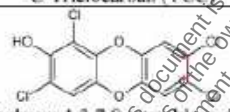
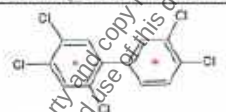
Table 1: Drug Structures and Physicochemical Properties.

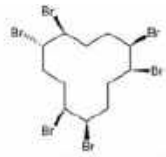
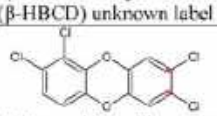
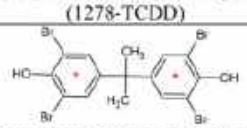
[a] Compound radioactively labeled with a directed label and specified on the structure with a red asterisk. An asterisk within a ring indicates a uniform label on the ring. Exceptions: IMI and *β*-HBCD carbon labels are unknown.

[b] SAs were adjusted depending on dose, as indicated. Values in parentheses are nominal concentrations for initial fortification.

[c] Average log *P* calculated from literature log *P* values accessed from www.chemspider.com, www.drugbank.ca, www.ebi.ac.uk/chembl/, and pubchem.ncbi nlm.nih.gov/ on 7/14/2017 using the predicted and experimental values were available.

[d] Values for log *D* at pH 6.8 were calculated using log *P* values from above sources and pK_a's from www.drugbank.ca, www.ebi.ac.uk/chembl/, www.druginfosys.com, pubchem.ncbi.nlm.nih.gov/, Johansson and Anlér [11] accessed on 7/14/2017.

Compound[a]	Class/ Use	M.W. S.A. (nCi/nmol)[b]	log P[c]	pKa[d]	log D[d]
 ¹⁴C-Glyphosate (GLY)	Herbicide/ pesticide	169.07 g/mol 50 (20 nM/ 200 nM) 5.0 (2000 nM)	-3.26 ± 1.52	5.89 0.40	-4.24 ± 1.49
 ¹⁴C-Imidacloprid (IMI) unknown label	Insecticide/ pesticide	255.66 g/mol 25.3 (20 nM/ 200 nM) 2.5 (2000 nM)	0.59 ± 0.59	5.28	-0.38 ± 0.59
 ¹⁴C-Bisphenol A (BPA)	Plasticizer	228.28 g/mol 53.5 (20nM/ 200nM) 6.05 (2000 nM)	3.60 ± 0.27		3.60 ± 0.27
 ¹⁴C-Estrone (E1)	Hormone	270.37 g/mol 51 (20nM/ 200nM) 5.7 (2000nM)	3.62 ± 0.45		3.62 ± 0.45
 ¹⁴C-3'-methylsulfone-2,2',4,5,5'-pentachloro biphenyl (3-MeSO$_2$-PCB-101)	PCB Metabolite	404.52 g/mol 53 (20/100/500nM)	4.62		4.62
 ¹⁴C-Triclocarban (TCC)	Antibacterial & disinfectant	315.58 g/mol 30 (20 nM/ 200 nM) 3.0 (2000 nM)	5.39 ± 0.45		5.39 ± 0.45
 ¹⁴C-2-hydroxy-1,3,7,8-tetrachloro dibenzo-*p*-dioxin (2-OH-TCDD)	TCDD Metabolite	337.97 g/mol 64.6 (20 nM/ 100 nM) 12.8 (500 nM)	6.15 ± 0.32		6.15 ± 0.32
 ¹⁴C-2,3',4,4',5-pentachlorobiphenyl (PCB-118)	Coolants/ plasticizers/ hydraulic fluids/ pesticides/ flame retardant	326.43 g/mol 10.3 (50 nM/ 200 nM) 2.5 (2000 nM)	6.78 ± 0.35		6.78 ± 0.35

 ^{14}C-β-hexachlorocyclododecane (β-HBCD) unknown label	Flame Retardant	641.69 g/mol 2 (200/500/2000 nM)	7.22 ± 0.65		7.22 ± 0.65
 ^{14}C-1,2,7,8-tetrachloro dibenzo-*p*-dioxin (1278-TCDD)	Industrial and incineration byproduct	321.97 g/mol 67.8 (20 nM/ 200 nM) 6.8 (2000 nM)	6.22 ± 0.72		6.22 ± 0.72
 ^{14}C-Tetrabromo Bisphenol A (TBBPA)	Flame Retardant	543.87 g/mol 25 (20 nM/ 200 nM) 3.7 (2000 nM)	6.69 ± 0.58		6.69 ± 0.58
 ^{14}C-2,4',4,5,5'-pentabromo diphenyl ether (BDE-99)	Flame Retardant	564.69 g/mol 49 (20 nM) 8.76 (200 nM) 0.98 (2000 nM)	7.31 ± 0.62		7.31 ± 0.62

Chemicals, supplies, and equipments

Raw (unpasteurised, nonhomogenised) cow milk was obtained from the bulk milk tank located at the North Dakota State University (Fargo, ND) Dairy farm within 48 h of milking. Non-radiolabeled chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO), U.S. Pharmacopeia (Rockville, MD), or other common vendors. Radiolabeled E1, GLY, PCB-118, and *β*-HBCD were procured through American Radio-labeled Chemicals, Inc. (ARC, St. Louis, MO). A mixture of the *β*- and *γ*-diastereoisomers of [^{14}C]-HBCD was identified in the ARC product. Flash chromatography on a silica gel column eluted with hexane containing increasing amounts of methylene chloride (0 − 50 %) was used to isolate [^{14}C]-*β*-HBCD. [^{14}C]-BPA and [^{14}C]-TCC were purchased from Moravek Inc. (Brea, CA). [^{14}C]-IMI was a gift from Bayer Crop Science (Research Triangle Park, NC). [UL-7,8-ring^{14}C]-1278-TCDD was purchased from ChemSyn Science Laboratories (Lenexa, KS). [^{14}C]-2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) was synthesised using published methods [23]. 2-OH-1378-TCDD was prepared in-house from [UL-7,8-ring^{14}C]-1278-TCDD by *in vitro* oxidation with human CYP1A1R Baculosomes (Cypex Ltd., Dundee, UK) and a glucose-6-phosphate dehydrogenase regenerating system according to manufacturer's instructions. [^{14}C]-2,2-bis(4-hydroxy-3,5-dibromophenyl)propane (TBBPA) was synthesised by brominating bis[^{14}C]-phenol A with 4.2 equivalents of bromine in 1:1 methanol/water; bis[^{14}C]-phenol A was prepared in-house from [UL-^{14}C]-phenol (2.0 mCi, 25 mCi/mmol) and acetone according to a published method [24]. 3′-[^{14}C]-MeSO$_2$-PCB-101 was synthesised de novo by Cadogan coupling as described in Haraguchi *et al.* [25] using sodium [^{14}C]-methyl thiolate for label introduction.

Silica gel plates were purchased from Analtech (Newark, DE). Scintillation cocktails were purchased from MP Biochemicals, LLC, (Ecolite; Solon, OH) or PerkinElmer (Waltham, MA; Carbosorb, and Permafluor). Amicon Ultra-15 centrifugal filters were purchased from Millipore (Billerica, MA). An Allegra X-14R centrifuge was obtained from Beckman-Coulter (Brea, CA). Liquid milk product fractions were mixed with scintillation fluid and assayed using a Tri-Carb 1900 liquid scintillation counter (LSC, Packard, Meriden, CT). Solid milk product samples were combusted using a Packard model 307 tissue oxidizer (Meriden, CT), trapped into Carbosorb, diluted with Permafluor, and then assayed by LSC. Sample purity was assessed by TLC and radioassay using a Bioscan AR-2000 Imaging Scanner for TLC (Washington, DC).

Determination of chemical purity and confirmation of test article stability

TLC analyses were used to assess chemical purities before and after the experiments, although for GLY, high-performance liquid chromatography instead of TLC was employed. Initial analyses were used to evaluate dose purity, whereas post-incubation analyses were used to evaluate whether chemical

degradation occurred during milk processing. TLC conditions and results are included in Table S3. GLY radiochemical purity (98.0 ± 0.4 %, n = 4) was determined based on Nagatomi *et al.* [26] using a Waters 2695 HPLC, a radiometric detector (Packard LFA 515TR, PerkinElmer, Waltham, MA), and a Dionex IonPac AS 12 column (4 × 200 mm, 9 µ m, Dionex Company, Sunnyvale, CA). The mobile phase was isocratic 0.2 % aqueous formic acid/acetonitrile (5/95, v/v), and the flow rate was 1 mL/min.

Milk processing and radiochemical analysis

The milk processing experiments consisted of three sequential phases. Specific details pertaining to preparation of phases are reported in Hakk *et al.* [7] and Shappell *et al.* [8]. Briefly, 12 tubes of raw milk (50 mL) were pasteurised at 63°C for 30 min. Triplicate tubes were fortified with each level of radiolabeled chemicals using three working solutions or with the appropriate solvent for blank milk, as described in **Table 2**. In phase 1, the fortified, pasteurised, whole milk samples were separated into milk fat and skim milk by centrifugation after equilibration; the partitioning of chemical between these phases was then determined by radiochemical detection methods. In phase 2, the skim milk originating from phase 1 was partitioned into curd and whey (enzymatically with rennet) and the distribution of the target chemical between these phases determined by radiochemical detection. In phase 3, the residual whey (15 mL) from phase 2 was separated into a protein-enriched fraction (~ 10 kD), retentate (~ 5 mL) and permeate (~ 10 mL) fractions using ultracentrifuge filters. To determine if degradation occurred during processing, milk fat, curd, and whey from the highest dose concentration were extracted and analyzed by TLC side by side with radiolabeled standards with the exception of GLY because no satisfactory TLC method was found. The main difference in the current study compared to the cited research [7-9] was that here the radiolabeled compounds were fortified only once into whole milk and not anew at the beginning of each phase (**Figure 5**), resulting in lower initial chemical concentrations in skim and whey fractions.

Table 2: Compound Associated with Casein or Whey Protein (nmol/mg Protein and Percent Association Based on Whole Milk).

[a] SA of some compounds required different doses, as indicated by bold text. Each fortified level contains three replicates.

[b] These data were derived from phase 2 data and have whey associated drug subtracted, using "0 % moisture curd" as described in text.

[c] These data were derived from phase 3 data as described in the text.

[d] Less than limit of quantitation (<LOQ). LOQ for PCB-118 is 1.92 nmol/L and for β-HBCD was 9.87 nmol/L.

[f] Inconsistent with other doses. No explanation.

compound	nominal conc. of whole milk[a] (actual) nM	nmol/mg casein protein[b]	nmol/mg whey protein[c]	conc. in casein/conc. in whey protein	mean % casein association based on whole milk[d]	mean % whey association based on whole milk[d]
GLY	20 (22)	0.08	0.15	0.53	7.92	3.68
	200 (217)	0.71	1.40	0.51		
	2000 (2059)	6.52	14.09	0.46		
IMI	20 (20)	0.21	0.11	1.91	15.43	3.19
	200 (201)	2.20	1.13	1.95		
	2000 (2066)	22.33	12.29	1.82		
BPA	20 (22)	0.44	0.23	1.91	45.76	6.68
	200 (216)	4.54	2.25	2.02		
	2000 (1992)	42.43	20.88	2.03		
E1	20 (20)	0.21	0.10	2.10	17.88	2.31
	200 (200)	1.66	0.96	1.73		
	2000 (1796)	15.89	8.70	1.83		
3-MeSO$_2$-PCB-101	20 (24)	0.06	0.04	1.50	4.25	0.99
	100 (79)	0.18	0.12	1.50		
	500(628)	1.60	1.08	1.48		
TCC	20 (19)	0.05	0.10	0.50	5.92	3.62
	200 (193)	0.54	0.95	0.57		
	2000 (1938)	5.68	9.72	0.58		
2-OH-1378-TCDD	20 (22)	0.16	0.64	0.25	17.39	16.79
	100 (107)	**0.77**	**3.26**	0.24		
	500 (556)	**4.71**	**16.16**	0.29		
PCB-118	**50 (60)**	**0.07**	<LOQ[e]		3.42	0.70
	200 (221)	0.35	0.24	1.46		
	2000 (2203)	2.90	2.37	1.22		
β-HBCD	**200 (229)**	0.38	<LOQ[e]		2.95	0.59
	500(656)	1.09	0.59	[illegible]		
	2000 (2067)	3.21	2.11	[illegible]		
1278-TCDD	20 (27)	0.11	0.06	1.83	4.14	1.29
	200 (184)	0.46	0.34	1.35		
	2000 (1784)	5.07	3.58	1.42		
TBBPA	20	0.27	0.08	0.34	18.01	22.96
	200 (234)	3.53	[illegible]	0.38		
	2000 (1815)	21.63	[illegible]	0.28		
BDE-99	20 (20)	0.12	0.03	4.0	6.66	1.20
	200 (178)	1.87	0.68	2.76		
	2000 (1690)	14.93	3.35	5.47		

Calculation of chemical associated with casein and whey Protein

The percentage of chemical associated with whey proteins was calculated according to Shappell *et al.* [8]. Briefly, the amount of free chemical measured in permeate (calculated by concentration and volume) was subtracted from the total amount of chemical present in retentate. The difference was assumed to be the amount of chemical associated with whey protein. Residual radioactivity on ultrafilters (measured by combustion analysis) was considered nonspecific binding and was subtracted from the fortified whey results; however, radioactivity present in filter washes was included with retentate radioactivity. Averaged Kjeldahl protein concentrations in curd from Shappell *et al.* [8] and Lupton *et al.* [9] and the resultant 0 % moisture curd radioactivity (see below) along with its SA were used to calculate nanomole per milligram casein protein association. Similarly, averaged Kjeldahl protein concentration in retentate from Shappell *et al.* [8] and Lupton *et al.* [9] and the protein associated radioactivity and its SA in retentate was used to calculate nanomole per milligram whey protein association.

Statistical analyses

Standard statistical methods were used to calculate means and variability and make inferences with respect to the significance of differences between means. Linear regression was used to assess dose dependence of the observed drug distribution log ratio of [chemical] milk fat /[chemical] skim milk or 0 % moisture [chemical] curd /[chemical] whey. Dose dependency was based on instances when the slope

differed ($P < 0.05$) from zero. Because curd is 70 % moisture and contains a small quantity of entrained whey, a 0 % moisture curd radioactivity value was calculated by subtracting entrained whey-associated radioactivity (calculated based on the percent moisture) from curd. The value representing entrained whey was added back to the whey fraction.
Coefficient of variation with respect to measured partition values across doses was typically much less than 10 %, whereas literature values for log *P* for a given chemical could sometimes differ by an order of magnitude or greater. Therefore, distribution data were modeled using mean log P values ± SD for each chemical. Mean log P values were calculated from predicted and measured entries included in Chemspider, DrugBank, ChemBL, and Pubchem databases. For 3′-$MeSO_2$-PCB-101, the log *P* value was derived from using conversion of chlorocyclohexatriene into *p*-chlorophenyl methyl sulfone as a model, which has log differences of 1.76. By using PCB-101 log *P* of 6.38 and subtracting 1.76, the log *P* of 3′-$MeSO_2$-PCB-101was derived as 4.62. Log *D* values were calculated as described by Scherrer and Howard [16] using a pH of 6.8 (reflecting the pH of milk); to obtain a theoretical range of log *D* values for each compound, the range of log P values derived from the above sources was used in conjunction with the range of pK_a values obtained from the same sources; log *D* values were averaged and SDs calculated. Relationships between the log distribution ratios and lipophilicity (log *D* and log *P*) were performed using linear function and included the 99 % CI and prediction interval by GraphPad Prism Version 7.03 (Graph-Pad Software, La Jolla, CA).

Results and Discussion

Chemical distribution from whole milk into milk fat and skim milk
Milk partitioning into lipid was highly reproducible, with typical coefficient of variance (CV) values of ≤ 5 %; exception was GLY with CV up to 19 % (Tables S5−S16). The high CV of GLY was due to its low partitioning into milk fat (Table S5). Similarly, CV of partitioning into skim milk was ≤ 5 %; exceptions were BDE, *β*-HBCD, 3′-$MeSO_2$-PCB-101, PCB_{28} and TCC because of low amounts in the skim milk. Recoveries (sum of total radioactivity in skim milk and milk fat) were > 90 %, ranging from ~ 91 % (for chemicals with log $D \geq 6.7$) up to 100 % for GLY (Figure 1 and Tables S5−S16). Distribution of chemical residues was not dose-dependent over the range of doses used (linear regression slope $P > 0.05$), suggesting that a chemical's distribution between skim milk and milk fat would be constant regardless of the concentration. In the absence of overt physiologic effects such as toxicity or effects on blood flow to the mammary gland, such results suggest that whole milk composition (*i.e.*, across species or breed types) would influence a chemical's presence in milk to a greater extent than the dose received.
For the 12 chemicals tested, distribution into milk fat ranged from < 3 % (0.95 % for GLY and 2.5 % for IMI) to > 80 % of the total amount added (3′-$MeSO_2$-PCB-101, TCC, PCB-118, *β*-HBCD, 1278-TCDD, and BDE-99). Intermediate distributions into milk fat occurred for phenolic compounds (BPA, 39 %; TBBPA, 46 %; 2-OH-1378-TCDD, 54 %; and E1, 74 %) (Tables S5−S16, **Figure 1**).

As would be anticipated, the data indicated that nonpolar chemicals concentrate into high lipid milk fractions. The concentration ratios in milk fat relative to whole milk for moderately polar phenolic compounds were about 10 (BPA, 8.2; TBBPA, 10.5; 2-OH-1378-TCDD, 11.2; and E1, 15.8) and were ~ 18−20 for highly nonpolar persistent environmental contaminants (BDE-99, *β*-HBCD, 3′-$MeSO_2$-PCB-101, PCB-118, TCC, and 1278-TCDD; Figure 1). Also as expected, polar chemicals partitioned to a large degree into skim milk, resulting in milk fat/whole milk concentration ratios of < 1 (GLY was 0.2, and IMI was 0.5; Figure 1). For the phenolic compound BPA, substitution of four phenyl hydrogens with bromines to form TBBPA (Table 1) increased lipophilicity (log D = 3.60 vs 6.69) and was reflected by TBBPA's milk fat/whole milk concentration ratio of 10.5 compared to that of 8.2 for BPA (**Figure 1**). Hydroxylation of a molecule decreases its relative lipophilicity with respect to its non-hydroxylated analogue, as is commonly observed during oxidative metabolism. Although 1278-TCDD and 2-OH-1378-TCDD have very similar log *D* values (6.15 and 6.22, respectively) hydroxylation resulted in reduced lipid solubility and a ~ 30 % reduction in milk fat distribution. However, the addition of a more polar functional group onto a pentachloro biphenyl molecule to form 3′-$MeSO_2$-PCB-101 did not shift the milk fat distribution pattern when compared to PCB-118. One possible explanation may be due to the change of chlorine substitution pattern.

Figure 1: Chemical distribution and relative concentration ratios from whole milk into skim milk and milk fat fractions. Bars represent percent mean of all concentrations (n = 3 concentrations, 3 replicates per concentration, replicate exceptions are n = 2 replicates each for 1278-TCDD 20 and 200 nM and n = 2 replicates for BDE-99 2000 nM) ± SD of the three dose means based on disintegrations per minute (dpm) of skim milk and milk fat fractions compared to whole milk dpm. Values on graph represent the mean ratio of the drug concentration in the fraction (milk fat or skim milk) to the initial drug concentration in whole milk ± SD of means between doses (n = 3 mean dose ratios). Sum of stack plot represents total chemical recovery. log *D* values given for each compound at bottom of plot.

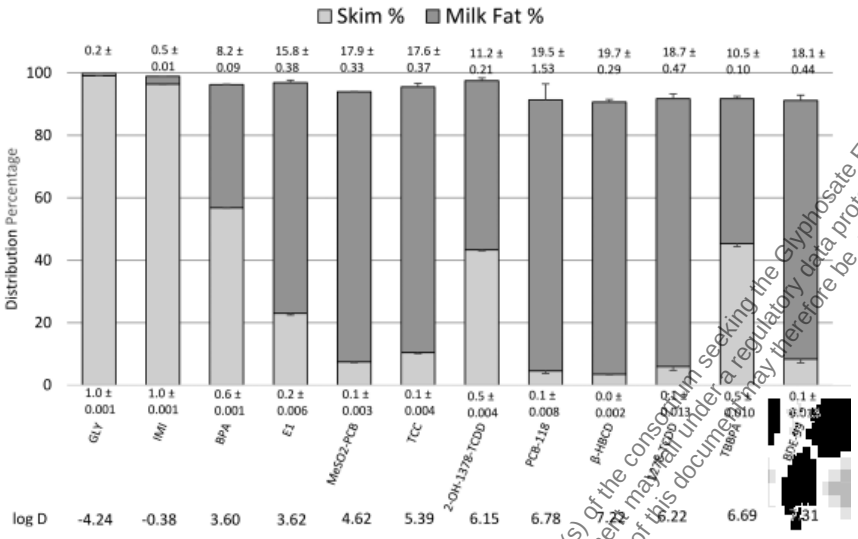


Table S5: GLY Phase 1 Average Distribution Data and Ratios.

Phase 1	Whole			Skim Milk				Milk Fat				Percent Recovery	Obs. Ratio
Mean	Spike DPM	Volume (mL)	Initial nM	Total DPM	Final Volume (mL)	Final nM	% GLY Dose	Total DPM	Mass (g)	Final nM (nmole/kg)	% GLY Dose	Total % based on Whole	[milk fat]/ [skim milk]
0nM		48.83		46.51					2.33				
20nM	117,903	48.90	21.72	46.58	22.59	99.10	948	2.31	3.69	0.80	99.90	0.16	
200nM	1,178,074	48.90	217.04	46.52	226.03	99.07	11,398	2.38	43.21	0.97	100.04	0.19	
2000nM	1,117,710	48.90	2,058.96	46.48	2,145.80	99.05	11,973	2.42	444.70	1.07	100.12	0.21	
St Dev													
0nM		0.08			0.03				0.05				
20nM	1,658	0.00	0.31	1,951	0.02	0.39	2.58	140	0.02	0.51	0.13	1,658.35	0.02
200nM	5,136	0.01	0.99	10,791	0.02	2.18	1.07	973	0.04	3.22	0.09	5,135.79	0.02
2000nM	11,354	0.01	21.18	4,072	0.08	7.45	1.11	2,330	0.07	73.05	0.20	11,354	0.03
% RSD													
0nM		0.16			0.06				2.07				
20nM	1.41	0.01	1.41	1.67	0.05	1.72	2.60	14.82	0.85	13.95	15.56	2.70	12.15
200nM	0.44	0.02	0.46	0.92	0.05	0.97	1.08	8.54	1.65	7.45	8.86	1.05	8.16
2000nM	1.02	0.03	1.03	0.37	0.17	0.35	1.12	19.46	3.01	16.43	18.54	0.92	16.51

Although literature describing the milk partitioning of the exact compounds studied here has not been found, there are several relevant studies available for comparison. For example, Jensen and Hummel [10] administered 2,4,5-trichlorophenoxy-acetic acid containing 2,3,7,8-TCDD to lactating dairy cows and found that 2,3,7,8-TCDD residues in cream exceeded those in milk by a factor of about 10. Although this is much lower than our reported ratio of ~ 19 for 1,2,7,8-TCDD (**Figure 1**), the difference could originate from the "medium heavy cream" used in the Jensen and Hummel study [10] which would have a fat content < 36 %. On the basis of our previous reports by Hakk *et al.* [7] and Lupton *et al.* [9], our milk fat

had an average fat content of 82 %. Regardless, our data confirmed those of Jensen and Hummel [10] in that the majority of dioxin residues would be associated with milk fat.

Compounds with a log *D* or *P* value of about 6 consistently concentrated in milk fat (or cream as cited in references). Concentrations of dichlorodiphenyltrichloroethane (DDT, Table S4) (log *D* 6.22 and log *P* = 5.92) in raw whole milk (5 % lipid), skim milk, and cream (70 % lipid) were reported as 7.5, 0.2, and 67.2 ppm, respectively, with a cream/whole milk ratio of 9.0 [12]. Pasteurisation produced a slight increase of the cream/whole milk distribution ratio, as pasteurised whole milk contained 6.0 ppm and cream contained 70.2 ppm DDT resulting in a cream/whole milk ratio of 12 [12]. Langlois *et al.* [13] reported the identical ratio of cream/whole milk for DDT in spite of a fat content for cream of only 37 %. Relative to the Mann [12] and Langois *et al.* [13] reports, higher milk fat/whole milk concentration ratios were found in this study for compounds having log *P* = ~ 6 (TCC, log *P* = 5.39, ratio 17.6; 1278-TCDD, log *P* = 6.22, ratio 18.7; PCB-118, log *P* = 6.78, ratio 19.5; **Figure 1**), which is also consistent with IVR (log *P* = 6.61, ratio 18.4) as reported by Hakk *et al.* [7]. The exception was 2-OH-1378-TCDD (log *P* = 6.15) which had a milk fat/whole milk concentration ratio of 11.2 in this study (**Figure 1**). These lower concentration ratios reported in the literature versus the current findings may be a reflection of differences in composition of the milk fat prepared here and the cream prepared in the cited reports.

A compound with a log *P* value similar to that of BPA (log *P* = 3.60) is the organophosphate cruformate (log *P* = 3.33, Table S4), which was fed to cows [14]. Similar to BPA, which concentrated eightfold in fat relative to whole milk, cruformate concentrated about fi vefold into cream [14]. If values were adjusted to reflect lipid mass yield (15 % of whole milk in their study, 10 % in ours) the fivefold concentration would increase to ~ 7.6-fold, in close agreement with the eightfold concentration found for BPA. For fenthion (log *P* = 3.21, Table S4), an organothiophosphate insecticide, the concentration ratio of fat/whole milk was ~ 5, with 80 − 90 % of the fenthion found in the fat fractions [15]. In the current work, the E1 (log *P* = 3.62) milk fat/whole milk concentration ratio was ~ 16 and 3′-MeSO$_2$-PCB (log *P* = 4.62, calculated) was ~ 18. Thus, the present results and those of O'Keeffe *et al.* [14, 15] suggested that factors in addition to log *P* also govern chemical disposition in milk.

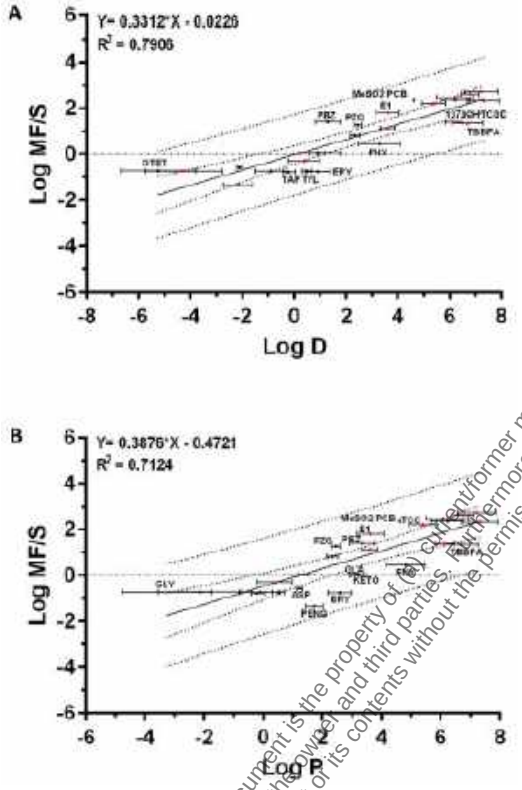
Similar to the studies done by Hakk *et al.* [7] and Lupton *et al.* [9], GLY and IMI (this study) distributed predominantly into the skim milk; thus, the concentration ratio between skim milk/whole milk was ~ 1, whereas the ratio of milk fat/whole milk was ~ 0.2 (Figure 1). Hakk *et al.* [7] observed similar distributions for compounds with low log *D* values, for example, OTET, PENG, and ERY, as did Lupton *et al.* [9] for ASP, CIPR, TAP, and TYL despite the diversity of chemical structures.

Using literature values of log *P* and pK$_a$ for each chemical (**Tables 1**, S1, and S2), mean and standard deviation (SD) log *D* values were calculated for ionizable compounds [16]. Relationships between log *D* or log *P* values and log [milk fat]/[skim milk] distributions, including 99 % confidence interval (CI) and prediction interval, are shown in **Figure 2A** (log *D*) and **2B** (log *P*). There are apparent uncertainties with respect to log *D* or log *P* for many of the studied compounds (**Figure 2A,B**). In general, distribution uncertainties with regard to log *D* or log *P* are much greater than the error associated with measurements of milk fat or skim partitioning. By combining the log [milk fat]/[skim milk] data of the current set with results obtained from those of Hakk *et al.* [7] and Lupton *et al.* [9], the linear regression with log *D* had a regression coefficient of 0.79 and with log *P*, the resulting linear regression had an r^2 = 0.71 (**Figure 2A,B**). The slightly better regression using log *D* data reinforces the conclusions of Hakk *et al.* [7] and Lupton *et al.* [9] that log D was a better predictor of the distribution between milk fat and skim milk than log *P*. Nevertheless, **Figure 2A** indicates that based on the 99 % CI for log *D*, numerous outliers were present when all 27 compounds were modeled. Outliers with respect to the 99 % CI for the log *D* plot (**Figure 2A**) included ERY, FNX, TAP, TBBPA, 2-OH-1378-TCDD, and TYL, compounds which distributed more toward skim than predicted. 2-OH-1378-TCDD likely would fall within the 99 % CI based on the SD of the calculated log *D*. Conversely, E1, 3′-MeSO$_2$-PCB-101, OTET, PBZ, and PZQ distributed more toward milk fat than predicted. Overall, the greatest limitation to predicting the behavior of any one chemical contaminant in milk seems to be the uncertainty associated with literature log *P* and pK$_a$ values used to calculate log *D* values in the model derivation.

Slopes of the linear log *D* and log *P* models were not 1, but 0.33 and 0.39, respectively (**Figure 2**). There was no reason to expect a 1:1 relationship between log *D* or *P* values of a chemical and its distribution between milk fat and skim milk. The lower slopes do indicate modeled chemicals that typically distribute

to a greater extent into skim milk than merely reflected by their log *D* or *P* values. Distribution data were not affected by the presence of degradates because none were detected by thin-layer chromatography (TLC) (Table S3). The model slopes highlight the differences between the simple, ideal, octanol/water partition system and the complex milk matrix which consists of water, lipid, protein, sugar, minerals, and micelles. We hypothesize that the presence of these additional milk components could account for the enhanced distribution into skim milk. For instance, milk proteins (casein, *β*-lactoglobulin, and lactalbumin) enhanced the solubilisation of DDT in water [17].

Figure 2: Regression analyses of log[chemical]$_{milk\ fat}$ /[chemical]$_{skim\ milk}$ (log *F/S*) vs log *D* and log *P* (pH 6.8). Plot A is the regression analysis of log *F/S* vs log *D*. Plot B is the regression analysis of log *F/S* vs log *P*. Error bars on the log *D* and log *P* for the chemicals reflect the variability of values reported in the literature. Compounds outside the 99 % CI but within 99 % of the prediction interval are labeled. Regressions are based on data from 27 chemicals. Red dots are chemicals of the current study, whereas black dots are chemicals published in Hakk *et al.* [7] and Lupton *et al.* [9].



Chemical distribution from skim milk into curd and whey.

Recoveries of radioactivity across tested chemicals were ≥ 95 % (sum of whey and curd), with the highest mean recovery (106.5 %) occurring for *β*-HBCD and the lowest recovery occurring for PCB-118 (90.7 %). The CVs for within dose replicates in whey and curd were generally < 4 % for the majority of chemicals tested; however, the CVs for the most lipophilic persistent organic pollutants, that is, 1278-TCDD, BDE-99, *β*-HBCD, 3′-MeSO$_2$-PCB-101, and PCB-118, were considerably higher, exceeding 3 % for whey (range 3.9 − 16.0 %) and 4 % for curd (range 4.3 − 10.0 %; **Figure 3** and Tables S17−S28). Higher CVs for these lipophilic chemicals in whey are to be expected, especially at lower concentrations, because of the small percentage of each compound that distributed into whey. Chemical distributions were generally not dose-dependent for 0 % moisture curd/whey ratios across the starting concentrations present in skim milk, although a dose dependency was apparent for BDE-99 ($p < 0.05$). An ~ 8 % increase in association with the curd fraction was measured with BDE-99 with each 10-fold increase in dose, that is, from 73 % to 80 % to 92 %, respectively. Initial concentrations in skim milk were 1.7, 13, and 204 nM (Table S28).

For the 12 compounds tested in the current study, chemicals retained in the curd fraction ranged from approximately 16.5 % for GLY to 86 % for β-HBCD when related to residual chemical in the skim milk of phase 1 (Tables S17 − S28). Distribution into curd was largely proportional to a chemical's lipophilicity. Of the most lipophilic compounds tested, ~ 80 % of chemical was distributed into curd (1278-TCDD, BDE-99, β-HBCD, and PCB-118). Compounds having moderate lipophilicity, that is, TBBPA, 3′-MeSO$_2$-PCB-101, 2-OH-1378-TCDD, and TCC, were more evenly distributed into both curd (40 − 60 %) and whey (35 − 60 %). Highly polar compounds had the lowest affinity for curd, for example, GLY (16.5 %) followed by IMI (23.7 %; **Figure 3**, Tables S17−S28).

Figure 3: Drug distribution and relative concentration ratios from skim milk into whey and curd fractions. Bars represent percent mean of all concentrations (n = 3 concentrations, n = 3 replicates per concentration, replicate exceptions are n = 2 replicates each for PCB-118 50 and 200 nM, n = 2 replicates each for β-HBCD 200 and 500 nM, n = 2 replicates each for 1278-TCDD 20 and 200 nM, and n = 2 replicates for BDE-99 20 nM) ± SD of all three dose mean percentages based on dpm of whey and curd (at 70 % moisture) fractions compared to forti fi ed skim milk dpm. Numerical values on the graph represent the mean ratio (n = 3) of the drug concentration in the fraction (curd or whey) to the initial drug concentration in skim milk ± SD. BDE-99 distribution was dose-dependent ($P < 0.05$). Sum of stacked plots represents total, unadjusted drug recovery values.

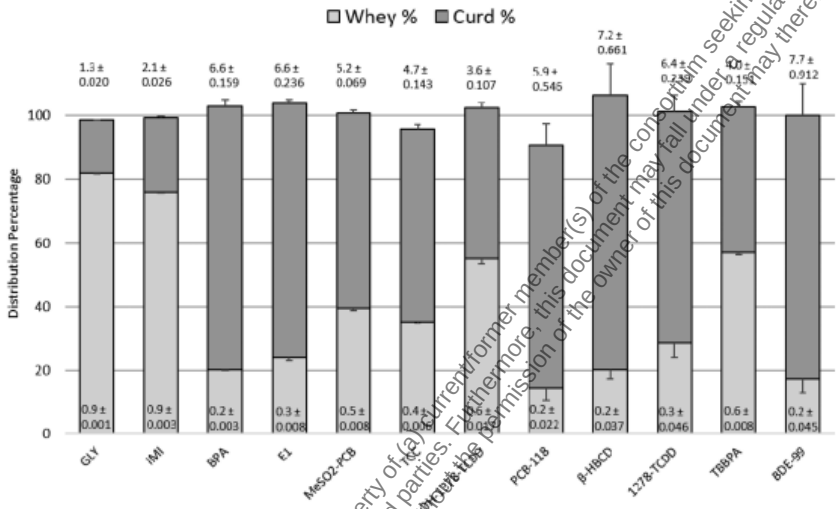


Table S17: GLY Phase 2 Average Distribution Data and Ratios.

Phase 2	Skim Milk			Whey Fraction					Curd Fraction					Percent Recovery	Obs. Ratio	Adj. Ratio
Average	Initial DPM	Volume (mL)	Mass (g)	Total DPM	Volume (mL)	Final nM	% GLY Dose	% GLY Dose (Corrected)	Total DPM	Mass (g)	Final nM (nmole/kg)	% GLY Dose	% GLY Dose (0% moisture)	Percent Recovery based on Skim Milk	$[GLY]_{curd}/[GLY]_{skim}$	$[GLY]_{w\&c curd}/[GLY]_{skim}$
0nM		48.38			39.59					6.01						
20nM	114,206	45.54	22.59	93,858	40.05	21.11	82.20	90.69	18,869	5.76	29.51	16.52	8.03	98.72	1.40	2.63
200nM	1,139,899	45.44	226.03	936,093	39.85	211.60	82.12	90.65	189,618	5.88	290.74	16.63	8.10	98.75	1.37	2.45
2000nM	1,081,533	45.41	2,145.80	885,328	39.83	2,002.63	81.86	90.37	177,067	5.87	2,715.70	16.37	7.86	98.23	1.36	2.38
St Dev																
0nM		0.08			0.37					0.29						
20nM	1,882	0.04	0.39	907	0.16	0.28	1.41	1.55	189	0.15	0.49	0.19	0.05	1.59	0.04	0.30
200nM	10,494	0.02	2.18	3,990	0.12	0.45	0.95	1.04	728	0.13	7.32	0.10	0.02	1.05	0.04	0.21
2000nM	3,918	0.07	7.45	2,693	0.02	5.56	0.06	0.07	4,651	0.09	38.25	0.45	0.44	0.50	0.02	0.07
%RSD																
0nM		0.18			0.94					4.74						
20nM	1.65	0.08	1.72	0.97	0.40	1.33	1.71	1.70	1.00	2.54	1.67	1.13	0.65	1.61	3.01	11.24
200nM	0.92	0.05	0.97	0.43	0.31	0.21	1.16	1.15	0.38	2.23	2.52	0.61	0.30	1.07	2.56	8.45
2000nM	0.36	0.15	0.35	0.30	0.05	0.28	0.08	0.08	2.63	1.52	1.41	2.74	5.59	0.51	1.54	2.94

When curd data (normally 70 % moisture) were expressed on a dry matter basis, the concentration ratios of 0 % moisture curd to whey (Tables S17−S28) were > 100 for the most lipophilic compounds, that is, 1278-TCDD (115), BDE-99 (327), *β*-HBCD (152), and PCB-118 (136), and for two of the phenolics, BPA (111) and E1 (104). Other phenolic compounds, that is, TBBPA and 2-OH-1378-TCDD, had much lower concentration ratios of 32 and 18, respectively, whereas 3′-MeSO$_2$-PCB-101 (56) and TCC (46) were also lower than the most lipophilic compounds. The 0 % moisture curd/whey concentration ratios for the most polar compounds ranged from 9.2 for IMI to 2.5 for GLY (Tables S17−S28).

Results for TBBPA were unexpected based on its structural similarity to BPA. The fire-retardant TBBPA is identical in the base structure to the plasticizer BPA with the exception that the 4-ortho hydrogens, with respect to the phenolic hydroxyls, are replaced by bromines. Bromination of the ortho-protons enhanced lipophilicity (log *P*) of TBBPA compared to BPA. In the 0 % moisture curd/whey, however, the concentration ratio decreased from 111 for BPA to 32 for TBBPA (Tables S17−S28). Based solely on lipophilicity (log *P*), the curd/whey concentration ratio would have been expected to increase for TBBPA relative to BPA. One possibility for the lower concentration ratio for TBBPA is that the much larger atomic radius of bromine (compared to hydrogen) resulted in steric hindrances for potential casein − TBBPA interactions.

Hydroxylation and methylsulfonation of chemicals altered distribution patterns in milk. Aromatic hydroxylation decreased lipophilicity slightly and thus increased distribution into skim milk for phase 1 and into whey for phase 2. For example, 2-OH-1378-TCDD had a greater distribution into the whey (~ 30 % greater) compared to 1278-TCDD. Comparison of PCB-118 and 3′-MeSO$_2$-PCB-101 also indicated that a methyl sulfone group decreased lipophilicity (log *D* 6.38 vs 4.62, respectively) and increased (> 25 %) distribution into whey. Despite a different chlorine substitution pattern between this pair of chemicals, the presence or absence of a methyl sulfone functional group likely plays a more important role in determining the effect on curd versus whey distribution. The full nature of this partitioning difference is undoubtedly based on more than hydrophobic interaction, for example, possible chemical/protein interactions or sequestration.

Published reports related to the partitioning of chemicals tested in this study into whey and curd are scant, but structures and characteristics of chemicals cited for comparison are provided in Table S4. For example, concentrations of the aromatic, chlorinated insecticide DDT (log *D* = 6.22 and log *P* = 5.92) were greater in cheddar cheese than in whey after milk processing, with cheese and whey concentrations of 47 and 0.5 ppm, respectively [12]. Similarly, Swiss-type cheese made from milk produced by dairy cows fed DDT contained ~ 8 times the original DDT concentration of whole milk, though DDT was not reported in whey [13]. In other studies, however, DDT was unstable during processing and 27 − 53 % of the starting DDT degraded to DDE and DDD [18] during the manufacturing of cheese. While DDT was not identified in whey at the dipping stage, it was measured in the whey pressed from curd [19]. Whey produced during the processing of raw whole milk had levels of DDE and DDD that increased twofold when measured at acidification, and concentrations were the same in the cheese product [19]. Similar concentrations of DDT were reported for whole milk and cheddar or Monterey cheese, indicating some net loss of DDT, as total cheese mass would be less than the original milk mass. No changes in DDT concentration were observed during storage.

Lipophilic compounds in this study concentrated in the curd to a greater extent than whey, but the lipophilic pesticide lindane (log *P* = 3.99), a cyclo-chlorinated structure with similarities to *β*-HBCD, did not concentrate in cheese or yogurt (produced from curd) made from contaminated raw milk [20]. The authors attributed the lack of concentration to heat treatment during pasteurisation which resulted in phenolic metabolite formation. Pasteurisation resulted in a 65 − 73 % reduction in lindane, with more losses during refrigeration of yogurt (1.4 − 8 % over 3 days) and cheeses (36.7 % in Ras cheese during 6 months in storage). Although the effects of pasteurisation and storage were not investigated in the current study, similar losses in *β*-HBCD might occur. Contrary to Abou-Arab [20], Langlois *et al.* [13] found that lindane concentration in curd (4.3 ppm) was approximately 12 times that measured in whey (0.34 ppm). In a second study, Langlois *et al.* [21] determined that curd concentrations of endrin (log *D* and *P* = 4.9) were about eight times those in whole milk (5.48 vs 0.7 ppm), whereas whey concentration was only 0.06

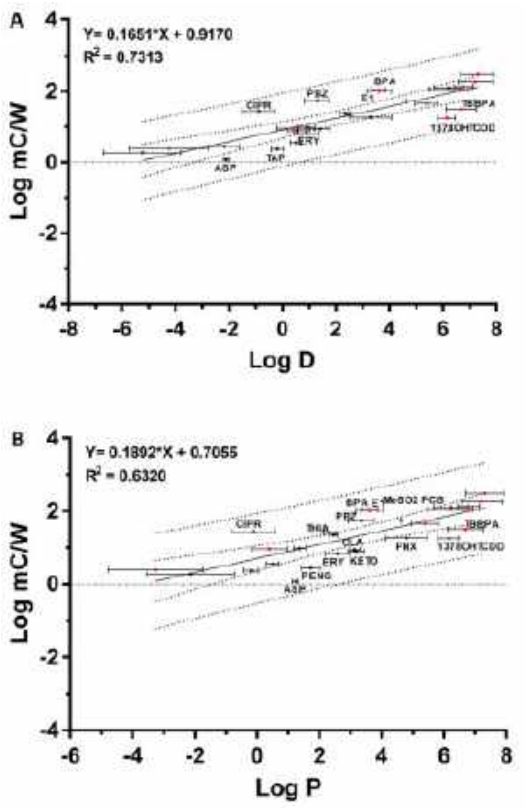
ppm (curd/whey concentration ratio = 91). Surprisingly, heptachlor (log *P* = 5.46), with higher lipophilicity than endrin, was present in whey (0.17 ppm) at approximately 1/20 the concentration measured in curd (3.77 ppm) (curd/whey concentration ratio = 22) [21]. Cruformate (log *P* = 3.33), which has a log *P* similar to BPA (3.60) and E1 (3.62), had a dose-dependent distribution. At a starting milk concentration of 0.07 ppm, cruformate was 22 times more concentrated in curd than that in whey (0.43 vs 0.02 ppm, respectively), but with a starting milk concentration of 0.16 ppm, the curd/whey concentration ratio was 31 (0.92 and 0.03 ppm, respectively) similar to that of BPA (29) and E1 (24) [14].

Hydrophilic compounds distributed more evenly between curd and whey. For example, the curd/whey concentration ratio for GLY (log *D* = − 4.24) was 1.4 and for IMI (log *D* = − 0.38) was 2.4, similar to SDMX (ratio 3.2), PENG (ratio 1.2), OTET (ratio 1.4), ERY (ratio 2.4), and KETO (ratio 2.4) as previously reported [8]. Given the diversity of chemical structures tested, the log *D* value of hydrophilic compounds does provide some predictive measure for curd and whey distribution. Similarly, TAP and TYL possessed fairly low curd/whey concentration ratios, that is, 1.3 and 1.5, respectively [9].

Figure 4A (log *D*) and **4B** (log *P*) shows the relationships between log D or log P values and log[0 % moisture curd]/[whey] concentration ratios, including 99 % CI and prediction interval. By combining the log[0 % moisture curd]/[whey] data of the current set with those of Shappell *et al.* [8] and Lupton *et al.* [9] the regression with log D had an $r^2 = 0.73$, whereas the log *P* regression had an $r^2 = 0.63$ (**Figure 4A,B**). The higher regression coe ffi cient obtained using log D data reinforces the previous conclusion that log *D* is a better predictor of the distribution between curd and whey than log *P* [8,9].

On the basis of the 99 % CIs for the log *P* regression, numerous outliers were present when all 27 compounds were modeled. Outliers for the curve fit on a log *D* basis (Figure 4A) included ASP, ERY, 2-OH-1378-TCDD, TAP, and TBBPA compounds which distributed more toward whey than predicted. Conversely, BPA, CIPR, E1, and PBZ distributed more toward curd than predicted. In the log *P* model (Figure 4B), four additional chemicals (CLA, KETO, FNX, and PENG) fell outside of the 99 % CIs.

Figure 4: Regression analyses of log[chemical]$_{0\ \%\text{moisture curd}}$ /[chemical]$_{\text{whey}}$ (log *mC/W*) vs log *D* and log *P* (pH 6.8). Plot A is the regression analysis of log *mC/W* vs log *D*. Plot B is the regression analysis of log *mC/W* vs log *P*. Error bars on the log *D* and log *P* for the chemicals reflect the variability of values reported in the literature. Compounds in between the 99 % CI and 99 % of the prediction interval are labeled. Red dots are chemicals of the current study, whereas black dots are chemicals published in Shappell *et al.* [8] and Lupton *et al.* [9]. Regressions are based on data from 27 chemicals.



Chemical distribution from whey into retentate and permeate.
In order to assess the percent of drug associated with the whey proteins, ultra filtration in conjunction with centrifugation was performed (phase 3; **Figure 5**). The expected volume of retentate was 33 % of the applied sample volume based on centrifugation time and speed, with the actual measured mean for all compounds being 37 ± 3.3 %. Mean recovery of radioactivity across all compounds was 100 ± 4.5 %. Mean non-specific binding of compounds to filters ranged from 0.2 % for GLY to 22.5 % for E1. Compounds with > 3 % filter binding include PCB-118 (6.4 %), BDE-99 (7.1 %), *β*-HBCD (8.2 %), BPA (13.4 %), and E1 (22.5 %) (Tables S29 − S40). Although compounds with high log *D* values could be expected to be "sticky" in the aqueous medium, four compounds with high log *D* values [TCC (log *D* = 5.39), 2-OH-1378-TCDD (log *D* = 6.15), 1278-TCDD (log *D* = 6.22), and TBBPA (log *D* = 6.69)] had filter binding of ≤ 2.4 %.

The associations of the 12 xenobiotics with whey protein, as determined by the percentage of compound measured in the retentate, revealed three groupings (**Figure 6**). The first was represented by GLY and IMI that have negative log *D* values (− 4.24 and − 0.38, respectively), where there was essentially no association with the whey protein (< 5 %) occurred (Tables S29 and S30). The second grouping was composed of BPA, E1, 3′-MeSO$_2$-PCB-101, 2-OH-1378-TCDD, and 1278-TCDD, which had moderate associations with whey protein, ranging from 33 to 76 % (Tables S31−S33, S35, and S38). Similar to our findings of ~ 64 % association of E1 with whey protein, Wolford and Argoudelis [22] reported 48 and 53 % of 17 *β*-estradiol and E1, respectively, associated with whey protein. The third grouping was composed of those compounds that were almost totally associated with retentate whey proteins (84 − 98 %; one outlier of 107 % for PCB-118 due extremely low starting radiocarbon in the whey). Chemicals in this grouping included BDE-99, *β*-HBCD, PCB-118, TBBPA, and TCC (Tables S34, S36, S37, S39, and S40). If present in whey, these compounds would concentrate in whey-derived protein products.

The percent of whole milk dose associated with either casein or whey proteins is reported in **Table 2**. About 25 % of TBBPA and 2-OH-1378-TCDD from whole milk distributed to whey, yet ~ 90 and 70 % (TBBPA and 2-OH-1378-TCDD, respectively) of that were associated with whey protein.

Figure 5: Scheme of milk partitioning processes that yielded cream and milk fat from whole milk (phase 1) curd and whey from skim milk (phase 2) and retentate and permeate from whey (phase 3).

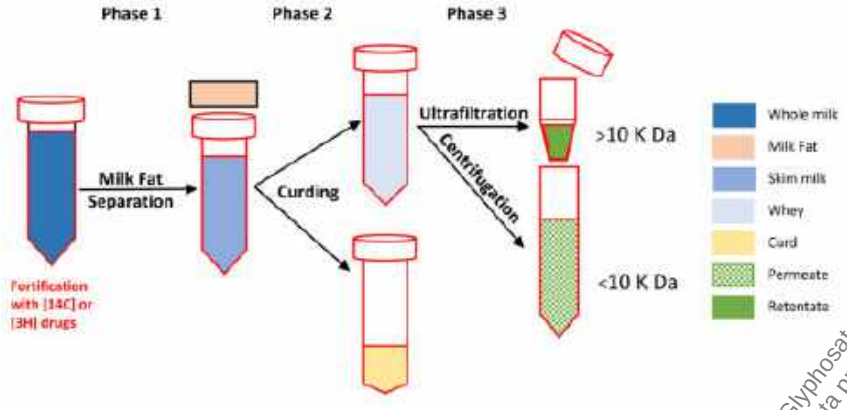


Figure 6: Drug distribution from whey into permeate, retentate, and filter fractions. Bars represent percent mean of all concentrations (n = 3 concentrations, concentration exceptions are PCB-118 and *β*-HBCD n = 2 concentrations; n = 3 replicates per concentration, replicate exceptions n = 2 replicates for TCC 20 nM, n = 2 replicates each for 1278-TCDD 200 and 2000 nM, n = 2 replicates for BDE-99 200 nM) ± SD of all three dose mean percentages based on dpm of permeate and retentate fractions compared to fortified whey dpm. Horizontal lines on each bar represent the actual retentate and permeate volume percentage after centrifugation. Sum of stacked plots represents total, unadjusted drug recovery values).

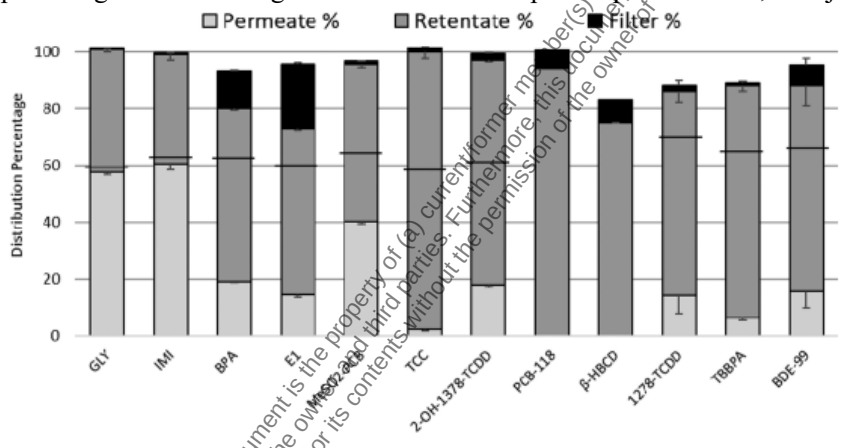


Table S29: GLY Phase 3 Average Distribution Data and Ratios.

Phase 3	Whey			Retentate Fraction				Permeate Fraction				Filter		Total Dose Recovery	Protein Association
Mean	Initial DPM	Volume (mL)	Initial nM	Total DPM	Volume (mL)	Final nM	% GLY Dose	Total DPM	Volume (mL)	Final nM	% GLY Dose	Total DPM	% GLY Dose	Percent Recovery based on Whey	% Associated
0nM		14.86			5.82				9.12						
20nM	34,827	14.86	21.11	15,225	6.16	22.25	43.73	19,722	8.78	20.24	56.62	57	0.16	101.12	86
200nM	349,549	14.88	211.60	148,612	6.00	223.16	42.52	202,487	8.96	203.51	57.93	490	0.14	101.20	4.4
2000nM	330,961	14.89	2,002.63	142,417	6.02	2,132.92	43.03	192,264	8.96	1,934.18	58.09	484	0.15	101.89	4.7
St Dev															
0nM		0.01			0.17				0.17						
20nM	458	0.01	0.28	231	0.10	0.06	1.19	371	0.10	0.23	0.33	11	0.03	0.85	0.6
200nM	864	0.01	0.45	4,001	0.14	1.20	1.24	3,503	0.14	1.02	0.91	26	0.08	0.71	0.3
2000nM	1,160	0.01	5.56	1,910	0.08	6.83	0.73	1,738	0.09	5.50	0.33	26	0.04	0.44	0.2
% RSD															
0nM		0.0			2.85				1.85						
20nM	1.31	0.1	1.33	1.52	1.70	0.25	2.72	1.88	1.13	1.13	0.58	18.57	18.79	0.84	12.4
200nM	0.25	0.04	0.21	2.69	2.26	0.54	2.92	1.73	1.59	0.50	0.57	5.37	5.52	0.71	6.7
2000nM	0.35	0.08	0.28	1.34	1.29	0.32	1.69	0.90	1.00	0.28	0.57	5.37	5.61	0.44	4.8

Chemical concentration based on protein mass for casein and whey proteins

Using 0 % moisture curd data from phase 2, the amount of chemical associated with caseins was calculated based on proteins present in curd and largely result from agglutination of casein (**Table 2**). Similarly, using phase 3 data, the amount of chemical associated with whey proteins can be calculated (**Table 2**). Chemical saturation of casein or whey protein was not observed because the mass of chemical per milligram protein increased as the concentration increased. In some instances, the initial expected fortification concentrations in whole milk differed from measured concentrations, as seen with 3′-MeSO$_2$-PCB-101 and *β*-HBCD. Whey protein association values for the lowest dose of BDE-99 are questionable because the starting skim milk contained < 2 nM and whey 0.3 nM. However, confidence in casein/whey protein association results is enhanced by the agreement found across doses (**Table 2**), exception was BDE-99, where ratios ranged from 2.8 to 5.5.

For the majority of chemicals tested (BDE-99, BPA, E1, *β*-HBCD, IMI, 3′-MeSO$_2$-PCB-101, PCB-118, and 1278-TCDD), the association with caseins was greater than that for whey proteins (ratio > 1, **Table 2**). The importance of methodology is evident when comparing ourfindings to those of Wolford and Argoudelis [22] that used equilibrium dialysis with E1 and the slightly more hydrophilic compound E2. They reported that E1 and E2 were largely (> 84 %) bound to protein when incubated in skim milk, and > 50 % of the bound estrogens was associated with whey proteins. These data are in contrast to our findings for E1, in which the association (nmol/mg protein) ratio was approximately 2 for casein/whey.

The difference between the results of the two studies was most likely the precipitation of curd caseins in the present work versus the presence of soluble caseins used for dialysis by Wolford and Argoudelis [22] (1979).

Other chemicals that preferentially associated with caseins relative to whey protein (ratio > 1) include THIA (2.5), IVR (2.0) [8], TYL (1.4), CIPR (2.0), and PZQ (1.5) [9]. Although the current work used a majority of chemicals with log *D* greater than 3.4, our previous reports described only one such chemical (IVR). The casein/whey protein association ratio of IVR was more similar to BPA (2.0), E1 (1.9), and IMI (1.9) (**Table 2**).

In spite of higher distribution of GLY into whey than curd (**Figure 3**), there was in fact very little preferential retention of GLY associated with whey protein (Figure 6). Similarly, TCC, 2-OH-1378-TCDD, and TBBPA also had casein/whey protein ratios <1. Although most of the total TCC dose was partitioned with milk fat (mean 85 %), the remainder distributed almost equally between whey and 0 % moisture curd (57 % curd, Table S22). TCC remaining in the whey was concentrated almost exclusively in the retentate (98 %) during ultracentrifugation (Table S34). The log *D* values of 2-OH-1378-TCDD

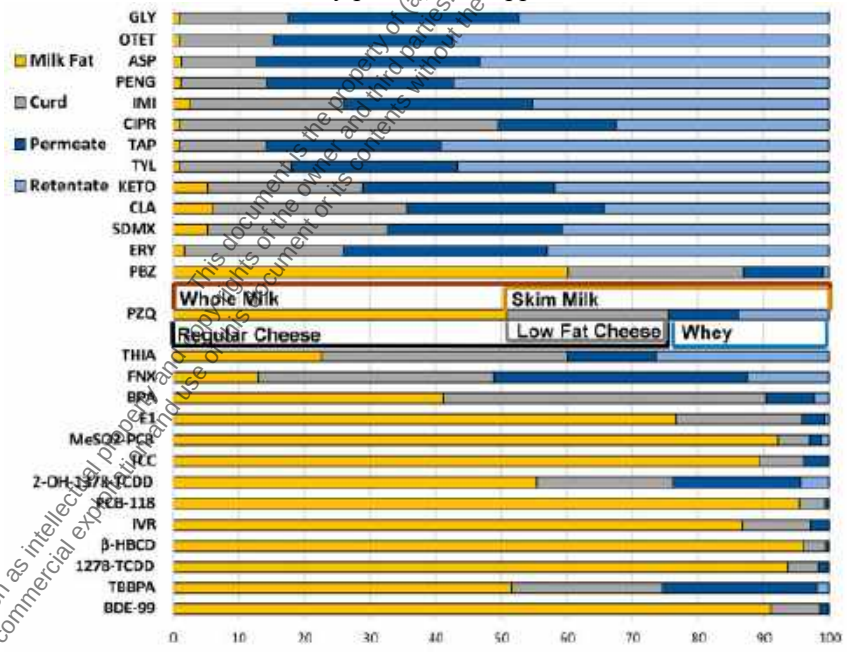
(6.15) and TBBPA (6.69) did not predict the respective mean casein/whey protein ratios of 0.26 and 0.33. Both chemicals also distributed to a lesser extent than predicted into milk fat. The common feature of both compounds is a hydroxyl moiety between two halogens (chlorines for 2-OH-1378-TCDD and bromines for TBBPA).

Previously studied chemicals that had higher association for whey proteins versus caseins were PENG (casein/whey ratio = 0.2), ERY (0.5), KETO (0.4), SDMX (0.8) [8]; TAP (0.5), CLA (0.4), and FNX (0.25) [9]. Although the distribution between lipid and aqueous phases was markedly dependent on the property of proteins, namely lipophilicity, small-molecule binding to proteins seems to be more dependent on specific functional groups within the protein. Identifying the specific functional groups and binding domains that can associate with studied chemicals within a plethora of whey and casein proteins lies outside the scope of the present research.

Relation to consumer products

To determine how the distributions of these compounds, if detected in whole milk, related to consumer products, the percent distributions intomilk fat, curd, retentate, and permeate were calculated in relation to the starting concentration in whole milk. **Figure 7** includes the experimentally derived percentages of each compound in high-fat products which would include butter, cream, and cheese; low-fat products would include skim milk, low fat cheese, yogurt, and low-fat derived whey protein products such as whey protein powders and baby formulas. Comparable to compounds previously tested [8,9], higher log *D* compounds (i.e., E1, 3 ′ -MeSO 2 -PCB-101, TCC, PCB-118, *β*-HBCD, 1278-TCDD, and BDE-99) generally distributed to high-fat products such as butter and cream. High-fat products that contain protein (*i.e.*, cheese) will concentrate both mid- to high-range log *D* molecules such as BPA, 2-OH-1378-TCDD, and TBBPA along with the higher log *D* compounds. Two compounds with low log *D*'s, that is, GLY and IMI, will primarily distribute into aqueous products, such as skim milk and whey.

Figure 7: Normalised percentages of chemicals calculated from whole milk to be in the milk end-products of milk fat, curd, permeate, and retentate based on data generated from the current studies as well as those reported in Hakk *et al.* [7], Shappell *et al.* [8], and Lupton *et al.* [9]. The PZQ bar has additional information on which milk end products comprise whole milk, skim milk, curd, low-fat curd, and whey, as a guide to where drug may partition during commercial milk processing. For percentage of chemical associated with whey protein see supplemental information tables S29 − S40).



Determining where a compound would concentrate in consumer products will also depend on the processing steps involved and what specific end product is being manufactured. For example, whole milk processed into skim milk and cream would generally have compounds with high log *D* values concentrated in butter and cream, whereas compounds with low log *D* values will be in skim milk. Compounds with mid-range log *D* values will be split between the higher fat products and skim milk. However, if whole milk is processed directly into cheese, then the mid-range and high-range log D value compounds will mainly concentrate in the cheese.

Conclusions

The partitioning of 12 environmental contaminants or metabolites into milk fractions was assessed. Partitioning between milk fat and skim milk and between 0 % moisture curd and whey was usually governed by the compound's lipophilicity. If a chemical was found in whey, the more nonpolar the compound the more likely it would be found in whey protein products. Phenolic compounds were the main chemicals that fell outside of the 99 % CIs of the models' regression analyses. These models provide a tool using log *D* as the primary chemical property to predict the distribution of chemicals into various milk products.

Supporting information
Supporting information with (Tables S1−S40) is available online:
http://pubs.acs.org/doi/suppl/10.1021/acsomega.8b00762/suppl_file/ao8b00762_si_001.pdf.
Only the tables relevant to glyphosate (S5, S17, S29) are shown in this summary.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The purpose of the described work was to investigate the partitioning of 12 environmental chemicals of diverse polarities into various milk fractions. One of the tested chemicals was glyphosate. The experiments were conducted with radio-labelled test materials which were fortified to raw (unpasteurised, non-homogenised) cow milk (3 fortification levels were investigated for each compound). Thereafter, the milk was processed into skim milk, milk fat, curd, whey, whey retentate and whey permeate. A linear model predicting the distribution of chemicals between skim milk and milk fat based on their lipophilicity was established. The distribution between curd and whey was also correlated with lipophilicity. Phenolic compounds had less predictable distribution patterns based on their lipophilicities.
During processing of whole milk to skim milk and milk fat, glyphosate partitioned essentially to skim milk (> 99 %). Only about 1 % of the glyphosate fortified to whole milk was recovered in milk fat. Following curding of the skim milk, most glyphosate remained in the whey fraction (> 80 %). The associations of glyphosate with whey protein (calculated by subtracting the amount present in permeate from the amount present in retentate) was very low (< 5 %). As expected due to its hydrophilicity, glyphosate primarily distributes into aqueous products, such as skim milk and whey. The distribution pattern between the various milk fractions was similar for the various amounts of glyphosate fortified to whole milk (range of ca. 0.004 mg/L to 0.348 mg/L).
Although the distribution of residues between skim milk and milk fat is not a data requirement for hydrophilic compounds like glyphosate, this information is considered relevant to risk assessment. Overall, the publication is deemed reliable. Normally, the distribution of residues between skim milk and milk fat should be investigated with raw milk containing incurred residues (in the context of metabolism or feeding studies) and not by (artificially) fortifying raw milk. However, due to the very low transfer of glyphosate-derived residues in milk, the approach used in the publication seems to be the best option to determine the distribution of parent glyphosate residues between skim milk and milk fat.

Relevant published articles from Literature Search Report

1. Information on the study

Data point	CA 6.4.2/005
Report author	Schnabel, K. *et al.*
Report year	2017
Report title	Effects of glyphosate residues and different concentrate feed proportions on performance, energy metabolism and health characteristics in lactating dairy cows
Document No.	DOI 10.1080/1745039X.2017.1391487 E-ISSN 1477-2817
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

The aim of this study was to examine the influence of glyphosate (GL) residues in feedstuffs on performance, energy balance and health-related characteristics of lactating dairy cows fed diets with different concentrate feed proportions. After an adaption period, 64 German Holstein cows (207 ± 49 d in milk; mean ± SD) were assigned to either groups receiving a GL contaminated total mixed ration (TMR) (GL groups) or an uncontaminated TMR (CON groups) during a 16 weeks trial. Contaminated feedstuffs used were legally GL-treated peas and wheat (straw and grain). GL and CON groups were subdivided into a "low concentrate" group (LC) fed on dry matter (DM) basis of 21 % maize silage, 42 % grass silage, 7 % straw and 30 % concentrate and a "high concentrate" group (HC) composed of 11 % maize silage, 22 % grass silage, 7 % straw and 60 % concentrate for ad libitum consumption. Body condition score, body weight, DM intake and milk performance parameters were recorded. In blood serum, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and glucose were measured and energy balance was calculated. Milk was analysed for GL residues.
At week 0, 7 and 15, general health status was evaluated by a modified clinical score. The average individual GL intake amounted for Groups CON_{LC}, CON_{HC}, GL_{LC} and GL_{HC} to 0.8, 0.8, 73.8 and 84.5 mg/d, respectively. No GL residues were detected in milk. GL contamination did not affect body condition score, body weight, DM intake, nutrient digestibility, net energy intake, net energy balance or BHB, glucose, NEFA and milk performance parameters; whereas concentrate feed proportion and time did affect most parameters. The clinical examination showed no adverse effect of GL-contaminated feedstuffs on cows' health condition. In the present study, GL-contaminated feedstuffs showed no influence on performance and energy balance of lactating dairy cows, irrespective of feed concentrate proportion.

Materials and Methods

Experimental design
Sixty-four German Holstein cows (207 ± 49 d in milk; mean ± SD) were used in a 17 weeks trial. The experiment was designed as a 2×2 factorial design with GL contamination and concentrate proportion in feed as the main factors. At the start of the experiment (week 0), all animals were fed with an energetically adequate total mixed ration (TMR), based on the recommendations of the Society of Nutrition Physiology (GfE 2001) consisting of 30 % maize silage, 30 % grass silage and 40 % concentrate on a dry matter (DM) basis. To provide equal conditions in the following 16 weeks of trial,

48 cows and 16 heifers were assigned to four different feeding groups by considering number of lactation (2.8 ± 0.7) and data that had been collected prior to the trial, presenting a 3-d mean of body weight (BW, 645 ± 21 kg), daily feed intake (40.9 ± 0.2 kg fresh matter of the ration) and fat corrected milk (FCM, 4 % fat; 29.1 ± 0.5 kg). Half of the animals received in their ration GL contaminated peas and wheat kernels, processed in concentrate and GL contaminated straw (Groups GL). As control group, the other half of cows received a non-contaminated ration (Groups CON). Both groups were subdivided into one group receiving a diet with a low concentrate proportion (LC) composed on DM basis of 21 % maize silage, 42 % grass silage, 7 % straw and 30 % concentrate, and another group receiving a diet with high concentrate proportion (HC) composed of 11 % maize silage, 22 % grass silage, 7 % straw and 60 % concentrate . TMR and water were provided *ad libitum*. Cows were kept in a free stall-barn, Groups GL and CON were separated by the feed alley, and within each group subgroups LC and HC were separated by fences inside the barn.

Feedstuff production, animal measurements and sample collection
Maize, grass, peas and wheat were grown on the acreage of the experimental station of the Friedrich-Loeffler-Institut (FLI), in Braunschweig, Germany, to generate equal growth and soil conditions. The acreage had not been treated before with GL for at least 3 years. Maize and grass were grown without GL-application. For GL contamination Roundup Record® (007525–60/MOT), Monsanto, Agrar Deutschland GmbH (Düsseldorf, Germany) was used as water-soluble granulate, containing as active ingredient 720 g GL per kg GL solution. A part of wheat and peas was treated with Roundup Record®, in pre-harvest application with 2.5 l/ha for wheat and 2 l/ha for peas, according to the legal regulations [Regulation (EC) No. 396/2005 of the European Parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC] while another part remained untreated and served for uncontaminated control feedstuffs. GL contaminated and non-contaminated feedstuffs were harvested and stored separately to avoid cross contamination.
During the trial, samples of maize and grass silage were taken twice a week, while samples of straw and concentrate were taken once a week and pooled over 4 weeks. Water and DMI were recorded daily by computerised feeding bins (type RIC; Insentec B.V., Marknesse, The Netherlands). Every second week the body condition score (BCS) was evaluated using a 5-point scale (Edmonson *et al.* 1989). Cows were milked twice daily beginning at 05:30 h and at 15:30 h and milk yield was recorded using automatic milk counters (Lemmer Fullwood GmbH, Lohmar, Germany). Morning and evening milk samples were collected twice a week. In week 0 and 16, an additional morning and evening milk sample was taken and pooled according to their proportion of total daily milk yield and frozen at −20°C. BW was recorded automatically by a scale after leaving the milking parlour. Blood samples were taken after morning milking from a jugular vein in serum tubes at week 0, 4, 8, 12 and 16.

Analyses
Feed samples were dried at 60°C before analysis for chemical composition according to the methods of the VDLUFA (1993) applying method number 3.1 (DM), 8.1 (crude ash), 4.1.2 (crude protein), 5.1.1 (ether extract), 6.1.1 (crude fibre), 6.5.1 (neutral detergent fibre without ash, amylase treated) and 6.5.2 (acid detergent fibre without ash). The TMR of each treatment group was tested for the apparent digestibility of crude nutrients and net energy for lactation (NE_L) content by using German Blackhead/SKF wethers according to the regulations published by the Society of Nutrition Physiology (GfE 1991).
GL and aminomethylphosphonic acid (AMPA) concentration in feed samples were measured by an accredited laboratory (Wessling GmbH, Altenberge, Germany). Samples were extracted with formic acid (0.1 %) and methylene chloride. Derivatisation was conducted with fluorenylmethoxycarbonyl chloride. After solid-phase extraction, GL and AMPA were determined by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). GL and AMPA were quantified using internal standards containing 1.2-$^{13}C_2{}^{15}N$ GL (1 µl/ml) and $^{13}C^{15}N$ AMPA (1 µl/ml). The limit of detection (LOD) and the limit of quantification (LOQ) for each substance were calculated from the signal-to-noise ratio amounting to 3 for the LOD and 10 for the LOQ, whereby the LOQ and LOD of the feed samples were 0.02 and

0.007 mg/kg for GL and AMPA, respectively. The recoveries for GL and AMPA analyses in feed samples were 70–120 % using an internal standard concentration of 0.625 mg/kg for feed analyses.
Milk samples were analysed for fat, protein, lactose and urea using an infrared milk analyser (Milkoscan FT 6000®; Foss Electric, Hillerød, Denmark). Somatic cell count (SCC) was detected by flow cytometric measurement (Fossomatic 500®, Hillerød, Denmark). GL was determined in milk samples by Federal Office of Consumer Protection and Food Safety (BVL, Marienfelde, Berlin). Based on QuPPe-Method (Anastassiades *et al.* 2015), the samples were homogenised, water content adjusted to 100 % and glyphosate $^{13}C_2{}^{15}N$ was added as internal standard. Afterwards, samples were extracted with MeOH/Cyclohexan. and purified with acetonitrile. After degreasing by freezing, derivatisation was conducted with 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl). GL was analysed by LC-MS/MS equipped with electrospray ionisation source (negative mode). Confirmation was performed by diagnostic ions (precursor ion (*m/z*): 390, 167.9; production (*m/z*): 390, 149.9. The LOQ was 0.01 mg/kg (recovery rate 104 %) which is according to (SANTE/11945/2015) the lowest spike level of the validation with recoveries between 70 and 120 % and a within laboratory reproducibility $RSD_{WR} \leq 20$ %.
Blood samples were analysed for serum concentrations of β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and glucose, after centrifugation, using an automatic analysing system, based on photometric measurement (Eurolyser®, Type VET CCA, Salzburg, Austria).

Clinical examination
At weeks 0, 7 and 15, the general health status of all cows was evaluated by a modified clinical score according to Seyboldt *et al.* (2015); Dirksen *et al.* (2012). Scoring was performed by two veterinarians who were unaware of the treatment group at the examination. The score system for most parameters was 0–2 (0, no symptom; 1, moderate symptom; 2, severe symptom); however, the locomotion system got a score of 0–3 (0, no symptom; 1, mild symptom; 2, moderate symptom; 3, severe symptom). Those parameters leading to yes or no answers were scored only 0–1 (0, no symptom, 1, symptom). For evaluation, the data were summarised to four different symptom complexes, namely respiration and cardiovascular system including 22 tested parameters (max score of 35), gastrointestinal tract including 13 tested parameters (max score of 30). Furthermore, udder and locomotion system score were counted separately for each quarter of the udder and each leg. Consequently, udder health included five tested parameters (max score of 29), and locomotion system included 20 tested parameters (max score of 238). If a cow reached the maximal symptom score in all parameters of one symptom complex, we postulated 100 % illness in that complex. For the total cumulative health score, each of the individual complexes then counted as 25 %. For example, if one cow would reach 100 % in one complex, the total illness would result in 25 % whereas a cow with 0 % in each complex would be considered as completely healthy.

Calculations
The energy content of the experimental TMR was calculated based on nutrient digestibility measured with wethers (GfE 1991) and on equations for calculation of energy content in feedstuffs published by the Society of Nutrition Physiology (GfE 2001), as well as net energy requirement for maintenance (NE M), feed content and requirement for NE_L and milk energy:

$$NE_M\,[MJ\,NE_L/d] = 0.293 \times BW^{0.75}\,[kg]$$
$$NE_L\,content\,[MJ/kg\,feed] = 0.6 \times [1 + 0.004 \times (q - 57)] \times ME\,[\,MJ/kg\,]$$
$$NE_L\,requirement\,[MJ\,NE_L/d] = [Milk\,energy\,output\,[MJ\,NE_L/d\,] + 0.086] \times Milk\,yield\,[\,kg/d\,]$$
$$Milk\,energy\,[MJ\,NE_L/kg] = 0.38 \times Milk\,fat\,[\%] + 0.21 \times Milk\,protein\,[\%] + 0.95$$

FCM (4 % fat) was calculated based on the equation of Gaines (1928):
$$FCM\,[kg/d] = [(Milk\,fat\,[\%] \times 0.15) + 0.4] \times Milk\,yield\,[\,kg/d\,]$$

Energy-corrected milk (ECM) was calculated based on the equation of Sjaunja *et al.* (1990):
$$ECM\,[kg/d] = Milk\,yield\,[\,kg/d\,] \times$$
$$[(38.3 \times Milk\,fat\,[\,g/kg\,] + 24.2 \times Milk\,protein\,[\,g/kg\,] + 16.54 \times Milk\,protein\,[\,g/kg\,] + 20.7) / 3140]$$

Net energy (NE) balance was calculated as follows:

$$\text{NE balance [MJ NE}_L\text{/d] = Energy intake [MJ NE}_L\text{/d] - \{NE}_M\text{ [MJ NE}_L\text{/d] + NE}_L\text{ [MJ NE}_L\text{/d]\}}$$

Statistical analyses

Before data analyses, data of DMI, BW, NE balance and milk performance were condensed to a 14 d mean. Variables were all tested for normal distribution via visual histogram plot, only the number of cell counts had to be given as decimal logarithmic value. All statistical analyses were performed using the Software SAS (Version 9.2; SAS Institute Inc., Cary, North Carolina, USA). Parameters were analysed using the MIXED procedure for repeated measures (Littell *et al.* 1998). In case the variable showed significant effect between the groups in week 0, week 0 of that variable was set as covariable. For each variable covariance, structure was tested for compound symmetry (CS), autoregressive (1) AR (1) and unstructured (UN), and the model which proved the best Akaike information criterion for a finite sample size (AICC) was chosen. The model contained GL contamination (GL), concentrate feed proportion (CFP) and time (t) measured in trial weeks as fixed effects and the interaction between GL and CFP, GL and t, CFP and t and GL, CFP and t. Effects were declared as a trend if p-values were ≤ 0.10 and as significant if p-values were ≤ 0.05 after Tukey's test. Results are presented as Least Square (LS) Means ± standard error (SE) of LS means unless otherwise stated.

Results

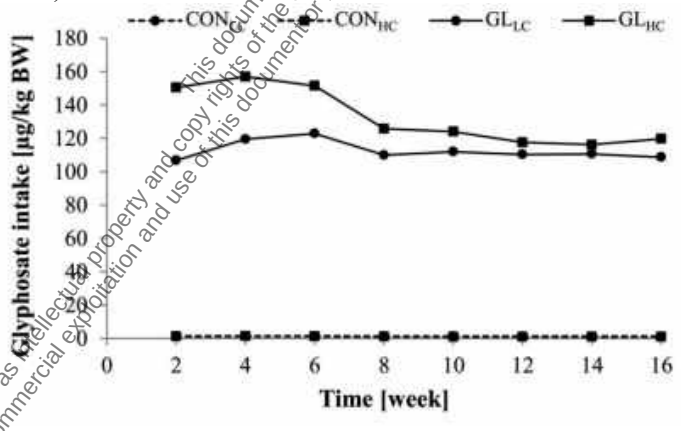
In total, 61 out of the initial 64 cows completed the entire trial. Two cows were excluded because of diseases not related to the experimental treatments. In the first week of the trial, one cow of group GL HC had an abomasal displacement. A cow of Group GL LC developed a general peritonitis in week 8. Another cow of Group GL LC became dry in trial week 11 and was excluded from the trial.
The chemical composition of the cows' ration components (Table 1) was within the normal range for the respective feedstuffs (DLG 1997). The average daily GL intake in Groups CON LC and CON HC was 0.8 and 0.8 mg/d, respectively, and in Groups GL LC and GL HC 73.8 and 84.5 mg/d, respectively. Figure 1 presents an overview of the cows' daily intake in the particular trial weeks in µg per kg BW).

Table 1: Ingredients of concentrate and chemical composition of the feedstuffs used in the total mixed ration (TMR).

	Concentrate composition[#]				Roughage[†]			
	Group CON_{LC}	Group CON_{HC}	Group GL_{LC}	Group GL_{HC}	Straw CON	Straw GL	Grass silage	Maize silage
Ingredients [% DM]								
Peas	29	29	-	-				
Peas GL treated	-	-	29	29				
Wheat	36	36	-	-				
Wheat GL treated	-	-	36	36				
Corn	26.8	30.8	26.8	30.8				
Urea	1.3	0.6	1.3	0.6				
Calcium carbonate	1.5	0.7	1.5	0.7				
Soybean oil	1	1	1	1				
Vitamin/mineral premix[5]	4.4	1.9	4.4	1.9				
Chemical composition								
Dry matter (DM) [g/kg]	881	884	885	881	899	895	324	345
Nutrients [g/kg DM]								
Crude ash	70	44	70	44	58	57	118	41
Crude protein	160	143	163	144	25	32	140	71
Ether extract	34	40	34	40	11	11	35	32
Crude fibre	34	34	34	35	436	440	298	230
$aNDF_{om}$[†]	106	116	111	115	806	808	550	402
ADF_{om}[♦]	43	45	42	46	504	507	326	237
Starch	589	610	591	612				322
Sugar	38	38	38	38				
Herbicide agent residue [mg/kg DM]								
Glyphosate	0.03	0.00	0.37	0.43	0.57	61.81	0.00	0.00
AMPA*	0.00	0.00	0.00	0.00	0.00	0.59	0.00	0.00

[#]CON, non-contaminated ration; GL, glyphosate contaminated ration; LC, low concentrate proportion (30% concentrate in TMR); HC, high concentrate proportion (60% concentrate in TMR); [†]composition (on DM basis of the TMR) in LC groups: 21% maize silage, 42% grass silage and 7% straw (GL or CON); in HC groups: 11% maize silage, 22% grass silage and 7% straw (GL or CON); [5]Provided per kg concentrate feed (according to manufacturer specification) for LC groups: 6.16 g Ca, 5.28 g Na, 3.52 g P, 2.2 g Mg, 0.31 g Zn, 0.21 g Mn, 0.06 g Cu; 4.4 mg I, 1.76 mg Se, 1.32 mg Co, 35 200 IU vitamin A, 4 400 IU vitamin D_3, 66 mg vitamin E; HC groups: 2.66 g Ca; 2.28 g Na; 1.52 g P; 0.95 g Mg; 0.13 g Zn; 0.09 g Mn; 0.02 g Cu; 1.9 mg I; 0.76 mg Se; 0.57 mg Co; 15 200 IU vitamin A; 1 900 IU vitamin D_3; 28.5 mg vitamin E. [†]$aNDF_{om}$, neutral detergent fibre without ash, amylase treated, [♦]ADF_{om}, acid detergent fibre without ash; *AMPA, aminomethylphosphonic acid (degradation product of GL). Values are presented as means.

Figure 1: Average daily glyphosate intake of the experimental groups per kg body weight (BW) (Values are presented as means). CON, non-contaminated ration; GL, glyphosate contaminated ration; LC, low concentrate proportion (30 % concentrate in TMR); HC, high concentrate proportion (60 % concentrate in TMR).



BCS, BW, water intake, DMI, NE intake and NE balance are shown in Table 2. These variables were not affected by GL treatment, no matter which CFP, while an interaction for CFP and t was observed ($p < 0.001$). The interactions were driven by the concentrate proportion in the ration presented with DMI in Figure 2. The mentioned performance parameters increased in HC groups and decreased in LC groups over the experimental time. This is also illustrated by the data of measured energy content, which was lower in LC groups (in Groups CON_{LC} and GL_{LC}, 6.6 and 6.6 NE_L MJ/kg DM, respectively) than in HC groups (CON_{HC}, and GL_{HC}, 7.1 and 7.2 NE_L MJ/kg DM, respectively). No interactions between GL and CFP were detected.

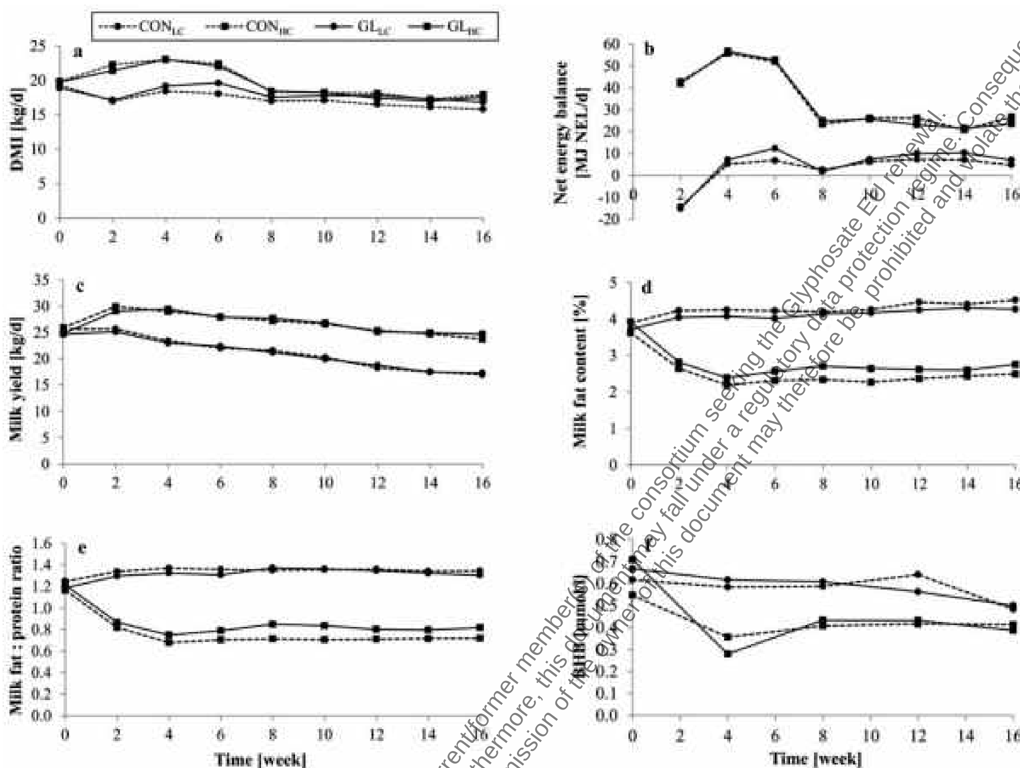
Table 2: Effects of glyphosate residues and concentrate feed proportion (CFP) in total mixed rations (TMR) on body condition score, body weight, dry matter intake (DMI), net energy intake and net energy balance.

	Control (CON)		Glyphosate (GL)		P-Value					
	LC[a] (n = 16)	HC[b] (n = 16)	LC (n = 14)	HC (n = 15)	GL	CFP	t	CFP × GL	CFP × t	GL × t
Body condition score	2.8 ± 0.1	3.2 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	0.469	0.018	<0.001	0.673	<0.001	0.689
Body weight [kg]	646 ± 5	675 ± 5	641 ± 5	669 ± 5	0.232	0.001	<0.001	0.240	<0.001	0.070
DMI [kg/d][c]	17.3 ± 0.3	19.7 ± 0.3	18.0 ± 0.3	19.4 ± 0.3	0.434	<0.001	<0.001	0.094	<0.001	0.767
Water intake [kg/d]	75.8 ± 2.7	82.3 ± 2.7	78.4 ± 2.9	84.1 ± 2.8	0.469	0.018	<0.001	0.619	0.007	0.281
Net energy intake [MJ NE_L/d]	112 ± 4	145 ± 4	112 ± 4	144 ± 4	0.990	<0.001	< 0.001	0.915	<0.001	0.845
Net energy balance [MJ NE_L/d]	3.3 ± 2.6	34.3 ± 2.6	5.1 ± 2.8	33.8 ± 2.7	0.769	<0.001	<0.001	0.631	<0.001	0.853
BHB[d] [mmol/l]	0.58 ± 0.02	0.43 ± 0.02	0.59 ± 0.02	0.45 ± 0.02	0.545	<0.001	<0.001	0.805	<0.001	0.222
Glucose [mg/dl]	59.6 ± 1.13	61.0 ± 1.13	60.8 ± 1.21	59.9 ± 1.17	0.987	0.937	<0.001	0.333	0.030	0.418
NEFA[e] [mmol/l]	0.22 ± 0.02	0.20 ± 0.02	0.22 ± 0.02	0.29 ± 0.02	0.912	0.175	<0.001	0.013	0.052	0.696

[a]LC, low concentrate proportion (30% concentrate in TMR); [b]HC, high concentrate proportion (60% concentrate in TMR); [c]time effect of trial week; [d]GL × CFP × t ($p > 0.05$) for all variables; [e]analysed with DMI week 0 as covariance factor; [f]BHB, β-hydroxybutyrate; [g]NEFA, non-esterified fatty acids. Values are presented as LS means ± SE (standard error).

The BHB concentrations in blood decreased in HC groups (CFP × t; $p < 0.001$), as presented in Figure 2. Glucose showed the same interaction (CFP × t; p = 0.030) but less pronounced, as presented in Table 2. NEFA concentrations in blood showed a trend for an interaction between CFP and t ($p = 0.052$). The higher NEFA concentrations in GL groups at the beginning of the trial and in Group GL_{HC} at the end of trial should not be interpreted due to the interaction between CFP and GL ($p = 0.013$) for this variable.

Figure 2: Effects of glyphosate residues and concentrate feed proportion in total mixed rations on dry matter intake (DMI) (A), net energy balance (B), milk yield (C), milk fat content (D), milk fat: protein ratio (E) and β-hydroxybutyrate (BHB) (F) during 16-weeks trial in established lactation period (Values are presented as LS means). CON, non-contaminated ration; GL, glyphosate contaminated ration; LC, low concentrate proportion (30 % concentrate in TMR); HC, high concentrate proportion (60 % concentrate in TMR).



Measurements of pooled milk samples revealed virtually no incidences of GL in milk (LOQ < 0.01 mg/kg). The time-dependent increase of milk yield in the HC groups and decrease in the LC groups resulted in an interaction (CFP × t; $p < 0.001$) presented in Figure 2. In contrast, LC groups increased and HC decreased in milk fat content and milk fat yield, milk protein yield, milk lactose yield, milk fat:protein ratio, milk urea (CFP × t; $p < 0.001$) and slightly in FCM (CFP × t; $p = 0.007$) and ECM (CFP × t; p = 0.076); all milk variables are presented in Table 3. The data of milk protein content was affected by t ($p < 0.001$) and CFP ($p = 0.036$) for all groups, while milk lactose content displayed a significant time-effect and a trend for an interaction between CFP and GL (CFP × GL; $p = 0.090$). An interaction between GL, CFP and t was found for milk yield and FCM ($p = 0.011$ and $p = 0.023$). Milk urea showed an interaction (GL × t; p = 0.004) between t and GL; this was due to slight differences between Groups CON_{HC} and GL_{HC} at the beginning of the experiment and disappeared in the course of the experiment. These differences were not significant but an impact on the detected interactions cannot be excluded.

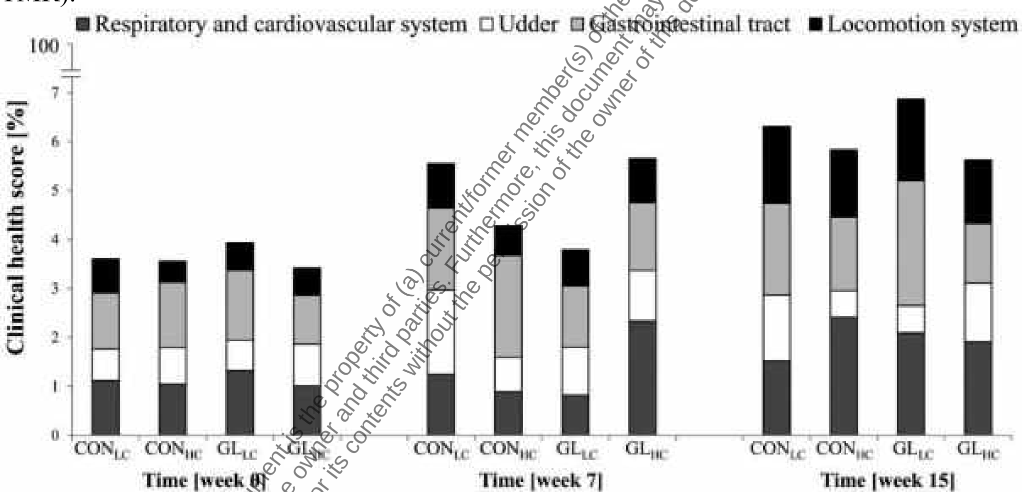
Table 3: Effects of glyphosate residues and concentrate feed proportion (CFP) in total mixed ration (TMR) on milk performance parameters.

	Control (CON)		Glyphosate (GL)		p-Value						
	LC[a] (n = 16)	HC[†] (n = 16)	LC (n = 14)	HC (n = 15)	GL	CFP	t[§]	CFP × GL	CFP × t	GL × t	GL × CFP × t
Milk yield [kg/d]	21.2 ± 1.0	26.7 ± 1.0	21.1 ± 1.1	26.7 ± 1.0	0.933	<0.001	<0.001	0.932	<0.001	0.855	0.031
Fat-corrected milk [kg/d]	22.6 ± 1.0	21.3 ± 1.0	21.9 ± 1.1	21.8 ± 1.0	0.905	0.523	<0.001	0.566	0.007	0.366	0.023
ECM[‡] [kg/d]	22.2 ± 0.9	22.1 ± 0.9	21.5 ± 1.0	22.7 ± 1.0	0.999	0.549	<0.001	0.484	0.076	0.696	0.179
ECM/dry matter intake [kg/kg][*]	1.26 ± 0.03	1.14 ± 0.03	1.22 ± 0.03	1.19 ± 0.03	0.945	0.036	<0.001	0.173	<0.001	0.931	0.080
Milk fat content [%]	4.27 ± 0.15	2.52 ± 0.15	4.11 ± 0.16	2.78 ± 0.15	0.749	<0.001	<0.001	0.170	<0.001	0.384	0.955
Milk fat yield [kg/d]	0.92 ± 0.04	0.69 ± 0.04	0.88 ± 0.05	0.74 ± 0.05	0.889	<0.001	<0.001	0.204	<0.001	0.612	0.910
Milk protein content [%]	3.19 ± 0.04	3.27 ± 0.04	3.13 ± 0.05	3.25 ± 0.05	0.343	0.036	<0.001	0.699	0.107	0.784	0.399
Milk protein yield [kg/d]	0.69 ± 0.03	0.88 ± 0.03	0.68 ± 0.03	0.87 ± 0.03	0.646	<0.001	<0.001	0.952	<0.001	0.033	0.240
Milk lactose content [%]	4.72 ± 0.05	4.69 ± 0.05	4.73 ± 0.05	4.86 ± 0.05	0.067	0.313	<0.001	0.090	0.249	0.194	0.221
Milk lactose yield [kg/d]	1.04 ± 0.05	1.27 ± 0.05	1.03 ± 0.05	1.31 ± 0.05	0.853	<0.001	<0.001	0.668	<0.001	0.709	0.509
Milk urea [mg/kg]	162 ± 7	77 ± 7	157 ± 7	88 ± 7	0.689	<0.001	<0.001	0.282	<0.001	0.004	0.453
Somatic cell count [log10/ml]	2.13 ± 0.10	2.31 ± 0.10	2.19 ± 0.10	2.28 ± 0.10	0.888	0.195	<0.001	0.649	0.289	0.770	0.911
Milk fat:protein ratio	1.34 ± 0.05	0.77 ± 0.05	1.31 ± 0.05	0.86 ± 0.05	0.520	<0.001	<0.001	0.230	<0.001	0.200	0.990

[a]LC, low concentrate proportion (30% concentrate in TMR); [†]HC, high concentrate proportion (60% concentrate in TMR); [§]t, time effect of trial week; [‡]ECM, energy corrected milk; [*]analysed with value of week 0 as covariance factor. Values are presented as LS means ± SE (standard error).

The average values of the total health score in Groups CON_{LC}, CON_{HC}, GL_{LC} and GL_{HC} were 5.2 ± 0.4, 4.5 ± 0.4, 4.9 ± 0.5 and 4.9 ± 0.5, respectively (LS means ± SE). The total health score (Figure 3) showed for all groups a time effect (t; $p < 0.001$) and an interaction between all three tested values (CFP × t × GL; $p = 0.010$).

Figure 3: Average daily glyphosate intake of the experimental groups per kg body weight (BW) (Values are presented as means). CON, non-contaminated ration; GL, glyphosate contaminated ration; LC, low concentrate proportion (30 % concentrate in TMR); HC, high concentrate proportion (60 % concentrate in TMR).



Discussion

GL is worldwide the most used active substance in non-selective herbicides in agriculture ([redacted] 2008). According to [redacted] *et al.* (2016), Krüger *et al.* (2013); Krüger *et al.* (2014a)) and Rulff *et al.* (2016) dairy cows are exposed to 0.08–0.9 mg GL per day due to GL contamination in common dairy cow rations. Up to now, the effects of GL on health of dairy cows was solely deduced from field observations and in vitro studies. Therefore, the present exact-feeding experiment on dairy cows in practical conditions was designed to investigate the effects of GL-contaminated feedstuffs which were generated by a legal application and represent a worst-case scenario. Here, the daily exposure was approximately four-fold higher than the maximum observed under average feeding conditions as outlined above.

The cows were fed two different crude fibre and concentrate proportions in their rations with the intention to investigate whether the overall effects of GL depend on different ruminal conditions as triggered by

different concentrate feed proportions. The average daily GL intake in GL groups amounted to 79.1 mg and in both groups straw formed the major GL source. GL was used by spray application, so that the plants' surface was the most contaminated part. This may explain the high straw contamination and the rather small contamination of peas and wheat kernels which are protected by their husks and pods. In CON groups, a small daily GL intake with an average value of 0.8 mg/d was observed. This corresponds to the average value of GL concentration in usual dairy rations (von Soosten *et al.* 2016). The half-life of GL soils residues varies between 2.8 and 500.3 d DT_{50} (50 % dissipation time) (EFSA 2015). Therefore, GL contamination in plants might be originated from soil residues. Overall, GL exposure in GL groups was about 100 times higher than in CON groups. In this study, 121 milk samples were analysed for GL and AMPA. No positive findings above the validated LOQ could be reported. These findings correspond to the study of von Soosten *et al.* (2016), who reported that milk was virtually free from GL and AMPA, while 8 % of daily consumed GL were excreted in urine and 61 % passed the digestive tract of dairy cows unmetabolised and were excreted with faeces. The high proportion of GL excreted with faeces also indicates a high concentration of unmetabolised GL in the gastrointestinal tract and the possibility for GL to interact with microorganisms within the ingesta passage time. The chemical composition of the cows ration offered the aimed components within the normal range for the respective feedstuffs with a high fibre content and low energy in LC groups and a low fibre content and high energy in HC feed groups (DLG 1997). Therefore, our experimental feeding design offered adequate conditions to test the effect of GL in rations with different concentrate parts on performance, energy metabolism and health characteristics of dairy cows.

Performance and health

In the present trial, the drop of DMI and the negative energy balance in LC groups at the beginning might be caused by the required adaptation to the experimental ration. The ME and NE_L concentrations of feed confirm the intended differences in energy supply between LC and HC groups, GL showed no influence on both of them and no differences between GL and CON groups were detectable.

Consequently, BW and BCS, NE intake and NE balance were affected by the concentrate feed proportion of the ration, but GL contamination had no influence on the parameters in both rations. The results are in accordance with the results of Donkin *et al.* (2003), who found a similar DMI of GL-tolerant RoundupReady corn sprayed with Roundup Ultra® (Monsanto Company, St. Lois, MO) and non-transgenic control corn.

Milk yield differed in accordance to the concentrate proportion of the ration and dropped slightly over time due to the advanced lactation period. Based on field observations, Krüger *et al.* (2014b) postulated milk yield decrease in GL fed cows; this could not be proven by our feeding trial. There was no change in the amount and composition of milk provable in GL groups compared to CON groups. Donkin *et al.* (2003) could not detect any influence of GL on milk components and on dairy cow performance. The lack of influence of GL on milk components might be related to the absence of GL residues in milk, demonstrating that milk is no major excretion pathway of GL. Consequently, direct effects of GL residues on synthesis of milk components in the mammary gland can be most probably excluded.

On the contrary, different dietary energy levels exhibited significant effects on concentrations and amounts of milk protein, milk fat content, milk lactose, milk urea, SSC and milk fat/protein ratio.

General energy metabolism was not adversely influenced by dietary treatments as blood NEFA, BHB and glucose values were in the normal reference range (Kraft and Dürr 2005). However, BHB levels were significantly influenced by dietary energy level. The overall blood BHB levels might result either from ketogenesis or from ruminal nutrient metabolism. Thus, the higher BHB levels in cows fed the LC diets might reflect a diet induced higher ruminal release of butyrate and/or a slight energy deficit compared to their HC-fed counterparts. Both dietary energy levels were not influenced by GL.

Regarding the putative health effects of GL, Rulff *et al.* (2016) considered GL being a part of pathogenesis of downer cow syndrome. Furthermore, Krüger *et al.* (2013, 2014b) and Ackermann *et al.* (2015) related possible symptoms of *C. botulinum* disease (drop of milk production, mobility disorders, retracted abdomen and forced respiration) to a forced production of BoNT, probably as a result from a decline of enterococci population in gastrointestinal tract. This effect should be more pronounced in high

fibre rations. Krüger *et al.* (2013) termed GL reasonable for the imbalance of the microorganisms, whereas Riede *et al.* (2016) could not show any effect of GL on microorganisms in their RUSITEC study. It should be noted that both were in vitro studies which are not able to represent realistic exposure conditions. But similar results were found in a study about the effect of GL contaminated feed on wethers, where GL showed no indication of an impairment of rumen bacteria or a shift in rumen microbial population, neither the group of cellulolytic bacteria, nor the group of amylolytic bacterial species (Hüther *et al.* 2005).

In our study, the general health status including the previously mentioned symptoms were evaluated. The general health status of cows is, among other factors, related to the health of the rumen microbiome (Zebeli *et al.* 2015). In our study, the cows showed less than 10 % symptoms in total clinical health score. Symptoms occurred without discernible pattern, for instance both GL groups were scored less than CON_{LC} but more than CON_{HC}. Despite the clear influence of the different concentrate proportions of the rations on several parameters, which probably caused very different gastro-intestinal microbial conditions, an effect of GL-residues in feed on performance, metabolism and health characteristics of dairy cows could not be observed in the present trial.

3. Assessment and conclusion

Assessment and conclusion by applicant:
About 30 cows (distributed in two subgroups) were fed with glyphosate-treated commodities for 17 weeks. During this period the exposure of these cows to parent glyphosate residues via feed was about 0.110-0.120 mg/kg bw/day (Figure 1). None of the analysed milk samples (presumably about 60 pooled samples from the two subgroups fed with glyphosate-treated commodities) showed residues of parent glyphosate or AMPA above the limit of quantification of 0.01 mg/kg. This is fully in line with the results of the GLP cow feeding studies submitted in the dossier, which also show that the transfer (if any) of glyphosate-derived residues in cow milk is extremely low. Although the residue analytical method and residue analyses are not reported with a high level of detail, the results are considered reliable since the general principle of the described analytical procedures is well known and the validity of the residue determination was obviously demonstrated by suitable fortification trials. The publication, therefore, is considered relevant and reliable.
However, the main objective of the publication was to investigate the impact of glyphosate residues in feed on health and performance of dairy cows. No significant effects were identified but this part of the publication is not considered relevant to the residue section.

1. Information on the study

Data point	CA 6.4.2/006
Report author	Von Soosten, D. *et al.*
Report year	2016
Report title	Excretion pathways and ruminal disappearance of glyphosate and its degradation product aminomethylphosphonic acid in dairy cows
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Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

From 6 balance experiments with total collection of feces and urine, samples were obtained to investigate the excretion pathways of glyphosate (GLY) in lactating dairy cows. Each experiment lasted for 26 d. The first 21 d served for adaptation to the diet, and during the remaining 5 d collection of total feces and urine was conducted. Dry matter intake and milk yield were recorded daily and milk and feed samples were taken during the sampling periods. In 2 of the 6 experiments, at the sampling period for feces and urine, duodenal contents were collected for 5 d. Cows were equipped with cannulas at the dorsal sac of the rumen and the proximal duodenum. Duodenal contents were collected every 2 h over 5 consecutive days. The daily duodenal dry matter flow was measured by using chromium oxide as a volume marker. All samples (feed, feces, urine, milk and duodenal contents were analyzed for GLY and aminomethylphosphonic acid (AMPA). Overall, across the 6 experiments (n = 32) the range of GLY intake was 0.08 to 6.67 mg/d. The main proportion (61 ± 11 %; ± SD) of consumed GLY was excreted with feces; whereas excretion by urine was 8 ± 3 % of GLY intake. Elimination via milk was negligible. The GLY concentrations above the limit of quantification were not detected in any of the milk samples. A potential ruminal degradation of GLY to AMPA was derived from daily duodenal GLY flow. The apparent ruminal disappearance of GLY intake was 36 and 6 %. In conclusion, the results of the present study indicate that the gastrointestinal absorption of GLY is of minor importance and fecal excretion represents the major excretion pathway. A degradation of GLY to AMPA by rumen microbes or a possible retention in the body has to be taken into account.

Materials and Methods

Six balance experiments with collection of total urine and feces were conducted at the experimental station of the Institute of Animal Nutrition, Friedrich-Loeffler-Institut, Brunswick, Germany. The experiments were approved by the Lower Saxony State Office for Consumer Protection and Food Safety, Oldenburg, Germany

Animals, Feeding, and Design of the Experiments

Overall, the 6 experiments included 32 lactating dairy cows of the German Holstein breed. For experiments 1 to 6, we used 5, 6, 5, 4, 6, and 6 animals per experiment, respectively. The animals were, on average, 90 DIM and in their second to fifth lactation. All cows were fitted with rumen and duodenum cannulas and were housed in a tiestall barn. Milking took place twice daily at 05:30 and 15:30 h. The animals were fed at the milking times. In all experiments the diet was based on maize silage (single forage component) and concentrates in different proportions (Table 1). The composition of the concentrates as well as the GLY and AMPA concentrations in the concentrates are shown in Table 2. Each balance experiment lasted 26 d. The first 21 d were allowed for equilibration to the experimental diet and the remaining 5 d were the sampling period. In experiments 1 and 2, the quantitative collection of urine and feces was followed by the quantification of daily duodenal dry matter flow (DMF) for 5 consecutive days

Table 1: Forage-to-concentrate ratio of the diet during the experiments.

Experiment	Maize silage (%)	Concentrate (%)
1	60	40
2	60	40
3	70	30
4	55	45
5	70	30
6	70	30

Measurements and Sample Collection

During the sampling period, DMI and milk yield were recorded in each individual animal daily. Feed samples for maize silage were taken twice and concentrate samples once during the sampling period.

Milk samples were taken once at morning and evening milking in the sampling periods. Total collection of feces and urine was conducted over 5 consecutive days. Cows were equipped with urine devices for separated drain of urine. The device was manufactured of artificial leather and was fitted and agglutinated around the vulva and pins. A polyvinylchloride tube drained the urine into a canister. The feces were collected in a stainless steel tub, which was positioned below a perforated floor at the end of the tiestall. The urine canister and feces tub were emptied once per day at the same time. Urine and feces were weighed and homogenised. Two percent of the daily fecal amounts were sampled and given into a pooled sample over the 5 consecutive days. A urine sample of 100 mL was taken from total urine volume each day. Urine and feces samples were stored at −20°C until analysis.

In experiments 1 and 2 a chromium oxide (Cr_2O_3) marker (19.8 % Cr_2O_3, 79.1 % wheat flour and 0.67 % aluminum sulfate) was introduced into the rumen via the rumen cannula and was used as a marker for quantitative measurement of the daily duodenal DMF. The administration of Cr_2O_3 was started 11 d before collection of duodenal chyme. Two portions of 50 g of Cr_2O_3 were administered every 12 h. During the duodenal chyme sampling period and 1 d before, 4 portions of 25 g of Cr_2O_3 were given every 6 h. Samples of duodenal contents were taken every 2 h during the 5 consecutive days of sampling. At each sampling, 100 mL of duodenal contents were collected and pooled over 24 h. The samples were stored at −20°C. The individual animal DMI was recorded and samples of the feedstuffs were retained according to the same pattern during the total collection period of urine and feces. Body weight was recorded before the start and after the end of an experiment.

Analyses
Samples of maize silage were dried at 60°C for 72 h. Duodenal contents and feces samples were freeze-dried for determination of DM. The feedstuffs and duodenal and fecal samples were ground through a 1-mm sieve. Aliquots of the morning and evening milk samples were pooled according to their proportion of total daily milk yield. The urine samples were thawed and pooled over the 5 consecutive sampling days according to their daily proportion of the total urine amount over the sampling period. In the daily duodenal samples the chromium concentrations were measured using an inductively coupled plasma optical emission spectrometer (Quantima, GBC Scientific Equipment Pty Ltd., Victoria, Australia) after sample preparation according to Williams *et al.* (1962). The chromium concentration was used to calculate the daily duodenal DMF. According to the daily duodenal DMF on the 5 sampling days, one aliquot pooled sample was generated per cow per 5 sampling days.
All samples were analyzed for GLY and AMPA in accredited laboratories. Feed and milk samples were analyzed by Wessling GmbH (Altenberge, Germany) and feces, urine, and duodenal chyme by Medizinisches Labor Bremen (Bremen, Germany). In milk and feed samples GLY and AMPA were extracted with formic acid (0.1 %) and methylene chloride. Derivatisation was conducted with fluorenylmethoxycarbonyl chloride. After solid phase extraction, GLY and AMPA were determined by using LC-MS/MS.

In feces, urine, and duodenal chime, GLY and AMPA were extracted with water. Derivatisation was conducted with trifluoroacetic anhydride and trifluoroethanol. Glyphosate and AMPA were determined by GC-MS/MS.

For all GLY and AMPA analyses an internal standard containing 1,2–$^{13}C_2$ ^{15}N GLY (1 µg/mL) and ^{13}C ^{15}N AMPA (1 µg/mL) was used. The limit of detection (LOD) and the limit of quantification (LOQ) for each substance was calculated form the signal-to-noise ratios. This ratio was 3 for the LOD and 10 for the LOQ. For both GLY and AMPA in feed samples, the LOQ and LOD was 0.02 and 0.007 mg/kg, respectively. For all other matrices the LOQ was 0.01 mg/kg and the LOD was 0.003 mg/kg. The recoveries for GLY and AMPA analyses in feed and milk samples were 70 to 120 % using an internal standard concentration of 0.625 mg/kg for feed analyses and 0.25 mg/kg for milk analyses. Recoveries for GLY in feces, urine, and duodenal content were 80 to 90, 98 to 101, and 96 to 102 %, respectively. Recoveries for AMPA analyses in feces, urine, and duodenal content were 80 to 95, 80 to 101, and 94 to 106 %, respectively. For determination of the recoveries in feces, urine, and duodenal content the internal standard concentration was 0.1, 0.001, and 5 mg/kg, respectively.

Calculations

Apparent GLY and AMPA retention was calculated with the following equation:

Apparent GLY/AMPA retention (mg/d) = GLY/AMPA intake (mg/d) – fecal excretion of GLY/AMPA (mg/d) – urinary excretion of GLY/AMPA (mg/d) – milk excretion of GLY/AMPA (mg/d).

Daily duodenal DMF and duodenal GLY/AMPA flow were calculated as follows:

DMF (kg/d) = [chromium application (mg/d)/duodenal chromium concentration (mg/g of DM)]/1000,

and

Daily duodenal GLY/AMPA flow (mg/d) = DMF (kg/d) × duodenal GLY/AMPA concentration (mg/kg DM).

Ruminal disappearance of GLY and AMPA was calculated with the following equation:

Ruminal disappearance of GLY/AMPA (mg/d) = GLY/AMPA intake (mg/d) – duodenal GLY/AMPA flow (mg/d).

Results

The determined GLY and AMPA concentrations of the individual sample matrices differed between the experiments. Only in experiment 4 could GLY be detected in the maize silage (0.035 mg/kg of DM). Maize silage in all other experiments contained GLY lower than the LOQ; AMPA was not detected in any of the maize silages. The GLY concentration in the concentrates ranged from 0.02 to 0.95 mg/kg of DM and AMPA concentrations ranged from a value lower than the LOQ to 0.65 mg/kg of DM. Therefore, the concentrates were the main source for exposure of GLY and AMPA in all experiments (Table 2). In urine and feces GLY concentrations ranged from 0.20 to 75.1 µg/L and 0.01 to 0.88 mg/kg of DM, respectively.

Table 2: Composition and concentrations of glyphosate and aminomethylphosphonic acid in the concentrates of the different experiments.

Component (%, unless noted)	Experiment					
	1	2	3	4	5	6
Soybean meal	20	20	25	15		
Rape seed meal			13			
Barley grain	22	22		14.7		
Wheat grain	22	22	36.5	29		
Wheat gluten					10	10
Maize grain	18	18		29	35	35
Sugar beet pulp, dried	15	15	18.3	8.4	48	48
Urea	2	2	2.5	1	3	3
Calcium carbonate/dicalcium phosphate			2.5	1.5	1.5	1.5
Sodium chloride			0.2	0.2	0.2	0.2
Mineral and vitamin-mix	1	1	2.0	1.2	2.3	2.3
Glyphosate (mg/kg of DM)	0.95	0.48	0.82	0.08	0.02	0.03
AMPA[1] (mg/kg of DM)	0.65	0.43	0.46	<LOQ[2]	<LOQ	0.02

[1]Aminomethylphosphonic acid (degradation product of glyphosate).
[2]<LOQ = AMPA concentrations in the samples were lower than the limit of quantification (LOQ).

In experiment 1 the highest GLY intake (6.7 mg/d) was observed. The lowest GLY intake (0.08 mg/d) was found in experiment 6 (Table 3). In accordance to the GLY intake, the excretion of feces (4.3 mg/d) and urine (0.44 mg/d) were highest in experiment 1. In experiment 5 the excretion of feces and urine were lowest, at 0.02 and 0.08 mg/d, respectively. In all milk samples the GLY concentration was below the LOQ.

Table 3: Intake and fecal and renal excretion of glyphosate in animals during the sampling period (means ± SD).

Experiment (animals)	Intake in feed (mg/d)	Excretion in feces (mg/d)	Excretion in urine (mg/d)
Experiment 1 (n = 5)	6.67 ± 0.02	4.34 ± 0.35	0.44 ± 0.08
Experiment 2 (n = 6)	3.36 ± 0.01	2.04 ± 0.24	0.26 ± 0.10
Experiment 3 (n = 5)	3.36 ± 0.00	1.69 ± 0.41	0.12 ± 0.02
Experiment 4 (n = 4)	0.53 ± 0.02	0.39 ± 0.05	0.04 ± 0.00
Experiment 5 (n = 6)	0.15 ± 0.21	0.02 ± 0.03	0.08 ± 0.10
Experiment 6 (n = 6)	0.08 ± 0.07	0.04 ± 0.06	0.13 ± 0.14
Experiment 1–6 (n = 32)	2.36 ± 2.61	1.42 ± 1.67	0.18 ± 0.15

The results for AMPA intake and excretion are presented in Table 4. The AMPA intake was on a lower level compared with GLY intake. The highest AMPA intake was observed in experiment 1 (4.57 mg/d) and the lowest in experiment 5 (lower than the LOQ; Table 4). Considerable excreted amounts of AMPA with feces and urine were only observed in experiments 1, 2, and 3. The excretion with feces was lower than the LOQ in experiments 4, 5, and 6. In the same experiments the excretion with urine (0.01 mg/d) was marginal; AMPA concentrations in milk were below the LOQ in all experiments.

Table 4: Intake and fecal and renal excretion of AMPA[1] in animals during the sampling period (means ± SD).

Experiment (animals)	Intake in feed (mg/d)	Excretion in feces (mg/d)	Excretion in urine (mg/d)
Experiment 1 (n = 5)	4.57 ± 0.21	2.25 ± 0.23	0.41 ± 0.05
Experiment 2 (n = 6)	3.03 ± 0.05	1.51 ± 0.21	0.36 ± 0.09
Experiment 3 (n = 5)	1.89 ± 0.00	0.83 ± 0.19	0.15 ± 0.03
Experiment 4 (n = 4)	<LOQ[2]	<LOQ	<LOQ
Experiment 5 (n = 6)	<LOQ	<LOQ	<LOQ
Experiment 6 (n = 6)	0.05 ± 0.04	<LOQ	<LOQ
Experiment 1–6 (n = 32)	1.30 ± 1.57	0.77 ± 0.89	0.16 ± 0.18

[1]AMPA = aminomethylphosphonic acid.
[2]<LOQ = AMPA concentrations in feed, feces or urine samples were lower than the limit of quantification (LOQ), and therefore the intake as well as the excretion with feces and urine was considered as zero.

The duodenal flows of GLY and AMPA in experiments 1 and 2 (measurement subsequent to total collection of feces and urine) are shown in Table 5. The intakes of GLY and AMPA during the duodenal sampling period were different in the 2 experiments, with highest intakes in experiment 1. However, the duodenal flows of GLY and AMPA were in a similar range. In both experiments an apparent ruminal disappearance occurred for both substances; 2.27 mg/d disappeared in experiment 1 and 0.19 mg/d of GLY disappeared in the rumen in experiment 2.

Table 5: Glyphosate and AMPA[1] intake and flow at the duodenum (means ± SD) during times of duodenal sampling followed after the balance experiment 1 and 2.

Experiment (animals)	Intake in feed (mg/d)	Flow at the duodenum (mg/d)	Apparent ruminal disappearance (mg/d)
Glyphosate			
Experiment 1 (n = 5)	6.24 ± 1.61	3.97 ± 0.83	2.27 ± 1.66
Experiment 2 (n = 6)	3.38 ± 0.14	3.20 ± 0.48	0.19 ± 0.34
AMPA			
Experiment 1 (n = 5)	3.54 ± 0.81	2.16 ± 0.57	1.38 ± 1.00
Experiment 2 (n = 6)	2.66 ± 0.11	2.32 ± 0.22	0.34 ± 0.18

[1]AMPA = aminomethylphosphonic acid.

Fecal, renal, and mammary excretion of GLY and the apparent retention of GLY, expressed as percentage of intake, are presented in Table 6. Due to very low intakes of GLY in experiments 5 and 6 these variables were not calculable. Overall, the ratio of GLY intake to excretion of GLY via feces or urine remained independent from the level of GLY intake and averaged 61 ± 11 % (fecal; mean ± SD) and 8 ± 3 % (renal). The mammary excretion was 0 % and the apparent retention 31 ± 13 %.

Table 6: Fecal, renal, and mammary glyphosate excretion as well as apparent retention expressed as proportion of glyphosate intake (means ± SD).

Experiment (animals)	Fecal (% of intake)	Renal (% of intake)	Mammary (% of intake)	Apparent retention (% of intake)
Experiment 1 (n = 5)	65 ± 5	7 ± 1	<LOQ[1]	28 ± 5
Experiment 2 (n = 6)	61 ± 7	8 ± 1	<LOQ	31 ± 10
Experiment 3 (n = 5)	50 ± 12	4 ± 1	<LOQ	46 ± 13
Experiment 4 (n = 4)	73 ± 10	8 ± 1	<LOQ	19 ± 10
Experiment 5 (n = 6)	NC[2]	NC	NC	NC
Experiment 6 (n = 6)	NC	NC	NC	NC
Experiment 1–4 (n = 20)	61 ± 11	8 ± 3	<LOQ	31 ± 13

[1]<LOQ = GLY concentrations in milk samples were lower than the limit of quantification (LOQ) and therefore the transfer into milk was considered as zero.
[2]NC = not calculated due to very low glyphosate intake in experiment 5 and 6 (<0.15 mg/d).

For AMPA the fecal, renal, and mammary excretion as well as apparent retention were not calculable for experiments 4, 5, and 6. In experiments 1, 2, and 3, the average fecal and renal excretion were 48 ± 8 and 10 ± 3 %, respectively. The mammary excretion was 0 % and the apparent retention was 42 ± 9 % (Table 7).

Table 7: Fecal, renal, and mammary AMPA[1] excretion as well as apparent retention expressed as proportion of AMPA intake (means).

Experiment (animals)	Fecal (% of intake)	Renal (% of intake)	Mammary (% of intake)	Apparent retention (% of intake)
Experiment 1 (n = 5)	50 ± 6	9 ± 1	0	42 ± 6
Experiment 2 (n = 6)	50 ± 6	12 ± 3	0	38 ± 9
Experiment 3 (n = 5)	44 ± 10	8 ± 2	0	48 ± 11
Experiment 4 (n = 4)	NC[2]	NC	NC	NC
Experiment 5 (n = 6)	NC	NC	NC	NC
Experiment 6 (n = 6)	NC	NC	NC	NC
Experiment 1–3 (n = 16)	48 ± 8	10 ± 3	0	42 ± 9

[1]AMPA = aminomethylphosphonic acid.
[2]NC = not calculated due to very low AMPA intake in experiment 4, 5, and 6 (<0.06 mg/d).

Discussion

The data from the present study represent the first results on GLY balance data in lactating cows and are therefore of high scientific relevance. In the present study a broad range of GLY exposition (0.08–6.7 mg/d) of the cows was measured. On average, cows were exposed daily to 4 µg of GLY/kg of BW. The maximum exposure of the cows was observed in experiment 1 (11 µg of GLY/kg of BW), and the minimum exposure was in experiment 6 (0.1 µg of GLY/kg of BW). The highest GLY contamination was observed in the concentrates of experiments 1 to 4. The average proportion of soybean meal in these concentrates was 22 % and the average GLY concentration was 0.58 mg/kg. If the greatest extent of GLY originated from soybean meal, the concentration of GLY in this ingredient should be 4.5 times higher than in the complete concentrate; this would result in values of approximately 3 mg/kg for soybean meal. This value is in the range of GLY concentrations (0.4–8.8 mg/kg) observed in genetically modified soybeans (Bøhn *et al.*, 2014) and leads to the assumption that GLY in the present investigation originated mainly from soybean meal.

In the present study 61 ± 11 % of the ingested GLY was excreted in feces and passed the gastrointestinal tract of the dairy cows unmetabolised. The excretion with urine (8 ± 3 % of daily intake) was the second important excretion pathway. In studies with rats, the elimination of ingested GLY with urine was approximately 30 % (Brewster *et al.*, 1991; Chan and Mahler, 1992). The difference in urinary

elimination of GLY between species might be explained by a possible higher gastrointestinal degradation of GLY to AMPA in dairy cows compared with rats. Gerlach *et al.* (2014) observed GLY concentrations of approximately 5 to 20 µg/L in urine samples of dairy cows. However, in Gerlach *et al.* (2014), neither the urine volume nor the GLY intake was measured and GLY excretion was not determined quantitatively. The GLY concentrations in urine of the present study ranged between values lower than the LOQ and 75.1 µg/L and suggest a representative range for conventional feeding conditions in dairy cows. A dietary intake lower than 10 mg/d as measured in the present study did not result in GLY excretion via milk. In all milk samples the GLY concentrations were below the LOQ. Under the conditions of the present study milk was no excretion pathway for GLY, but these results should be verified by further investigations, especially with higher daily GLY intakes.

For the remaining 31 ± 13 % of GLY that was not excreted with feces and urine, degradation by rumen microbes could be relevant. For experiments 1 and 2, with the highest GLY intake per day, the duodenal flow of GLY was 36 and 6 % lower compared with the daily intake, respectively. These results suggest that GLY might have been degraded in the rumen. Jacob *et al.* (1988) and Heitkamp *et al.* (1992) described microbes originating from soil (Pseudomonas sp. strain LBr) with the ability to degrade GLY to AMPA. In contrast to dairy cows, metabolism of GLY in rats was 7 % (Anadón *et al.*, 2009) or less than 1 % (Brewster *et al.*, 1991). The relevance of rumen microbes for degradation of GLY has to be clarified in further studies.

The fact that a high proportion of GLY (61 ± 11 %) passes the rumen and intestine unmetabolised is important regarding potential effects of GLY on microbes in the gastrointestinal tract. In recently published studies, a relationship of GLY to the development of chronic visceral botulism in dairy cows was hypothesised (Krüger *et al.*, 2013; Gerlach *et al.*, 2014). For ruminants, only a few studies are available regarding the effects of GLY on microbial community and ruminal fermentation parameters. Riede *et al.* (2014) found no effects of GLY on ruminal fermentation and microbial community in vitro. These results agreed with results of a study in wethers by Hüther *et al.* (2005), which showed no effects of GLY on pH value and concentration of VFA in rumen fluid. Further research is necessary to clarify whether the unmetabolised GLY in the gastrointestinal tract may affect rumen microbes.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The publication describes a series of 6 experiments in which dairy cows (n = 4-6 per experiment) were fed with glyphosate-treated feed for 26 days and where the excretion of parent glyphosate and AMPA residues via feces, urine and milk was investigated during the last 5 days of the experiments (i.e. at a time when steady state can be assumed). The intake of parent glyphosate residues ranged between < 0.001 mg/kg bw/day (experiments 4, 5 and 6) and 0.011 mg/kg bw/day (experiment 1) while the intake of AMPA residues ranged between < 0.001 mg/kg bw/day (experiments 4, 5 and 6) and about 0.008 mg/kg bw/day (experiment 1). These intake levels are far below the dose levels investigated in the goat metabolism studies and cow feeding studies submitted in the dossier (since the applicable guidelines require that the dose levels be higher) but are likely to reflect "typical" intake levels of dietary cows. In the experiments it was found that 50-73 % of ingested glyphosate was excreted in feces and 4-8 % in urine. Similarly, 44-50 % of ingested AMPA was excreted in feces and 8-12 % in urine (these figures assume that no glyphosate is metabolised to AMPA in the cows). These results are consistent with the results of the submitted goat metabolism studies which show that 47-78 % of the administered radioactivity is excreted via feces and 4.7-23 % via urine. The residues of parent glyphosate and AMPA in milk were below the limit of quantification of 0.01 mg/kg, which is consistent with the results of the GLP cow feeding studies submitted in the dossier. Although the residue analytical method and residue analyses are not reported with a high level of detail, the results are considered reliable since the general principle of the described analytical procedures is well known and the validity of the residue determination was obviously demonstrated by suitable fortification trials. The publication, therefore, is considered relevant and reliable.

CA 6.4.3 Pigs

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.4.3/001
Report author	[redacted]
Report year	1987
Report title	Residue determination of Glyphosate and AMPA in swine tissues following a 28 day feeding study
Report No	[redacted]-6627
Document No	M-651049-01-1
Guidelines followed in study	US EPA: Subdivision O, Pesticide Assessment Guidelines for Residue Chemistry
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 505: • Age and breed of the pigs not reported • Only 2 animals per treatment group • For meat other pieces than loin, flank or hind-leg collected (triceps, gracilis, and longissimus dorsi muscle) • Depuration phase with only 1 interval instead of 3 intervals • Insufficient detail provided in the study report to determine the interval of sample frozen storage before extraction and analysis.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate (N-phosphonomethyl glycine) and AMPA (aminomethylphosphonic acid) in tissues of swine dosed with glyphosate and AMPA for a period of 28 consecutive days, and 28 days after dosing ended (i.e., after a withdrawal period of 28 days).

Glyphosate and AMPA (in a 9:1 ratio) were administered to swine through dietary intake for a period of 28 consecutive days with use of a feed diet that was fortified with glyphosate and AMPA at each of three levels (1X, 3X, and 10X treatment groups). The nominal concentrations of glyphosate in the diet for the 1X, 3X, and 10X treatment groups were 36 ppm (mg/kg feed), 108 mg/kg feed, and 360 mg/kg feed, respectively. The nominal concentrations of AMPA in the diet for the 1X, 3X, and 10X treatment groups were 4.0 mg/kg feed, 12 mg/kg feed, and 40 mg/kg feed, respectively. Measured levels of glyphosate and AMPA attained in the swine diet were near nominal values. Actual levels of glyphosate (not corrected for recovery) in the 1X, 3X, and 10X treatments groups in feed on a dry weight basis averaged 33.4 mg/kg, 105.2 mg/kg, and 340.2 mg/kg, respectively. Expressed on a body weight basis, the average dose levels of glyphosate in the 1X, 3X, and 10X groups were 0.98 mg/kg bw/day (1.12 mg/kg bw/day without animal

M00142, which was sacrificed early due to health issues), 3.24 mg/kg bw/day, and 11.13 mg/kg bw/day, respectively. The actual levels of AMPA (not corrected for recovery, expressed as AMPA) in the 1X, 3X, and 10X treatments groups in feed on a dry weight basis averaged 3.6 mg/kg, 11.0 mg/kg, and 36.8 mg/kg, respectively. Expressed on a body weight basis, the average dose levels of glyphosate (expressed as glyphosate) in the 1X, 3X, and 10X groups were 0.11 mg/kg bw/day, 0.34 mg/kg bw/day, and 1.20 mg/kg bw/day, respectively.

The analytical method LOQ for glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) was 0.05 mg/kg in fat, muscle, liver, and kidney.

The residue values presented in the summary of the study report had been corrected for recovery. The residue values described below were not corrected for recovery.

Residues of glyphosate and AMPA in all fat samples (days 1–56) from the 1X, 3X, and 10X treatment groups were below the LOQ (<0.05 mg/kg).

Residues of glyphosate and AMPA in all muscle samples (days 1–56) from the 1X, 3X, and 10X treatment groups were below the LOQ (<0.05 mg/kg) except for the day 28 samples of the 10X treatment, which contained 0.054 mg/kg glyphosate on average.

The average levels of glyphosate in liver samples at the end of the 28-day dosing period in the 3X and 10X treatment groups were 0.163 mg/kg and 0.598 mg/kg, respectively; residues of glyphosate from the 1X treatment group were below the LOQ (<0.05 mg/kg). Glyphosate residues in liver were below the LOQ in samples collected after a 28-day withdrawal period for the 1X, 3X, and 10X treatment groups. AMPA levels were below the LOQ (<0.05 mg/kg) in liver samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average levels of AMPA were 0.100 mg/kg and 0.337 mg/kg, respectively. AMPA residues in liver were below the LOQ in samples collected after a 28-day withdrawal period for the 1X, 3X, and 10X treatment groups.

The average levels of glyphosate in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups were 0.365 mg/kg, 2.53 mg/kg, and 7.63 mg/kg, respectively. Glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for the 1X treatment group, and were 0.072 mg/kg and 0.178 mg/kg in the 3X and 10X treatment groups, respectively. The average levels of AMPA in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups were 0.063 mg/kg, 0.264 mg/kg, and 0.872 mg/kg, respectively. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for all the treatment groups.

Test facilities

Study directory: [redacted]

In-Life phase: [redacted]

Analytical phase: [redacted]

I. Materials and Methods

A. Materials

Two test materials, glyphosate and AMPA, were administered to the treated animals in this study. Further information on the test materials is listed in the tables below.

1. Test materials

Test material number 1:

Description:	Glyphosate
Batch number:	Not reported
HLA sample number:	50501936
Active ingredient(s):	Glyphosate (N-phosphonomethyl glycine)
CAS number:	1071-83-6
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	97.6 %
Formulation type:	NA
Appearance/colour:	Powdered solid
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in screw-top glass jar
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Test material number 2:

Description:	AMPA
Batch number:	Not reported
HLA sample number:	50501935
Active ingredient(s):	AMPA (aminomethylphosphonic acid)
CAS number:	1066-51-9
Content of a.s. nominal:	Not specified
Content of a.s. analysed:	97.0 %
Formulation type:	NA
Appearance/colour:	Powdered solid
Certificate of analysis:	Not reported
Expiry date:	Not reported
Storage conditions:	Stored at room temperature in screw-top glass jar
Purity and composition:	All specifications of purity and composition of the test item were provided by the sponsor

Cross-bred swine were the test animals used in this study. Details are listed in the table below.

2. Test animals

Species:	Pig (*Sus scrofa)*
Gender:	Male and Female
Breed:	Not provided
Source:	[redacted]
Age:	Not provided
Weight at dosing, (Day-1):	Ranged from 65-81 kg
Number of animals:	16 swine selected out of a group of 20: (4 in untreated control group, and 4 in each of 3 treated groups (1X, 3X, and 10X dose levels). Each group contained two barrows (castrated males) and two gilts (nulliparous females).

Animal Identification:	Uniquely numbered ear tag
Animal health / observations:	Physical examination of each animal by staff veterinarian at the beginning of acclimation, at the beginning of the test period (Day -1), and just before sacrifice (Days 25 [Animal M00142], 29, and 57). The animals were approved for use in the study by the staff veterinarian on 17-Jun-1985.
Acclimation period:	15 days (12 days for Animal M00146).
Diet:	The basal diet was composed of Ralston Purina Farm Blend Hog Chow®, Lot No. 4751431 (18.5 %) and ground yellow corn (81.5 %). This diet was fed *ad libitum*. There were no known contaminants in the basal diet which would interfere with the conduct or outcome of this study.
Water:	Water was supplied *ad libitum*.
Housing:	The animals were housed in an insulated concrete block building with a sloping concrete floor. The floor was flushed daily with water to remove urine and feces. The animals were confined to individual 5-ft x 10-ft stalls during acclimation and test periods. Each stall was equipped with a feeder and automatic watering nipple or trough waterer.

The environmental conditions at the test facility during the in life phase of the study are summarised in the table below.

3. Environmental conditions

Temperature:	Ambient; ranged from 18–30 °C
Humidity:	Ranged from 50–86 %
Air change:	Not reported
Photoperiod:	Not reported

B. Study Design and Methods

The study included 4 treatment groups, an untreated control and 3 treated groups (1X, 3X, and 10X dose levels). The 1X dose level was chosen by utilizing the RAC diet that would yield the highest expected residue level. This chosen level (40 mg/kg) actually represents the average of the maximum expected residue in the feed diet for swine. Exaggerated dose levels (3X and 10X) were also included in the study, in accordance with guidelines for swine feeding studies. The animals were assigned to treatment groups in the acclimation period. Animals were randomised to treatments using a table of random numbers. Four swine were assigned to the untreated control group and to each of the three treated groups. Each group contained two barrows and two gilts.

The control group was fed a non-treated diet while the three treated groups were fed rations containing both glyphosate and AMPA in a 9:1 ratio. Dosing of treated animals continued for 28 consecutive days. Upon completion of dosing, one male and one female from each treatment group were sacrificed and tissue samples were collected. The remaining swine were retained for use in a withdrawal phase of the study to evaluate reduction in any residues in tissues after dosing ended. The remaining eight swine (two from each treatment group) were sacrificed at 28 days after the end of the dosing period (i.e. Study Day 56).

Further details on the dosing regimen, including target dose levels, are summarised in the table below.

1. Dosing regimen

Route:	Oral via dietary intake
Vehicle:	Farm Blend Hog Chow Concentrate and ground corn which was fortified with glyphosate and AMPA
Timing / frequency per day:	Test diet was added to feeders as necessary
Duration:	28 consecutive days
Treatment groups (dose levels):	4 treatment groups; untreated control and 3 dose levels (dry feed basis): 1X: nominal at 36 mg/kg glyphosate + 4 g/kg AMPA in total diet 3X: nominal at 108 mg/kg glyphosate + 12 mg/kg AMPA in total diet 10X: nominal at 360 mg/kg glyphosate + 40 mg/kg AMPA in total diet

The blend of Hog Chow concentrate and ground corn was fortified with glyphosate and AMPA by addition of the powdered solid test materials. Glyphosate and AMPA were used as received in the Week 1 and Week 2 feed mixes. A series of hand mixing and blending steps achieved a uniform concentration of glyphosate and AMPA. For Week 3 and Week 4 feed mixes, glyphosate was pre-ground to a finer powder prior to use while AMPA was used as received. A series of blending steps achieved a uniform concentration of glyphosate and AMPA.

Fortified feed samples were collected and analysed to confirm that the blending procedure produced a uniform concentration of the test materials throughout the treated batch. Samples were collected from the top, bottom, left and right positions of the mixing bowl for the three dose levels. Results from analysis of the samples confirmed that uniform distribution of the test materials in the feed concentrate was achieved. Additionally, stability of glyphosate and AMPA in the feed diet was evaluated. Analysis of fortified feed indicated no significant decrease in glyphosate or AMPA concentrations when stored for 12 days at 25 °C. The batches of treated diets used to administer the test materials to the swine in this study were stored no longer than 7 days before use. Therefore, the period of demonstrated test material stability in the feed diet covers the maximum period of storage experienced in the study.

Samples (200 g each) were collected from each batch of feed provided to the swine and were analysed to determine levels of glyphosate and AMPA.

2. Daily observations and animal data collection

All animals were observed daily for general condition and behaviour. The amount of feed offered and refused by each animal was determined daily. Feed in the feeders was completely changed each week when a new batch of test diet was mixed. Body weight was recorded weekly during the acclimation, test, and withdrawal periods.

3. Tissue sample collection

At the time of tissue sample collection, specified animals were euthanised (stunning gun followed by exsanguination). Samples of fat (composite of equal amounts of omental and subcutaneous fat), muscle (composite of equal amounts of triceps, gracilis, and longissimus dorsi muscle), liver, and kidney were collected from animals individually upon completion of the 28-day dosing period (within 1 day of administration of the final dose) or during the withdrawal phase of the study, 28 days after the end of the dosing period (Study Day 56). Gross necropsy was performed on sacrificed animals.

Tissue samples were initially stored frozen (<-20 °C) in polyethylene containers at the In-life facility, Hazleton Laboratories, and then shipped to the Analytical Phase facility (Monsanto, St. Louis, Missouri, USA) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table 6.4.3-1: Tissue sampling information

Commodity	Timing (Study Days when samples collected)	Quantity / sample
Muscle[1]	End of dosing: Study Day 28 Withdrawal phase: Study day 56	~ 500 g/animal[3]
Fat[2]		~ 500 g/animal[3]
Liver		~ 550 g/animal[3]
Kidney		~ 500 g/animal[3]

1 Composite of equal amounts of triceps, gracilis, and longissimus dorsi muscle.
2 Composite of equal amounts of omental and subcutaneous fat.
3 Duplicate samples were collected; one shipped for analysis and one held as a reserve sample.

4. Analytical phase

Analysis of feed samples as well as tissue samples was conducted at the Analytical Phase facility, Monsanto Company, St. Louis, Missouri, USA.

An analytical methodology was developed and validated for the determination of glyphosate and AMPA in the feed diet. The procedure consisted of extracting the feed diets with an aqueous/organic partition extraction (2:1 deionised water and chloroform) on a shaker, centrifuging, and ion exchange resin clean up. Quantitation was achieved by using a liquid chromatograph equipped with an Aminex A-9 analytical column, an o-phthalaldehyde (OPA) post-column reactor, and a fluorescence detector. The limit of validation/quantitation (LOQ) of the method was 4 mg/kg. Each feed diet was analysed in duplicate.

Recovery results with feed fortified with glyphosate and AMPA demonstrate that the intended dose concentration was achieved and are summarised in the table below.

Table 6.4.3-2: Recovery results: glyphosate and AMPA in feed

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Results/Range (%)	Mean (%)	Standard deviation[1] (%)	Relative standard deviation[1] (%)	Number analyses (n)
Feed	Glyphosate	36 (1X)	101, 102, 103, 99.7, 95.5, 96.6, 97.3, 97.7, 81.9, 83.1, 94.6, 93.6	95.5	*6.7*	*7.1*	12
		108 (3X)	93.6, 93.8, 89.6, 94.8, 90.2, 89.3, 103, 99.2	94.2	*4.8*	*5.1*	8
		360 (10X)	98.8, 98.0, 99.4, 100, 101, 99.1, 98.0, 100, 95.0, 97.6, 94.6, 93.1	97.9	*2.4*	*2.5*	12
		Overall	81.9-103	96.1	5.1	*5.3*	32
	AMPA	4 (1X)	94.0, 94.6, 99.2, 94.0, 93.6, 93.5, 91.8, 91.6, 81.4, 83.7, 90.2, 89.0	91.4	*4.9*	*5.3*	12
		12 (3X)	92.7, 91.6, 92.8, 95.0, 89.3, 84.9, 91.1, 80.2	89.7	*4.9*	*5.4*	8
		40 (10X)	97.7, 99.6, 87.9, 86.8, 91.8, 91.5, 83.0, 85.9, 85.2, 88.9, 89.3, 87.2	89.6	*4.9*	*5.5*	12
		Overall	80.2-99.6	90.3	4.8	*5.3*	32

Table 6.4.3-2: Recovery results: glyphosate and AMPA in feed

1 Standard deviation for individual fortification levels as well as all relative standard deviation values were calculated for this summary and are shown in italics.

Another analytical methodology was developed and validated for the determination of glyphosate and AMPA in swine fat, muscle, liver, and kidney tissues. All samples were analysed using the analytical method based on the well-established method DFG 405 (refer to CA 4.1.2). The procedure used an aqueous/organic partition extraction (2:1 deionised water and chloroform). Glyphosate and AMPA were isolated from swine fat, muscle, liver, and kidney extracts by elution through Chelex 100 resin in the Fe(III) form. Glyphosate and AMPA were eluted from the resin with hydrochloric acid and the iron was removed using anion exchange resin. After concentration to dryness to remove the hydrochloric acid, samples were analysed using a two column switching high pressure liquid chromatograph equipped with an OPA post-column reactor and a fluorescence detector.

The limit of validation/quantitation (LOQ) was 0.05 mg/kg each for glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) in fat, muscle, liver, and kidney. Each tissue was analysed in duplicate with a typical analytical set consisting of 2 control samples, 2 fortified controls, and 8 treated samples. Recovery results with samples of fat, muscle, liver, and kidney fortified with glyphosate and AMPA are summarised in the table below.

Table 6.4.3-3: Recovery results: glyphosate and AMPA in tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results/Range (%)	Mean (%)	Standard deviation[1] (%)	Relative standard deviation[1] (%)	Number analyses (n)
Glyphosate	Fat	0.05	103, 110, 95.1, 93.7, 89.1, 90.3	96.9	*8.1*	*8.3*	6
		0.10	96.7, 95.5	96.1	-	-	2
		Overall	89.1-110	96.7	6.8	7.0	8
	Muscle	0.05	102, 104, 89.0, 83.3, 81.5, 79.8, 77.7, 105	90.3	12	13	8
	Liver	0.05	65.8, 68.1	67.0	-	-	2
		0.10	80.9, 80.9, 82.1, 82.7	81.7	*0.9*	*1.1*	4
		0.50	89.7, 86.8	88.3	-	-	2
		Overall	65.8-89.7	79.6	8.4	11	8
	Kidney	0.05	93.6, 88.6	91.1	-	-	2
		0.25	100, 102	101	-	-	2
		1.0	101, 101	101	-	-	2
		2.5	96.6, 98.9	97.8	-	-	2
		Overall	88.6-102	97.7	4.6	4.7	8

Table 6.4.3-3: Recovery results: glyphosate and AMPA in tissues

Analyte	Matrix	Fortification level (mg/kg)	Recovery				
			Results/Range (%)	Mean (%)	Standard deviation[1] (%)	Relative standard deviation[1] (%)	Number analyses (n)
AMPA	Fat	0.05	97.5, 100, 81.8, 81.3, 82.8, 85.8	88.2	*8.4*	*9.5*	6
		0.10	90.3, 88.9	89.6	-	-	2
		Overall	81.3-100	88.6	7.1	8.0	8
	Muscle	0.05	92.0, 89.1, 92.5, 93.8, 89.4, 87.7, 87.0, 88.7	90.0	2.4	2.7	8
	Liver	0.05	82.4, 83.2	82.8	-	-	2
		0.10	88.5, 88.0, 86.2, 85.7	87.1	*1.4*	*1.6*	4
		0.50	83.7, 81.1	82.4	-	-	2
		Overall	81.1-88.5	84.9	2.7	3.2	8
	Kidney	0.05	99.3, 101	100	-	-	2
		0.25	86.2, 86.4	86.3	-	-	2
		1.0	91.6, 91.8	91.7	-	-	2
		2.5	86.3, 87.3	86.8	-	-	2
		Overall	86.2-101	91.2	6.0	6.6	8

1 Standard deviation and relative standard deviation values for individual fortification levels were calculated for this summary and are shown in italics.

II. Results and Discussion

A. Dose levels

As indicated previously, feed was fortified with glyphosate and AMPA at specified levels as the vehicle to administer the test materials through dietary intake to swine in the treated dose group. The nominal concentrations of glyphosate in the diet for the 1X, 3X, and 10X treatment groups were 36 mg/kg, 108 mg/kg, and 360 mg/kg, respectively. The nominal concentrations of AMPA (expressed as AMPA) in the diet for the 1X, 3X, and 10X treatment groups were 4.0 mg/kg, 12 mg/kg, and 40 mg/kg, respectively.

Analysis of samples of feed collected during the dosing phase of the study confirmed that actual dose levels were close the nominal/targeted dose levels. A results summary of analysis of feed to determine actual dose levels of glyphosate and AMPA (not corrected for recovery) is shown in the table below.

Table 6.4.3-4: Actual dose levels of glyphosate and AMPA in feed (not corrected for recovery)

Dose Level	Week number	Average Glyphosate (mg/kg feed/day)[1]	Average AMPA (mg/kg feed/day)[1]
1X Glyphosate (nominal): 36 mg/kg AMPA (nominal): 4 mg/kg	1	*29.7*	*3.7*
	2	*32.0*	*3.7*
	3	*36.2*	*3.6*
	4	*35.9*	*3.4*
	Overall average:	*33.4±3.1*	*3.6±0.1*
3X Glyphosate (nominal): 108 mg/kg AMPA (nominal): 12 mg/kg	1	*114.0*	*11.2*
	2	*101.9*	*11.5*
	3	*103.0*	*11.0*
	4	*101.7*	*10.2*
	Overall average:	*105.2±5.9*	*11.0±0.6*

Table 6.4.3-4: Actual dose levels of glyphosate and AMPA in feed (not corrected for recovery)

Dose Level	Week number	Average Glyphosate (mg/kg feed/day)[1]	Average AMPA (mg/kg feed/day)[1]
10X Glyphosate (nominal): 360 mg/kg AMPA (nominal): 40 mg/kg	1	*339.4*	*36.5*
	2	*329.7*	*36.8*
	3	*346.5*	*36.8*
	4	*345.3*	*37.0*
	Overall average	*340.2±7.7*	*36.8±0.2*

1 Average values were calculated for this summary and are shown in italics. Overall averages and standard deviations are calculated from the four weekly average values because an unequal number of individual samples were collected per week.

The results showed that the actual levels of glyphosate and AMPA in each of the 3 dose levels were close to the nominal/target levels. The overall averages for glyphosate in the total diet on a dry feed basis (not corrected for recovery) in the 1X, 3X, and 10X treatments groups were 33.4 mg/kg, 105.2 mg/kg, and 340.2 mg/kg, respectively. The overall averages for AMPA in the total diet on a dry feed basis (not corrected for recovery) in the 1X, 3X, and 10X treatments groups were 3.6 mg/kg, 11.0 mg/kg, and 36.8 mg/kg, respectively.

Additionally, in a second table below, dosage was calculated and expressed on the basis of subgroup average animal body weight (i.e. mg test material / kg bw/day). These results were calculated for this summary using the subgroup average intake of glyphosate and AMPA and average body weight of each subgroup during the dosing phase of the study. The overall averages for glyphosate dosage on a body weight basis in the 1X, 3X, and 10X treated groups were 0.98 mg/kg bw/day (1.12 mg/kg bw/day without animal M00142, which was sacrificed early due to health issues), 3.24 mg/kg bw/day, and 11.13 mg/kg bw/day, respectively. The overall averages for AMPA dosage on a body weight basis in the 1X, 3X, and 10X treated groups were 0.11 mg/kg bw/day, 0.34 mg/kg bw/day, and 1.20 mg/kg bw/day, respectively.

Table 6.4.3-5: Actual dose levels of glyphosate and AMPA administered to swine for 28 days expressed on basis of body weight (bw) and concentration in total diet (dry feed)

Nominal dose level	Animal Number	Average body weight during dosing (kg)[1]	Average daily dry feed consumption (kg)[1]	Glyphosate dose/day[1]		AMPA dose/day[1]	
				mg/kg bw	mg / animal	mg/kg bw	mg / animal
1X[2] [36 mg/kg glyphosate + 4 mg/kg AMPA in dry feed (total diet)]	142[3]	*82*	*1.4*	*0.57*	*47.1*	*0.06*	*5.1*
	144	*80*	*2.8*	*1.16*	*92.0*	*0.12*	*9.9*
	140	*97*	*2.9*	*1.00*	*96.1*	*0.11*	*10.3*
	138	*97*	*3.5*	*1.21*	*117.1*	*0.13*	*12.6*
	Average[4]:	*89*	*2.6 (3.0)*	*0.98 (1.12)*	*88.1 (101.7)*	*0.11 (0.12)*	*9.5 (10.9)*
3X[5] [108 mg/kg glyphosate + 12 mg/kg AMPA in dry feed (total diet)]	135	*90*	*2.4*	*2.77*	*249.8*	*0.29*	*26.1*
	137	*82*	*2.3*	*2.97*	*241.9*	*0.31*	*25.3*
	141	*93*	*3.2*	*3.64*	*339.2*	*0.38*	*35.4*
	130	*99*	*3.4*	*3.58*	*355.0*	*0.37*	*37.1*
	Average:	*91*	*2.8*	*3.24*	*296.5*	*0.34*	*31.0*
10X[6] [360 mg/kg glyphosate + 40 mg/kg AMPA in dry feed (total diet)]	146	*88*	*2.7*	*10.31*	*910.1*	*1.11*	*98.4*
	129	*89*	*2.8*	*10.67*	*944.2*	*1.15*	*102.1*
	145	*96*	*3.5*	*12.20*	*1173.8*	*1.32*	*126.9*
	132	*94*	*3.1*	*11.34*	*1063.2*	*1.23*	*114.9*

Table 6.4.3-5: Actual dose levels of glyphosate and AMPA administered to swine for 28 days expressed on basis of body weight (bw) and concentration in total diet (dry feed)

	Average:	*92*	*3.0*	*11.13*	*1022.8*	*1.20*	*110.6*

1 All values were calculated for this summary and are thus shown in italics.
2 Average daily dose for 1X group was 33.44 mg glyphosate/kg feed and 3.60 mg AMPA/kg feed (uncorrected for recovery).
3 Animal consumed much less feed during last 2.5 weeks of test and was sacrificed on Day 25 due to stomach ulcers.
4 Average values shown in parentheses for 1X group exclude animal 142.
5 Average daily dose for 3X group was 105.18 mg glyphosate/kg feed and 10.98 mg AMPA/kg feed (uncorrected for recovery).
6 Average daily dose for 10X group was 340.24 mg glyphosate/kg feed and average was 36.78 mg AMPA/kg feed (uncorrected for recovery).

B. Animal health and daily observations

There were no findings concerning animal health or behavior that were considered to be test related. Animal 142 showed decreased feed consumption and dark feces from Days 16 through 25. On Day 25, this animal's condition worsened. The staff veterinarian diagnosed the animal as having bleeding stomach ulcers. The animal was then sacrificed on Day 25. The necropsy findings supported the diagnosis. Feed consumption for all animals (except animal 142) in each test group remained essentially stable during the test period. Body weight fluctuations seen during the study were considered normal for adult animals. Following animal sacrifice, necropsy/pathology evaluation indicated no macroscopic or microscopic observations that appear treatment related.

C. Residue levels in tissues

The residue values presented in the summary of the study report had been corrected for recovery. The residue values in the tables and text below were not corrected for recovery.

The residues of glyphosate (expressed as glyphosate) and AMPA (expressed as AMPA) in tissues (fat, muscle, liver, and kidney) collected from untreated control animals were below the LOQ (<0.05 mg/kg).

Frozen storage stability of glyphosate and AMPA in swine matrices (fat, muscle, liver and kidney) was evaluated in a separate study completed subsequent to this feeding study. No significant degradation of glyphosate or AMPA in swine fat, muscle, liver, or kidney was observed for 460 days, which was the maximum period of frozen storage evaluated.

The residues of glyphosate and AMPA in all fat samples (days 1–56) from the 1X, 3X, and 10X treatment groups were below the LOQ (<0.05 mg/kg).

The residues of glyphosate and AMPA in all muscle samples (days 1–56) from the 1X, 3X, and 10X treatment groups were below the LOQ (<0.05 mg/kg) except for the day 28 samples of the 10X treatment, which contained 0.054 mg/kg glyphosate on average.

Table 6.4.3-6: Residues of glyphosate and AMPA in muscle

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
10X Glyphosate (average): *340.2 mg/kg in feed;* *11.13 mg/kg bw* AMPA (average): *36.8 mg/kg in feed;* *1.20 mg/kg bw*	146	28	1	0.057	*0.057*	<0.050	*<0.050*
			2	0.057		<0.050	
	129	28	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	Study Day 28, 10X treatment group average:				*0.054*		*<0.050*

1 Study Day 28 is at the end of the 28-day dosing period.
2 LOQ (limit of quantitation): 0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater. Averages of uncorrected residues were calculated for this summary and thus are shown in italics.

The average levels of glyphosate in liver samples at the end of the 28-day dosing period in the 3X and 10X treatment groups were 0.163 mg/kg and 0.598 mg/kg, respectively. The residues of glyphosate from the 1X treatment group were below the LOQ (<0.05 mg/kg). The glyphosate residues in liver were below the LOQ in samples collected after a 28-day withdrawal period for the 1X, 3X, and 10X treatment groups. The AMPA levels were below the LOQ (<0.05 mg/kg) in liver samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average levels of AMPA were 0.100 mg/kg and 0.337 mg/kg, respectively. The AMPA residues in liver were below the LOQ in samples collected after a 28-day withdrawal period for the 1X, 3X, and 10X treatment groups.

The average levels of glyphosate in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups were 0.365 mg/kg, 2.53 mg/kg, and 7.63 mg/kg, respectively. The glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for the 1X treatment group, and were 0.072 mg/kg and 0.178 mg/kg in the 3X and 10X treatment groups, respectively. The average levels of AMPA in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups were 0.063 mg/kg, 0.264 mg/kg, and 0.872 mg/kg, respectively. AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for all the treatment groups.

Table 6.4.3-7: Residues of glyphosate and AMPA in liver

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
3X Glyphosate (average): *105.2 mg/kg in feed;* *3.24 mg/kg bw* AMPA (average): *11.0 mg/kg in feed;* *0.34 mg/kg bw*	135	28	1	0.175	*0.173*	0.109	*0.110*
			2	0.171		0.110	
	137	28	1	0.154	*0.154*	0.092	*0.090*
			2	0.153		0.088	
	Study Day 28, 3X treatment group average:				*0.163*		*0.100*
	141	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	130	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	Study Day 56, 3X treatment group average:				*<0.050*		*<0.050*

Table 6.4.3-7: Residues of glyphosate and AMPA in liver

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
10X Glyphosate (average): *340.2 mg/kg in feed;* *11.13 mg/kg bw* AMPA (average): *36.8 mg/kg in feed;* *1.20 mg/kg bw*	146	28	1	0.709	*0.719*	0.440	*0.452*
			2	0.729		0.463	
	129	28	1	0.471	*0.477*	0.218	*0.222*
			2	0.483		0.225	
	Study Day 28, 10X treatment group average:				*0.598*		*0.337*
	145	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	132	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	Study Day 56, 3X treatment group average:				*<0.050*		*<0.050*

1 Study Day 28 is at the end of the 28-day dosing period; Study Day 56 is during the withdrawal period, 28 days after the end of dosing, respectively.
2 LOQ (limit of quantitation): 0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater. Averages of uncorrected residues were calculated for this summary and thus are shown in italics.

Table 6.4.3-8: Residues of glyphosate and AMPA in kidney

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
1X Glyphosate (average): *33.4 mg/kg in feed;* *0.98 mg/kg bw* AMPA (average): *3.6 mg/kg in feed;* *0.11 mg/kg bw*	142	25[5]	1	0.130	*0.136*	<0.050	*<0.050*
			2	0.141		<0.050	
	144	28	1	0.603	*0.595*	0.076	*0.076*
			2	0.587		0.075	
	Study Day 28, 1X treatment group average:				*0.365*		*0.063*
	140	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	138	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	Study Day 56, 1X treatment group average:				*<0.050*		*<0.050*
3X Glyphosate (average): *105.2 mg/kg in feed;* *3.24 mg/kg bw* AMPA (average): *11.0 mg/kg in feed;* *0.34 mg/kg bw*	135	28	1	2.87	*2.91*	0.300	*0.308*
			2	2.94		0.316	
	137	28	1	2.14	*2.15*	0.219	*0.221*
			2	2.15		0.222	
	Study Day 28, 3X treatment group average:				*2.53*		*0.264*
	141	56	1	0.094	*0.095*	<0.050	*<0.050*
			2	0.095		<0.050	
	130	56	1	<0.050	*<0.050*	<0.050	*<0.050*
			2	<0.050		<0.050	
	Study Day 56, 3X treatment group average:				*0.072*		*<0.050*

Table 6.4.3-8: Residues of glyphosate and AMPA in kidney

Treatment Group	Animal No.	Study Day[1]	Analysis replicate	Residue found[2, 3, 4] (mg/kg)			
				Glyphosate	Glyphosate, average	AMPA	AMPA, average
10X Glyphosate (average): *340.2 mg/kg in feed;* *11.13 mg/kg bw* AMPA (average): *36.8 mg/kg in feed;* *1.20 mg/kg bw*	146	28	1	9.25	*9.12*	1.01	*0.977*
			2	8.98		0.943	
	129	28	1	6.15	*6.14*	0.778	*0.767*
			2	6.12		0.756	
	Study Day 28, 10X treatment group average:				*7.63*		*0.872*
	145	56	1	0.163	*0.162*	<0.050	*<0.050*
			2	0.160		<0.050	
	132	56	1	0.197	*0.195*	<0.050	*<0.050*
			2	0.192		<0.050	
	Study Day 56, 10X treatment group average:				*0.178*		*<0.050*

1 Study Day 28 is at the end of the 28-day dosing period; Study Day 56 is during the withdrawal period, 28 days after the end of dosing, respectively.
2 LOQ (limit of quantitation): 0.05 mg/kg
3 Residue values are uncorrected for recovery.
4 For purposes of calculating averages, residue values of <0.05 mg/kg were assigned a value of 0.05 mg/kg if being averaged with a value of 0.05 mg/kg or greater. Averages of uncorrected residues were calculated for this summary and thus are shown in italics.
5 Animal sacrificed on Day 25 due to stomach ulcers.

III. Conclusion

The residues of glyphosate and AMPA in all fat samples (days 1–56) from the 1X, 3X, and 10X treatment groups were below the LOQ (<0.05 mg/kg).

The residues of glyphosate and AMPA in all muscle samples (days 1–56) from the 1X, 3X, and 10X treatment groups were below the LOQ (<0.05 mg/kg) except for the day 28 samples of the 10X treatment, which contained 0.054 mg/kg glyphosate on average.

The average levels of glyphosate in liver samples at the end of the 28-day dosing period in the 3X and 10X treatment groups were 0.163 mg/kg and 0.598 mg/kg, respectively. The residues of glyphosate from the 1X treatment group were below the LOQ (<0.05 mg/kg). The glyphosate residues in liver were below the LOQ in samples collected after a 28-day withdrawal period for the 1X, 3X, and 10X treatment groups. The AMPA levels were below the LOQ (<0.05 mg/kg) in liver samples at the end of the 28-day dosing period in the 1X treatment group. In the 3X and 10X treatment groups, the average levels of AMPA were 0.100 mg/kg and 0.337 mg/kg, respectively. The AMPA residues in liver were below the LOQ in samples collected after a 28-day withdrawal period for the 1X, 3X, and 10X treatment groups.

The average levels of glyphosate in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups were 0.365 mg/kg, 2.53 mg/kg, and 7.63 mg/kg, respectively. The glyphosate residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for the 1X treatment group, and were 0.072 mg/kg and 0.178 mg/kg in the 3X and 10X treatment groups, respectively. The average levels of AMPA in kidney samples at the end of the 28-day dosing period in the 1X, 3X, and 10X treatment groups were 0.063 mg/kg, 0.264 mg/kg, and 0.872 mg/kg, respectively. The AMPA residues in kidney were below the LOQ (<0.05 mg/kg) in samples collected after a 28-day withdrawal period for all the treatment groups.

Residues of glyphosate and AMPA, when found in tissues at the end of the dosing period decreased significantly during the 28-day withdrawal period when dosing was discontinued, indicating that these residues do not accumulate irreversibly under the conditions tested.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the residues of glyphosate and AMPA in swine (pig) tissues (fat, muscle, liver, and kidney) has previously been evaluated at EU level. The study is considered acceptable for use in determining the level of glyphosate and AMPA residues that may transfer from the swine diet to edible swine tissues. The study was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 505 and OECD Guidance Document on Residues in Livestock (Series on Pesticides No. 73) with a few deviations.

Age and breed of the pigs is not reported, but it can be assumed that pigs with representative age and breed were used in this study.

Tissue samples of only 2 animals per treatment group were analysed. Meat samples were composed of triceps, gracilis, and longissimus dorsi muscle instead of loin, flank or hind-leg. The period for which samples were stored frozen before extraction/analysis is not provided. The dates of first and last sacrifice are 16.07.1985 and 13.08.1985. The report was finalised in September, 1987. Thus, the maximum storage time could be estimated as 806 days (27 months). The storage stability of glyphosate and AMPA upon frozen storage was demonstrated in cow kidney, liver, fat, and muscle for a minimum of 671 days (22 months) in a feeding study resented within this chapter (CA.6.4.2/003 [redacted] 1987). Thus, the storage stability is not covered. However, it has to be kept in mind, that the storage period calculated based on the study finalisation date and not the date of last analysis is likely to be a huge overestimation.

Depuration phase was performed with only 1 interval instead of 3 intervals.

The study is considered valid as these deficits are not expected to significantly impact the quality or reliability of the study.

Assessment and conclusion by RMS:

CA 6.4.4 Fish

The intended uses supported in the EU are not used as fish feed items according to SANCO/11187/2013 rev. 3. Therefore, no data are required in support of this application.

CA 6.5 Effects of Processing

CA 6.5.1 Nature of the residue

The nature of residues of glyphosate and its metabolites AMPA and N-acetyl AMPA in processed commodities was investigates in three studies.

Table 6.5.1-1: Parameters for hydrolysis conditions

Annex point	Study	Study type	Substance(s)	Status	Remark
CA 6.5.1/001	[redacted], 2020	High temperature hydrolysis	AMPA, *N*-acetyl AMPA	Valid	-
CA 6.5.1/002	[redacted] 2010	High temperature hydrolysis	Glyphosate	Valid	-
CA 6.5.1/003	[redacted] 2006	High temperature hydrolysis	Glyphosate	Valid	-

Study previously not submitted to the EU

1. Information on the study

Data point:	CA 6.5.1/001
Report author	[redacted]
Report year	2020
Report title	AMPA and *N*-Acetyl AMPA Hydrolysis under typical conditions (pH, temperature and time) of processing
Report No	S19-22457
Document No	M-680101-01-1
Guidelines followed in study	OECD Guideline for the Testing of Chemicals, 507 European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 507: • Test material AMPA and *N*-acetyl AMPA used in the study were not radiolabelled
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive summary

The purpose of this study was to investigate the hydrolytic transformation/degradation of AMPA and *N*-acetyl AMPA. The experiments were carried out under laboratory conditions, which are representative for food processing operations of raw agricultural commodities (RACs).

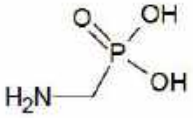
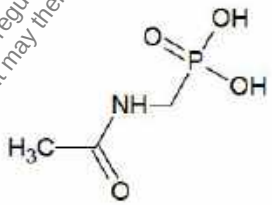
The following conditions were tested:

Pasteurisation	90°C at pH 4 for 0 and 20 min
Baking, brewing, boiling	100°C at pH 5 for 0 and 60 min
Sterilisation	120°C at pH 6 for 0 and 20 min

The recovery for the high temperature hydrolysis tests ranged from 90.8 % to 100 % for AMPA and from 101.9 % to 103.1 % for *N*-acetyl AMPA.
For all the tests conducted, no significant change in the concentration of AMPA and *N*-acetyl AMPA was detected in all samples at the end of the incubation period.
Based on these results, AMPA and *N*-acetyl AMPA were found to be stable to hydrolysis in the pH range tested when subjected to high temperatures. AMPA and *N*-acetyl AMPA are expected to be stable during common processing practices such as pasteurisation, sterilisation and baking/brewing/boiling.

I. Materials and Methods

A. Materials

Test material	Aminomethylphosphonic acid	*N*-Acetyl AMPA
Chemical structure:		
Batch number:	107466	787490
Purity analysed	98.5 % w/w	95.1 % w/w
CAS No:	1066-51-9	57637-97-5
Appearance / colour:	solid / white	solid / white

B. Study design

1. Preparation of stock and test solutions

All aqueous buffered solutions were prepared using citric acid monohydrate dissolved in demineralised water and adjusted to pH 4.0, 5.0 and 6.0 with 2 M sodium hydroxide. For sterilisation, all buffers were autoclaved after preparation.

The test solutions for hydrolysis were prepared by adding buffer solution to the test vessel, followed by adding the respective 100 mg/L test item solution. The test vessel was closed with a PTFE sealed cap. Two replicate samples were prepared per test item and set of hydrolytic conditions.

Sterility of the test solutions (before hydrolysis) was checked by their application to sterile agar plates and incubation at 37° C for 2 days. In addition, a negative and a positive control were incubated under the same conditions. The colonies developed on the plates were counted.

2. Experimental conditions

The samples were prepared in duplicate for each buffer system using sterile glass vials.

Duplicate samples were analysed immediately for time zero where no heat was used. Two additional samples at pH 4 ± 0.1 were placed in an oven and maintained at 90°C ± 5°C for 20 minutes. Two samples at pH 5 ± 0.1 were placed in an oven and maintained at 100°C ± 5°C for 60 minutes and two samples at

pH 6 ± 0.1 were placed in an autoclave and maintained at sterilizing conditions at 120°C) ± 5°C for 20 minutes.

Table 6.5.1-2: Parameters for hydrolysis conditions

pH	Temperature [°C]	Test period [min]	Representative Process
4.0 ± 0.1	90 ± 5	0 and 20 min	Pasteurisation
5.0 ± 0.1	100 ± 5	0 and 60 min	Baking, brewing and boiling
6.0 ± 0.1	120 ± 5	0 and 20 min	Sterilisation

3. Sampling

Duplicate samples were collected at time 0 and one subsequent time point after application. At sampling, duplicate samples were retrieved from the respective oven or autoclave. The pH of the solution was measured and recorded. Aliquots of 0.05 mL were taken from the test vessel before and after the respective processing and were diluted 20-fold with water + 0.1 % formic acid prior to LC-MS/MS analysis. After analysis, samples were stored in a freezer at ≤-18°C.

4. Analytical phase

The buffer solutions were analysed for AMPA and *N*-Acetyl AMPA residues by high performance liquid chromatography coupled with mass spectrometry (LC-MS/MS) in multiple reaction monitoring (MRM) mode using AMPA and *N*-Acetyl-AMPA standards in diluted buffer solutions for calibration. Quantification was performed by using linear regression with additional correction for bracketing standards. Injections of diluted samples were interspersed with injections of standard solutions after maximum 5 samples to verify the detector response and to adjust the calculated concentration.

The method was validated within this study. Fortification experiments with AMPA and *N*-Acetyl AMPA at fortification levels of 0.05 mg/L (LOQ) and 1.1 mg/L were performed. The results are summarised in the table below.

Table 6.5.1-3: Method recovery results

Matrix	Analyte	Fortification level (mg/L)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)[1]	Relative standard deviation (%)	Number analyses (n)
Buffer pH 4	AMPA	0.05	87.4 - 107.2	96.9	8.5	8.8	5
		1.1	89.0 - 100.0	95.5	5.4	5.6	5
		Overall	**87.4 - 107.2**	**96.2**	**6.8**	**7.1**	**10**
	N-Acetyl AMPA	0.05	102.4 - 108.4	105.0	2.5	2.3	5
		1.1	94.4 - 100.7	99.0	2.6	2.7	5
		Overall	**94.4 - 108.4**	**102.0**	**3.9**	**3.9**	**10**
Buffer pH 5	AMPA	0.05	89.4 - 105.6	96.7	6.1	6.3	5
		1.1	93.4 - 110.9	100.3	7.0	7.0	5
		Overall	**89.4 - 110.9**	**98.5**	**6.5**	**6.6**	**10**
	N-Acetyl AMPA	0.05	96.4 - 100.4	98.5	1.4	1.4	5
		1.1	94.0 - 101.8	98.6	2.9	2.9	5
		Overall	**94.0 - 101.8**	**98.6**	**2.2**	**2.2**	**10**

Table 6.5.1-3: Method recovery results

Matrix	Analyte	Fortification level (mg/L)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)[1]	Relative standard deviation (%)	Number analyses (n)
Buffer pH 6	AMPA	0.05	89.2 - 108	103.1	1.6	1.6	5
		1.1	86.4 - 105.4	94.5	7.0	7.4	5
		Overall	**86.4 - 105.4**	**98.8**	**6.6**	**6.7**	**10**
	N-Acetyl AMPA	0.05	100 - 105.2	102.5	2.0	1.9	5
		1.1	100.0 - 104.4	102.4	2.1	2.1	5
		Overall	**100.0 - 105.2**	**102.4**	**1.9**	**1.9**	**10**

1 Standard deviation values for individual fortification levels were calculated for this summary.

II. Results and Discussion

The pH of the samples was measured at each sampling time. The pH results for all sets of samples indicated that the buffering capacity was maintained in the solution during the study period. Sterility assay for pH 4, 5 and 6 samples showed no growth for any of the samples tested, indicating that sterility was preserved throughout the study.
The hydrolysis of AMPA and *N*-Acetyl AMPA test substances were examined at pH 4, pH 5 and pH 6 at 90°C, 100°C and 120°C, respectively.
Aliquots of all test solutions were analysed in duplicate by LC-MS/MS before and after hydrolysis. Under the tested conditions representative for food processing, no hydrolysis of AMPA and *N*-Acetyl AMPA were observed.

The recoveries for the high temperature hydrolysis tests ranged from 90.8 % to 100 % for AMPA and from 101.9 % to 103.1 % for *N*-acetyl AMPA of the applied dose for all solutions showing that AMPA and *N*-Acetyl AMPA did not degrade at temperatures ranging from 90°C to sterilizing conditions 120°C in any of the buffer systems tested. Detailed results are provided below.

Table 6.5.1-4: Recovery results for AMPA and *N*-acetyl AMPA before and after processing

Hydro-lysis conditions	AMPA			*N*-Acetyl AMPA		
	Recovery before processing [%][1]	Recovery after processing [%][1]	Recovery after processing with respect to initial measured concentration [%]	Recovery before processing [%][1]	Recovery after processing [%][1]	Recovery after processing with respect to initial measured concentration [%]
pH 4, 90°C, 20 min	97.0	90.8	93.7	104.2	101.9	97.8
pH 5, 100°C, 60 min	93.0	100.0	107.5	104.3	103.1	98.8
pH 6, 120°C, 20 min	102.7	95.8	93.3	103.3	102.3	99.0

1 With respect to fortification level of 1 mg/L

III. Conclusion

Under hydrolysis conditions representative of pasteurisation (pH 4), baking, brewing and boiling (pH 5) and sterilisation (pH 6) there was no significant change in the concentration of test items, sample weights or pH-values. It can be concluded that AMPA and *N*-Acetyl AMPA are stable under these test conditions or, if degradation products are formed, they altogether represent less than 10 % of the amount of test item prior to hydrolysis (maximum of 6.7 % estimated for AMPA under conditions representative of sterilisation).

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the high temperature hydrolysis of AMPA and *N*-Acetyl AMPA has been previously not evaluated at EU level. It was performed under GLP. The study was conducted with non-labelled material. This is a deviation to OECD Guideline for the Testing of Chemicals, 507 as the high temperature hydrolysis is usually conducted with radio labelled material. The result of the study shows, that there was no significant change in the concentration of test items. Therefore, the study is considered to be reliable. It complies with current requirements as laid down in Reg. (EU) No 283/2013.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.1/002
Report author	[redacted]
Report year	2010
Report title	Nature of [^{14}C]Glyphosate Residues in Processed Commodities – High Temperature Hydrolysis
Report No	1925W-001
Document No	MSL0023072
Guidelines followed in study	OECD Guideline for the Testing of Chemicals, 507
Deviations from current test guideline	No deviation from OECD Guideline for the Testing of Chemicals, 507
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The purpose of this study was to investigate the hydrolytic transformation/degradation of *N*-phosphono-(^{14}C-methyl)glycine (^{14}C-glyphosate). The experiments were carried out under laboratory conditions, which are representative for food processing operations of raw agricultural commodities (RACs).

The following conditions were tested:

Pasteurisation	90°C at pH 4 for 0 and 20 min
Baking, brewing, boiling	100°C at pH 5 for 0 and 60 min
Sterilisation	120°C at pH 6 for 0 and 20 min

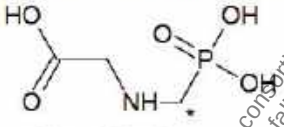
The recovery for the high temperature hydrolysis tests ranged from 95.6 % to 99.4 % of applied radioactivity.
For all the tests conducted, no significant change in the concentration of glyphosate was detected in all samples at the end of the incubation period.
Based on these results, glyphosate was found to be stable to hydrolysis in the pH range tested when subjected to high temperatures. Glyphosate is expected to be stable during common processing practices such as pasteurisation, sterilisation and baking/brewing/boiling.

I. Materials and Methods

A. Materials

Test material N-(phosphono-^{14}C-methyl)glycine (= ^{14}C-glyphosate)

Chemical structure:



* Position of the radio label

Radiochemical purity:	98.7 % (HPLC prior experimental start)
Specific activity:	10.28 MBq/mg (6.17 x 10^5 dpm/µg) (test substance supplied)
CAS No:	1071-83-6
Log $P_{o/w}$:	-3.2
Water solubility:	10.5 g/L

B. Study design

1. Preparation of stock and test solutions

The active substance *N*-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) was received as neat compound and was stored in a freezer (< -10°C) when not in use. A stock solution of ^{14}C-glyphosate was prepared by dissolving the neat ^{14}C-glyphosate in 1 mL of HPLC grade water. HPLC analysis of this solution showed that the test substance was < 95 % pure and needed further purification. The test substance was purified by HPLC. The purified stock ^{14}C-glyphosate was dissolved in water. Radiochemical purity of the test substance determined was 98.7 % prior to use in the study. The concentration of the purified glyphosate stock solution was 7.498 x 10^4 dpm/µL.

All aqueous buffered solutions were prepared using sterile 0.1 M potassium biphthalate. Buffered solutions (0.1 M potassium biphthalate) were prepared and adjusted to pH 4.0, 5.0 and 6.0 and solutions were sterilised by passing through sterile cellulose acetate membrane filter into previously autoclaved vials/bottles. Prior to application, nitrogen was bubbled for at least 5 minutes through each buffer via sterile bacterial air filter to avoid the effects of oxygen on the test systems.

Aliquots of the test systems were taken at time 0 and final time point and were cultured on plates of Trypticase Soy Agar (TSA) in an incubator at 35 °C for sterility assay. After at least 48 hours of incubation, the cultures were evaluated for microbial growth.

Dose solutions were prepared by transferring aliquots of ^{14}C-glyphosate stock solution to sterile bottles containing buffer solution (pH 4.0, 5.0 or 6.0). Each dose solution was mixed well, and aliquots were

radioassayed by liquid scintillation counting (LSC) to determine the concentration. The concentration of glyphosate in buffered solutions before hydrolysis ranged from 1.07 mg/L to 1.15 mg/L.

Aliquots of the dosing solutions were also taken after subsampling of each buffer solution to determine homogeneity of the solutions during the dosing process. Stability of the dosing solutions under conditions of administration was demonstrated by HPLC analysis after dosing and during analysis of time 0 samples.

2. Experimental conditions

The samples were prepared in duplicate for each buffer system using sterile amber glass vials. The test systems were dosed under aseptic conditions in a biological-hood flow cabinet.

Duplicate samples were analysed immediately for time zero where no heat was used. Two additional samples at pH 4 ± 0.1 were placed in an oven and maintained at 90°C ± 5°C for 20 minutes. Two samples at pH 5 ± 0.1 were placed in an oven and maintained at 100°C ± 5°C for 60 minutes and two samples at pH 6 ± 0.1 were placed in an autoclave and maintained at sterilizing conditions (approximately 121 °C) for 20 minutes.

Table 6.5.1-5: Parameters for hydrolysis conditions

pH	Temperature [°C]	Test period [min]	Representative Process
4.0 ± 0.1	90	0 and 20 min	Pasteurisation
5.0 ± 0.1	100	0 and 60 min	Baking, brewing and boiling
6.0 ± 0.1	~121	0 and 20 min	Sterilisation

3. Sampling

Duplicate samples were collected at time 0 and one subsequent time point after application. At sampling, duplicate samples were retrieved from the respective oven or autoclave. The pH of the solution was measured and recorded. Triplicate aliquots (3 x 0.1 mL) were taken for LSC analysis. All solutions were analysed by HPLC within two days of sampling.

4. Analytical phase

Each dose solution was mixed well, and radioactivity measurement was carried out by liquid scintillation counting (LSC) to determine the concentration.

^{14}C-glyphosate and its potential degradates were analysed and quantitated based on cation-exchange HPLC analysis with LSC analysis of the collected eluent fractions. The identity of ^{14}C-glyphosate was based on co-chromatography with glyphosate reference standard upon HPLC analysis. Reference standards were co-chromatographed with all samples. Confirmatory analysis was done by strong anion-exchange chromatography with LSC analysis of the collected eluent fractions.

Aliquots of the ^{14}C aqueous test samples were co-injected with glyphosate standard solution. HPLC radiochromatograms were produced from the fraction collection of the HPLC eluent (0.5 minutes fractions) employing a fraction collector with subsequent quantitation of the fractions by LSC.

For radiochemical purity checks, aliquots of the diluted solutions of test substance were co-injected with reference standard for analysis.

II. Results and Discussion

Aliquots of the dose solutions taken throughout the dosing processes showed that all dose solutions were homogeneous during the application processes. The pH of the samples was measured at each sampling time. The pH results for all sets of samples indicated that the buffering capacity was maintained in the solution during the study period. Sterility assay for pH 4, 5 and 6 samples showed no growth for any of the samples tested, indicating that sterility was preserved throughout the study.

The hydrolysis of glyphosate test substance was examined at pH 4, pH 5 and pH 6 at 90°C, 100°C and approximately 121°C, respectively.

Aliquots of all test solutions were analysed in duplicate by HPLC before and after hydrolysis. Under the tested conditions representative for food processing, no hydrolysis of ^{14}C-glyphosate was observed.

Radiocarbon recoveries for the high temperature hydrolysis tests ranged from 95.6 to 99.4 % of the applied dose for all solutions showing that glyphosate did not degrade at temperatures ranging from 90°C to sterilizing conditions (~121°C) in any of the buffer systems tested. Detailed results are provided below.

Table 6.5.1-6: Recovered radioactivity ^{14}C-glyphosate before and after hydrolysis in sterile buffer solutions

Hydro-lysis conditions	Sample	Re-plicate	Mass balance (% applied dose)	Glyphosate		Other peaks	
				% in HPLC chromatogram	% applied dose	% in HPLC chromatogram	% applied dose
pH 4, 90°C, 20 min	0 min	A	96.1	99.7	95.8	0.3	0.3
		B	95.7	99.6	95.3	0.4	0.4
	20 min	A	95.6	99.5	95.1	0.5	0.5
		B	95.9	99.4	95.3	0.6	0.6
pH 5, 100°C, 60 min	0 min	A	96.7	99.8	96.5	0.2	0.2
		B	96.6	99.7	96.3	0.3	0.3
	60 min	A	98.9	99.5	98.4	0.5	0.5
		B	99.3	99.5	98.8	0.5	0.5
pH 6, 120°C, 20 min	0 min	A	98.3	99.5	97.8	0.5	0.5
		B	97.6	99.7	97.3	0.3	0.3
	20 min	A	98.3	98.4	96.7	1.6	1.6
		B	99.4	98.5	97.9	1.5	1.5

C. Storage stability

All samples were analysed by HPLC within two days of sampling. All samples and standard solutions were stored frozen (< 0°C) when not in use. Repeated injections of the glyphosate standard solution showed no degradation of the reference substance throughout the study.

III. Conclusion

The hydrolytic degradation behaviour of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) under conditions representative for food processing operations (pasteurisation, baking, brewing, boiling and sterilisation) was investigated.
The recovery for the high temperature hydrolysis ranged from 95.6 % to 99.4 % of the applied dose for all solutions. The experiments showed that glyphosate did not degrade at temperatures ranging from 90°C to sterilizing conditions (~121°C) in any of the buffer systems tested, indicating that glyphosate should be stable in/on processed commodities during common processing practices.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the high temperature hydrolysis of glyphosate has been previously evaluated at EU level. It was performed under GLP. The study complies with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 507. Therefore the study is considered to be reliable.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.1/003
Report author	[redacted]
Report year	2006
Report title	High temperature hydrolysis of [^{14}C]IN-MCX20 in buffered aqueous solution at pH 4, 5, and 6
Report No	DuPont-19797
Document No	-
Guidelines followed in study	European Commission Working Document 1607/VI/97 Rev. 2, June 1999 (Appendix E, Processing Studies, 7035/VI/95 Rev. 5, July 1997)
Deviations from current test guideline	• It is reported that an oven (instead of an autoclave) was used to reach the temperature of 120 °C, simulating sterilisation, but the "oven" is not listed in the instrumental list • Only representative, not-integrated HPLC chromatograms are shown in the study report. Co-chromatography (fortification of samples with radiolabelled reference standard) was only performed on selected samples (only one is shown). • Minor peaks (other peaks) were < 10 % AR, but >0.01-0.05 mg/kg and > 0.05 mg/kg, respectively. The other peaks were detected both in control samples (taken before hydrolysis, at 0 min) as after hydrolysis were comparable. Further characterisation of these minor peaks was not attempted • The average material balance of control samples at pH 5 are slightly below 90 %. • Sterility of the buffers was not determined in this study. However, *N*-acetyl-glyphosate remained stable throughout the study in all test systems and no difference was observed between heated and

	control samples • Purity of the radiolabelled test substance was not determined as part of this study prior to day 0. Samples were stored at -10 °C
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive summary

The purpose of this study was to investigate the hydrolytic transformation/degradation of [^{14}C]-*N*-acetyl-glyphosate. The experiments were carried out under laboratory conditions, which are representative for food processing operations of raw agricultural commodities (RACs).

The following conditions were tested:

Pasteurisation	90°C at pH 4 for 0 and 20 min
Baking, brewing, boiling	100°C at pH 5 for 0 and 60 min
Sterilisation	120°C at pH 6 for 0 and 20 min

Solutions of [^{14}C]-*N*-acetyl-glyphosate were prepared in 0.01 M citrate buffer (pH 4, 5, and 6) at a nominal test concentration of 1.0 µg/mL, which is not more than one-half of the water solubility of *N*-acetyl-glyphosate in these buffers. At the end of the incubation period, samples were analysed by LSC to determine the quantity of radioactivity present in each sample. Radioactivity was quantitatively recovered from each test solution.

Average radiocarbon recoveries for the high temperature hydrolysis tests generally ranged from 95.3 to 98.6 % of the applied radioactivity (AR) for all solutions, except for pH 5 control samples that accounted for 87.7 % AR, due to cracking of two vials during freezing.

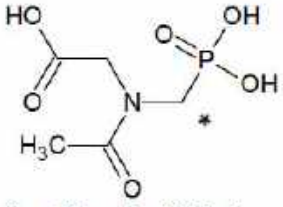
Test solutions were subject to chromatographic analysis (HPLC) to investigate the nature of any hydrolysis products formed. In all samples, the majority of applied radioactivity was recovered as *N*-acetyl-glyphosate. In all samples, no significant degradation occurred during incubation. LC-MS analysis was performed on selected samples to confirm identifications of *N*-acetyl-glyphosate made using HPLC.

Based on these results, *N*-acetyl-glyphosate was found to be stable to hydrolysis in the pH range tested when subjected to high temperatures. *N*-acetyl-glyphosate is expected to be stable during common processing practices such as pasteurisation, sterilisation and baking/brewing/boiling.

I. Materials and Methods

A. Materials

Test material N-acetyl-[phosphonomethylene-^{14}C-]glycine
[^{14}C]- *N*-acetyl-glyphosate

Chemical structure:	
	* position of radiolabel
Radiochemical purity:	>96 % (97.2 %, HPLC; assay conducted by Perkin Elmer Life and Analytical Sciences)
Specific activity:	0.51 MBq/mg (13.83 µCi/mg)
Lot number:	3562-059
CAS No:	129660-96-4 (non-radiolabeled)
Log $P_{o/w}$:	Log P_{ow} = -6.29 at 25 °C (at pH 5) Log P_{ow} = -6.26 at 25 °C (at pH 7) Log P_{ow} = -6.86 at 25 °C (at pH 9)
Solubility in water	Not specified within the report

B. Study design

1. Preparation of stock and test solutions

The active substance *N*-acetyl-[phosphonomethylene-^{14}C]glycine ([^{14}C]-*N*-acetyl-glyphosate) was received as neat compound and was stored in a freezer (≤ -10 °C) when not in use. A stock solution of ^{14}C-*N*-acetyl-glyphosate was prepared by dissolving 0.150 mL of the radioactive test substance in 5 mL of HPLC grade water. Radiochemical purity of the test substance determined was >96 %. The concentration of the purified glyphosate stock solution was 1708.8 µg/mL.

A buffer concentration of 0.01 M was selected to minimize possible catalytic effects. All solutions were prepared by combining 0.1 M citric acid solution and 0.1 M trisodium citrate solution with distilled grade water. Buffered solutions were adjusted to pH 4.0, 5.0 and 6.0.

Since *N*-acetyl-glyphosate remained stable throughout the study in all test systems and no difference was observed between heated and control samples, sterility of the buffers was not determined.

Test solutions were prepared by transferring aliquots of the radiolabelled stock solution to buffer solution (pH 4, 5 or 6) to obtain a nominal concentration of approximately 1.0 µg/mL ^{14}C- *N*-acetyl-glyphosate. The pH of each buffer solution was measured at the time of preparation, after addition of the test item and after sampling and was deemed acceptable.

Each test solution was mixed well and aliquots were radioassayed by liquid scintillation counting (LSC) to determine the concentration. The concentration of *N*-acetyl-glyphosate in buffered solutions before hydrolysis ranged from 1.176 mg/L to 1.184 mg/L.

2. Experimental conditions

The samples were prepared in triplicate for each buffer system using sterile glass vials.

Triplicate samples were analysed immediately for time zero where no heat was used. Three additional samples at pH 4 ± 0.1 were placed in an oven and maintained at 90 °C ± 5°C for 20 minutes. Three samples at pH 5 ± 0.1 were placed in an oven and maintained at 100 °C ± 5°C for 60 minutes and three samples at pH 6 ± 0.1 were placed in an oven and maintained at sterilizing conditions (approximately 121 °C) for 20 minutes.

Table 6.5.1-7: Parameters for hydrolysis conditions

pH	Temperature [°C]	Test period [min]	Representative Process
4.0 ± 0.1	90 ± 5	0 and 20 min	Pasteurisation
5.0 ± 0.1	100 ± 5	0 and 60 min	Baking, brewing and boiling
6.0 ± 0.1	120 ± 5	0 and 20 min	Sterilisation

Temperatures were recorded before and after exposure to the test conditions. Control samples were placed at room temperature for the same time period as the corresponding heat-treated samples. At the end of the incubation period, the vessels containing samples were allowed to cool to room temperature before being analysed.

3. Sampling

Triplicate samples were collected at time 0 and one subsequent time point after application. At sampling, triplicate samples were retrieved from the respective oven. The pH of the solution was measured and recorded. Triplicate aliquots (3 x 1 mL) were taken for LSC analysis. All samples were initially analyzed via HPLC on the sampling day. Samples were stored frozen, after the initial analysis, at less than *ca* -10 °C. LSC analyses were conducted on thawed samples that had been frozen overnight.

4. Analytical phase

Each dose solution was mixed well, and radioactivity measurement was carried out by liquid scintillation counting (LSC) to determine the concentration.
^{14}C- *N*-acetyl-glyphosate and its potential degradates were analysed and quantitated based on reverse phase HPLC analysis with LSC analysis of the collected eluent fractions. The identity of ^{14}C- *N*-acetyl-glyphosate was based on co-chromatography with *N*-acetyl-glyphosate reference standard upon HPLC analysis:
Unchanged *N*-acetyl-glyphosate in samples was identified using HPLC by comparing the retention time of the radioactive peak with that of an authentic standard. Representative samples were fortified with the radiolabelled reference standard and analysed using HPLC. The limit of quantification was 0.4 % AR. The identification of *N*-acetyl-glyphosate was confirmed by the analysis of selected samples using a second analytical method (LC-MS analysis). *N*-acetyl-glyphosate was identified by comparing LC/MS profiles to that of an authentic unlabelled reference standard under the same conditions.

II. Results and Discussion

The pH of the samples was measured at each sampling time. The pH results for all sets of samples indicated that the buffering capacity was maintained in the solution during the study period.
The hydrolysis of *N*-acetyl-glyphosate test substance was examined at pH 4, pH 5 and pH 6 at 90 °C, 100 °C and approximately 120 °C, respectively.
Aliquots of all test solutions were analysed in triplicate by HPLC before and after hydrolysis. Under the tested conditions representative for food processing, no hydrolysis of ^{14}C- *N*-acetyl-glyphosate was observed.
Average radiocarbon recoveries for the high temperature hydrolysis tests generally ranged from 95.3 to 98.6 % of the applied radioactivity (AR) for all solutions, except for pH 5 control samples that accounted for 87.7 % AR. The lower recoveries observed in pH 5 control samples result from the cracking of two of the vials during the overnight freezing prior to LSC analysis. The replicate sample that did not crack had a material balance of 98.8 % AR. Since *N*-acetyl-glyphosate remained stable throughout the study and the pH 5 heated samples showed acceptable recoveries, the pH 5 control samples were not retested. It was shown that *N*-acetyl-glyphosate did not degrade at temperatures ranging from 90 °C to sterilizing conditions (120 °C) in any of the buffer systems tested.

The amount of *N*-acetyl-glyphosate in the pH 4 samples incubated at 90 °C was 93.2 % AR (1.096 μg equiv/mL) after 20 min. Other minor radiolabelled components were detected, which collectively accounted for less than 4 % AR. The amount of *N*-acetyl-glyphosate in the corresponding control samples was 91.4 % AR (1.075 μg equiv/mL). Other minor components were detected, which collectively accounted for less than 4 % AR.
The amount of *N*-acetyl-glyphosate in the pH 5 samples incubated at 100 °C was 92.1 % AR (1.091 μg equiv/mL) after 60 min. Other minor radiolabelled components were detected, which collectively accounted for less than 5 % AR. The amount of *N*-acetyl-glyphosate in the corresponding 5 control samples was 84.0 % AR (0.994 μg equiv/mL). Other minor components were detected, which collectively accounted for less than 5 % AR.
The amount of *N*-acetyl-glyphosate in the pH 6 samples incubated at 120 °C was 93.9 % AR (1.105 μg equiv/mL) after 20 min. Other minor radiolabelled components were detected, which collectively accounted for less than 5 % AR. The amount of *N*-acetyl-glyphosate in the corresponding control samples was 93.1 % AR (1.096 μg equiv/mL). Other minor components were detected, which collectively accounted for less than 5 % AR.
No hydrolysis products formed in concentrations of ≥ 5 % of the initial applied radioactivity. Therefore, these minor products were not identified. Detailed results are provided in the table below.

Table 6.5.1-8: Recovered radioactivity ^{14}C- *N*-acetyl-glyphosate before and after hydrolysis in sterile buffer solutions

Hydrolysis conditions	Sample	Re-plicate	Mass balance (% applied dose)	*N*-acetyl-glyphosate	Other peaks[1]
				% applied dose	% applied dose
pH 4, 90°C, 20 min	0 min	1	99.7	96.8	2.9
		2	87.0	83.6	3.4
		3	99.3	93.8	5.5
		Mean	95.3	91.4	3.9
	20 min	1	92.0	89.5	2.5
		2	99.9	96.9	2.9
		3	98.1	93.2	4.9
		Mean	96.7	93.2	3.4
pH 5, 100°C, 60 min	0 min	1	86.5	80.9	5.6
		2	77.7	74.8	2.9
		3	98.8	96.3	2.5
		Mean	87.7	84.0	3.7
	60 min	1	98.7	94.5	4.2
		2	99.3	91.7	7.5
		3	92.5	90.2	2.2
		Mean	96.8	92.1	4.7
pH 6, 120°C, 20 min	0 min	1	98.4	91.7	6.7
		2	99.1	92.6	6.6
		3	98.4	95.0	3.4
		Mean	98.6	93.1	5.5
	20 min	1	97.8	94.2	3.7
		2	98.0	91.2	6.8
		3	98.2	96.3	1.9
		Mean	98.0	93.9	4.1

1 sum of minor peaks (individually <5 % AR)

C. Storage stability

All samples were analysed by HPLC on the day of sampling. A reference standard was analysed with each HPLC run, which verified proper column and instrument operation and degradation of the reference substance throughout the study was not reported. All samples and standard solutions were stored frozen (< -10 °C) when not in use. Samples were analysed by LSC after being frozen overnight and allowed to thaw.

III. Conclusion

This study demonstrated that *N*-acetyl-glyphosate remained stable under simulated pasteurisation (pH 4, 90°C, 20 minutes), baking, brewing or boiling (pH 5, 100°C, 60 minutes), and sterilisation (pH 6, 120°C, 20 minutes) conditions.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the high temperature hydrolysis of *N*-acetyl-glyphosate has been previously evaluated at EU level. It was performed under GLP. The study complies with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 507, with minor deficits: It is reported that an oven (instead of an autoclave) was used to reach the temperature of 120 °C, simulating sterilisation, but the "oven" is not listed in the instrumental list; Only representative, not-integrated HPLC chromatograms are shown in the study report. Co-chromatography (fortification of samples with radiolabelled reference standard) was only performed on selected samples (only one is shown). Minor peaks (other peaks) were < 10 % AR, but >0.01-0.05 mg/kg and > 0.05 mg/kg, respectively. However, the other peaks were detected both in control samples (taken before hydrolysis, at 0 min) as after hydrolysis and were comparable (3.7-5.5 % AR before hydrolysis and 3.4 – 4.7 % AR after hydrolysis). Further characterisation of these minor peaks was therefore not attempted; The average material balance of control samples at pH 5 are slightly below 90 %. However, the lower recoveries were only observed in two of the three vials, because they had cracked during the overnight freezing prior to LSC analysis. The replicate sample that did not crack had a material balance of 99 % AR. Since *N*-acetyl-glyphosate remained stable throughout the study and the pH 5 heated samples showed acceptable recoveries, the pH 5 control samples were not retested; Sterility of the buffers was not determined in this study. However, *N*-acetyl-glyphosate remained stable throughout the study in all test systems and no difference was observed between heated and control samples; Purity of the radiolabeled test substance was not determined as part of this study prior to day 0. It was however determined after sample analysis and found to be >96 %. There was no impact on the study; Samples were stored at -10 °C, but analysed by HPLC on the day of sampling and by LSC after being frozen overnight and allowed to thaw.
The study is considered to be reliable.

Assessment and conclusion by RMS:

CA 6.5.2 Distribution of the residue in inedible peel and pulp

Glyphosate concentrates primarily in processed fractions such as hulls and bran of cereals and citrus peel due to surface residues; in meal after removal of oil fractions; and in concentrated liquid fractions such as molasses. Details are presented in the chapter below.

CA 6.5.3 Magnitude of residues in processed commodities

In the supervised residue studies in orchards and vegetables residues of glyphosate and AMPA were always below the LOQ of 0.05 mg/kg. The dietary risk assessments show that the chronic and acute risk

is <10 % of the ADI and of the ARfD, respectively for any European consumer group diet. Therefore, processing studies are not needed.

Nevertheless, data on the magnitude of residue of glyphosate and AMPA in processed commodities were generated for citrus, olives and potatoes.

Table 6.5.3-1: Studies on processing of glyphosate and its metabolite AMPA

Annex point	Study	Crop	Processed commodities	Status	Remark
CA 6.5.3/001	[redacted] 1986	Citrus	Juice, peel, feed meal, press liquor	Valid	Recalculation of results of the study conducted in 1975
CA 6.5.3/002	[redacted] 1975	Citrus	Juice, peel, feed meal, press liquor	Valid	
CA 6.5.3/003	[redacted] 1988	Potato	Chips, wet peel (chips), flakes, wet peel (flakes), dry peel (flakes) and granules	Valid	
CA 6.5.3/004	[redacted] 1996	Olives	Oil, raw and refined	Valid	
CA 6.5.3/005	[redacted] 1993	Olives	Oil, unrefined	Valid	
CA 6.5.3/006	[redacted], 1992	Olives	Oil, unrefined	Valid	

Citrus fruit

For citrus, the essential processing product is juice, peel, feed meal and press liquor.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.3/001
Report author	[redacted]
Report year	1986
Report title	Determination of Glyphosate and Aminomethylphosphonic acid residues in citrus fruit and process fractions following post-directed treatment with Roundup herbicide
Report No	MSL-6194
Document No	-
Guidelines followed in study	OECD GLP FAO Guidelines
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 508: • The test formulation used in the trials is not described. • A description of the test facility is not provided.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

In the report the results of the processing study in citrus conducted in 1975 (CA 6.5.3/002) were recalculated.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.3/002
Report author	[redacted]
Report year	1975
Report title	CP 57573, Residue and metabolism part 27: Determination of CP 67573 and CP 50435 residues in citrus process fractions
Report No	377
Document No	-
Guidelines followed in study	No test guidelines cited in the report
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 508: • The test formulation used in the trials is not described. • A description of the test facility is not provided. • The mean recoveries for the higher fortification ranges are below 70 %
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in citrus (fruit) and processed fraction juice, peel, press liquor and feed meal after three applications of Roundup.

The study included 6 field trials with processing in the USA. The citrus were treated three times, to the soil under the citrus trees at rates of 4.48 kg glyphosate per hectare or 8.97 kg glyphosate per hectare. Citrus fruit samples for processing were taken 1, 7 and 21 days after the last application.
Residues of glyphosate in whole fruit sampled 1-21 days after the last application ranged from <0.05 mg/kg to 0.22 mg/kg.
No residues of AMPA above the LOQ were found in any whole fruit taken 1, 7 and 21 days after the last application or in the processed commodities.

For juice the mean processing factor for glyphosate was 0.78, indicating that there was no concentration of glyphosate residue into juice relative to the raw commodity whole fruit. For citrus peel, feed meal and press liquor the mean processing factors for glyphosate were 2.43, 3.08 and 1.92, respectively.

I. Materials and Methods

A. Materials

1. Test material

Description:	Roundup
Batch number:	Not reported
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	Not reported
Content of a.s. analysed:	Not reported
Formulation type:	Not reported

B. Methods

1. Field phase

Six residue trials were conducted on different citrus fruit (orange, lemon, grapefruit) during 1972 in the USA. Three applications of Roundup was performed onto the soil under the citrus trees at 4.48 kg a.s./ha or 8.97 kg a-s./ha. The main application parameters are outlined in the table below.

Table 6.5.3-2: Application information

Trial no.	Crop	Scientific names	Variety	Application rate kg a.s./ha
California, Riverside/ USA / 1973	Orange	*Citrus paradisi*	Navel	3 x 4.48
				3 x 8.97
Florida, Lake Alfred USA / 1973	Orange	*Citrus paradisi*	Pineapple	3 x 8.97
Florida, Lake Alfred USA / 1973	Orange	*Citrus paradisi*	Valencia	3 x 8.97
California, Riverside/ USA / 1973	Lemon	*Citrus limon*	not specified	3 x 4.48
				3 x 9.97
Texas, Lake Alfred USA / 1973	Grapefruit	*Citrus paradisi*	Ruby Red	3 x 8.97
Florida, Lake Alfred USA / 1973	Grapefruit	*Citrus paradisi*	Marsh	3 x 8.97

Regions, varieties and cultivation were typical for the cultivation of citrus fruit.

2. Sampling

Specimens of citrus fruit were taken by hand from treated and untreated plots at 1, 7, and 21 days after the last application. Control specimens were taken before treated specimens.

Field samples composited from replicates were processed and frozen shortly after sampling and kept frozen until they were ready for analysis. Whole citrus fruits were kept frozen until processing.

3. Processing

Processing was performed to obtain the processed fractions of juice, peel, feed meal and press liquor. The technology used was a lab-scale process similar to the industrial process.

The citrus fruit were defrosted and washed. After juicing the peel were grinded in a food grinder. After adding calcium hydroxid and mixing the peel was allow to stand until it hardened and a clear serum could be readily squeezed out.
Using a tapered screw press, the peel was pressed. The products of this operation were a dilute emulsion of peel oil in the peel serum and a wet pulp. The wet the pulp was dried overnight in a vented oven at 60° to 65° C.

4. Analytical phase

Glyphosate and AMPA were isolated from citrus specimen by aqueous extraction followed by ion exchange chromatography. After derivatisation to the N-trifluoroacetyl methyl esters the samples were subjected to GLC using a phosphorus specific flame photometric detector. For both analytes the limit of detection was 0.05 mg/kg in most fractions and 0.025 mg/kg in pre-wash and wash water samples.

The method was validated within this study, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ), 0.025 mg/kg for prewash water and after wash water and higher level were performed. Some of the mean recoveries are below 70 % and therefore outside the guidance requirements. These low mean recovery values are mainly found for the higher forticfication level. The lower fortification levels are sufficiently validated. The results are summarised in the table below.

Table 6.5.3-3: Recovery results

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Juice	Glyphosate	0.1	55-104	71	14	19	22
		0.2	50-89	64	9.9	16	21
		Overall	**50-104**	**68**	**12**	**18**	**43**
	AMPA	0.1	53-106	75	14	19	24
		0.2	53-99	72	16	22	24
		Overall	**53-106**	**73**	**15**	**20**	**48**
Peel	Glyphosate	0.05	59-98	80	20	25	4
		0.1	52-95	71	11	16	22
		0.2	53-80	66	8.4	13	21
		Overall	**52-98**	**70**	**11**	**16**	**47**
	AMPA	0.05	55-95	66	19	29	4
		0.1	41-110	67	16	24	21
		0.2	44-99	66	14	21	19
		Overall	**41-110**	**67**	**15**	**23**	**44**

Table 6.5.3-3: Recovery results

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Press liquor	Glyphosate	0.05	106	106	N/A	N/A	1
		0.1	71-92	80	8.3	10	6
		0.2	56-85	74	10	14	8
		0.4	73	73	N/A	N/A	1
		Overall	**56-106**	**78**	**12**	**15**	**16**
	AMPA	0.05	108	108	N/A	N/A	1
		0.1	80-106	93	9.2	9.9	7
		0.2	57-102	81	16	19	8
		0.4	85	85	N/A	N/A	1
		Overall	**57-108**	**88**	**14**	**16**	**17**
Feed meal	Glyphosate	0.05	106	106	N/A	N/A	1
		0.1	59-93	76	13	18	10
		0.2	58-86	72	10	14	7
		Overall	**58-106**	**76**	**14**	**18**	**18**
	AMPA	0.1	48-98	63	15	23	10
		0.2	43-65	56	7	13	8
		Overall	**43-98**	**60**	**12**	**20**	**18**
Oil	Glyphosate	0.05	77-87	82	6.7	8.2	2
		0.1	56-80	67	8.2	12	8
		0.2	66	66	N/A	N/A	1
		Overall	**56-87**	**70**	**9.4**	**14**	**11**
	AMPA	0.05	77-80	78	2.3	2.9	2
		0.1	76-95	83	6.8	8.1	8
		0.2	96	96	N/A	N/A	1
		Overall	**76-96**	**84**	**7.2**	**8.7**	**11**
Molasses	Glyphosate	0.1	58-81	71	9.7	13.7	5
		0.2	58-70	66	5.2	7.8	5
		Overall	**58-81**	**69**	**7.7**	**11**	**10**
	AMPA	0.1	71-96	87	9.8	11	5
		0.2	61-86	77	12	15	5
		Overall	**61-96**	**82**	**12**	**14**	**10**
Grapefruit pulp, rag, seed	Glyphosate	0.1	83-95	89	8.3	9.4	2
		0.2	80-94	87	10	12	2
		Overall	**80-95**	**88**	**7.8**	**8.8**	**4**
	AMPA	0.1	91-97	94	4.2	4.5	2
		0.2	76-85	81	6.3	7.8	2
		Overall	**76-97**	**87**	**8.8**	**10**	**4**

Table 6.5.3-3: Recovery results

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Finisher pulp	Glyphosate	0.1	81-115	95	18	19	3
		0.2	72-93	81	11	13	3
		Overall	**72-115**	**88**	**15**	**17**	**6**
	AMPA	0.1	96-112	103	8.4	8.1	3
		0.2	87-88	88	0.6	0.7	3
		Overall	**87-112**	**95**	**9.9**	**10**	**6**
Peel frits	Glyphosate	0.1	57-71	62	8.0	13	3
		0.2	56-63	59	3.6	6.0	3
		Overall	**56-71**	**61**	**5.7**	**9.5**	**6**
	AMPA	0.1	62-69	64	3.7	5.8	3
		0.2	68-90	80	11	14	3
		Overall	**62-90**	**72**	**11**	**16**	**6**
Citrus pulp	Glyphosate	0.1	86-89	88	2.1	2.4	3
		0.2	73-83	78	5.0	6.4	3
		Overall	**73-89**	**83**	**6.6**	**8.0**	**6**
	AMPA	0.1	79-83	80	2.6	3.2	3
		0.2	79-86	81	3.8	4.7	3
		Overall	**79-86**	**81**	**3.0**	**3.7**	**6**
Oil emulsion water	Glyphosate	0.1	93-96	94	1.8	1.9	3
		0.2	69-90	78	11	14	3
		Overall	**69-96**	**86**	**11**	**13**	**6**
	AMPA	0.1	87-97	88	10	11	3
		0.2	75-89	82	7.2	8.8	3
		Overall	**75-97**	**85**	**8.4**	**9.9**	**6**
Pre-wash water	Glyphosate	0.03	74-97	88	12	14	3
		0.05	82-93	88	5.9	6.6	3
		Overall	**74-97**	**88**	**8.5**	**9.6**	**6**
	AMPA	0.03	63-93	79	16	20	3
		0.05	73-84	77	5.6	7.2	3
		Overall	**62-93**	**78**	**11**	**14**	**6**
After wash water	Glyphosate	0.03	91-96	94	3.1	3.3	3
		0.05	96	96	N/A	N/A	1
		0.1	82-96	89	10	11	2
		Overall	**82-96**	**93**	**5.9**	**6.3**	**6**
	AMPA	0.03	84-90	88	3.5	3.9	3
		0.05	90	90	N/A	N/A	1
		0.1	84-90	87	4.2	4.9	2
		Overall	**84-90**	**88**	**3.0**	**3.5**	**6**

Table 6.5.3-3: Recovery results

Matrix[1]	Analyte	Fortification level (mg/kg)	Recovery[2]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)

1 Values for matrices from orange, lemon and grapefruit were combined.
2 Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.
N/A Not applicable

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA in processed commodities after usage of Roundup when applied as per the study.

Residues of glyphosate in whole fruit sampled 1-21 days after the last application ranged from <0.05 mg/kg to 0.22 mg/kg.
No residues of AMPA above the LOQ were found in any whole fruit taken 1, 7 and 21 days after the last application or in the processed commodities.

For juice residues of glyphosate raged from <0.05 mg/kg to 0.10 mg/kg resulting in processing factors from 0.45 to <1, indicating that there was no concentration of glyphosate residue into juice relative to the raw commodity whole fruit.
For peel, feed meal and press liquor residues of glyphosate raged from <0.05 mg/kg to 0.69 mg/kg, from <0.05mg/kg to 0.39 mg/kg and from <0.05 mg/kg to 0.37 mg/kg, respectively. The resulting in processing factors for peel, feed meal and press liquor ranged from 1.1 to 3.1, from 1.4 to 5.3 and from <0.83 to 2.7, respectively.

In the cases where no residues of glyphosate and AMPA in the whole fruit above the LOQ of 0.05 mg/kg were found, the calculation of a processing factor was not possible.

Detailed residue levels are shown in the table below.

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
California, Riverside/ USA / 1973	Orange / Navel upper level	3 x 4.48	1	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Press liquor	<0.05	-	<0.05	-
			7	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	0.06 0.05 Mean=0.06	>1.2	<0.05	-
				Press liquor	<0.05	-	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Press liquor	<0.05	-	<0.05	-
		3 x 8.97	1	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05 <0.05 0.06 <0.05 Mean=0.05	>1.0	<0.05	-
				Feed meal	0.06 0.08 Mean=0.07	>1.4	<0.05	-
				Press liquor	<0.05	-	<0.05	-
			7	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Press liquor	<0.05	-	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Press liquor	<0.05	-	<0.05	-

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
California, Riverside/ USA / 1973	Orange / Navel lower level	3 x 4.48	1	Whole fruit[4]	0.05 <0.05 0.06 0.05 Mean=0.05	-	<0.05	-
				Juice	<0.05	<1.0	<0.05	-
				Peel	0.16 <0.05 0.18 0.16 Mean=0.14	2.8	<0.05	-
				Feed meal	0.27 0.26 Mean=0.27	5.3	<0.05	-
				Press liquor	0.12 0.11 Mean=0.12	2.3	<0.05	-
			7	Whole fruit[4]	0.05 0.05 0.08 0.06 Mean=0.06	-	<0.05	-
				Juice	<0.05	<0.83	<0.05	-
				Peel	<0.05 0.11 0.33 0.25 Mean=0.19	3.1	<0.05	-
				Feed meal	0.08 0.13 Mean=0.11	1.8	<0.05	-
				Press liquor	0.11 0.12 Mean=0.12	1.9	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	0.06 <0.05 0.08 0.08 Mean=0.07	>1.4	<0.05	-

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
		3 x 8.97	1	Whole fruit[4]	0.05 0.17 0.50 0.17 Mean=0.22	-	<0.05	-
				Juice	<0.05 <0.05 0.23 <0.05 Mean=0.10	0.45	<0.05	-
				Peel	0.13 0.64 1.31 0.69 Mean=0.69	3.1	<0.05	-
				Feed meal	0.38 0.39 Mean=0.39	1.8	<0.05	-
				Press liquor	0.36 0.37 Mean=0.37	1.7	<0.05	-
			7	Whole fruit[4]	<0.05 0.11 <0.05 <0.05 Mean=0.07	-	<0.05	-
				Juice	<0.05	<0.71	<0.05	-
				Peel	0.09 0.45 0.05 <0.05 Mean=0.16	2.3	<0.05	-
				Feed meal	0.35 0.34 Mean=0.35	4.9	<0.05	-
				Press liquor	0.13 0.17 Mean=0.15	2.1	<0.05	-

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
			21	Whole fruit[4]	0.10 0.17 <0.05 <0.05 Mean=0.09	-	<0.05	-
				Juice	0.09 0.11 <0.05 <0.05 Mean=0.08	0.83	<0.05	-
				Peel	0.14 0.38 0.15 0.42 Mean=0.20	2.2	<0.05	-
				Feed meal	0.24 0.35 Mean=0.30	3.3	<0.05	-
				Press liquor	0.25 0.24 Mean=0.25	2.7	<0.05	-
Florida, Lake Alfred USA / 1973	Orange/ Pineapple	3 x 8.97	1	Whole fruit[4]	<0.05	-	<0.05	-
				Washed	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Pulp	<0.05	-	<0.05	-
				Dried pulp	<0.05 0.05 Mean=0.05	>1.0	<0.05	-
				Oil	<0.05	-	<0.05	-
				Molasses	0.05 <0.05 Mean=0.05	>1.0	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Washed	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
Florida, Lake Alfred USA / 1973	Orange/ Valencia	3 x 8.97	1	Whole fruit[4]	<0.05	-	<0.05	-
				Washed	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Washed	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Pulp	<0.05	-	<0.05	-
				Dried pulp	<0.05	-	<0.05	-
				Oil	<0.05	-	<0.05	-
				Molasses	<0.05	-	<0.05	-
California, Riverside/ USA / 1973	Lemon/ not specified	3 x 4.48	1	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05 <0.05 0.09 <0.05 Mean=0.06	>1.2	<0.05	-
				Feed meal	0.18 0.12 Mean=0.15	>3.0	<0.05	-
				Press liquor	0.05 0.06 Mean=0.06	>1.2	<0.05	-
			7	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	0.05 <0.05 Mean=0.05	>1.0	<0.05	-
				Press liquor	<0.05	-	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Press liquor	<0.05	-	<0.05	-

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
		3 x 8.97	1	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	0.06 <0.05 0.09 <0.05 Mean=0.06	>1.2	<0.05	-
				Feed meal	0.09 0.07 Mean=0.08	>1.6	<0.05	-
				Press liquor	<0.05		<0.05	-
			7	Whole fruit[4]	<0.05 <0.05 0.08 <0.05 Mean=0.06	-	<0.05	-
				Juice	<0.05	<0.83	<0.05	-
				Peel	<0.05 <0.05 0.11 <0.05 Mean=0.07	1.1	<0.05	-
				Feed meal	0.09 0.08 Mean=0.09	1.4	<0.05	-
				Press liquor	<0.05	<0.83	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	0.07 0.05 Mean=0.06	>1.2	<0.05	-
				Press liquor	<0.05	-	<0.05	-
Texas, Lake Alfred USA / 1973	Grapefruit, Ruby Red	3 x 8.97	1	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Pulp, rag, seeds	<0.05	-	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Feed meal	<0.05	-	<0.05	-
				Pulp, rag, seeds	<0.05	-	<0.05	-

Table 6.5.3-4: Residues of glyphosate and AMPA in citrus processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors[3]	Residues found (mg/kg)	Processing factors
Florida, Lake Alfred USA / 1973	Grapefruit/ Marsh	3 x 8.97	1	Whole fruit[4]	<0.05	-	<0.05	-
				Washed	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-
				Pulp	<0.05	-	<0.05	-
				Dried pulp	<0.05	-	<0.05	-
				Oil	<0.05	-	<0.05	-
			21	Whole fruit[4]	<0.05	-	<0.05	-
				Washed	<0.05	-	<0.05	-
				Juice	<0.05	-	<0.05	-
				Peel	<0.05	-	<0.05	-

1 Days after last application
2 In the case all replicates were <0.05 mg/kg only the mean value of <0.05 mg/kg is given
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample
4 Calculated value based on juice yield x residue in juice + peel yield x residue in peel

III. Conclusion

The median processing factors of glyphosate for citrus juice, peel, feed meal and press liquor were 0.83, 1.8, 1.8 and 1.9, respectively. Glyphosate does not concentrate in matrices destined for human consumption.
For AMPA no residues above the LOD were present in the raw agricultural commodity. Therefore, a calculation of processing factors was not possible.

III. Conclusion

The median calculated processing factors of glyphosate for citrus juice, peel, feed meal and press liquor were 0.83, 1.8, 1.8 and 1.9, respectively. Glyphosate does not concentrate in matrices destined for human consumption.
Processing factors could be calculated for oranges and lemon processed commodities. For grapefruit, the residues of glyphosate and AMPA in whole fruit and all processed commodities were always below the LOQ and no processing factors could be calculated.
For AMPA no residues above the LOD were present in the raw agricultural commodity. Therefore, a calculation of processing factors was not possible.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was not performed under GLP. Some of the mean recoveries for AMPA are below 70 % and therefore outside the guidance requirements. These low mean recovery values are mainly found for the higher forticfication level. The lower fortification levels are sufficiently validated. The residue studies in orchards show that the residues of glyphosate and AMPA are both below the LOQ of 0.05 mg/kg. Since this low residue levels are sufficiently validated the deviation from the guideline can regarded as minor. Even though there are some minor deviations from the current test guideline (test formulation used in the trials is not described; description of the test facility is not provided) the study is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 508. It adequately supports the representative processing processes for glyphosate and AMPA in citrus fruit.

Assessment and conclusion by RMS:

Summary of citrus processing factors

The processing factors or glyphosate residues in citrus processed fractions are summarised in the table below.

Table 6.5.3-5: Overview of processing factors for glyphosate residues in citrus processed fractions

Source: DocID (trial reference)	PHI	Citrus juice	Citrus peel	Feed meal	Press liquor
Orange / Navel (lower level) 3 x 4.48 kg a.s./ha	1	<1.0	2.8	5.3	2.3
	7	<0.83	3.1	1.8	1.9
	21	-	>1.4		
Orange / Navel (upper level) 3 x 8.97 kg a.s./ha	1	-	>1.0	-	-
Orange / Navel (lower level) 3 x 8.97 kg a.s./ha	1	0.45	3.1	1.8	1.7
	7	<0.71	2.3	4.9	2.1
	21	0.83	2.2	3.3	2.7
Lemon 3 x 4.48 kg a.s./ha	1	-	>1.2	>3.0	>1.2
	7	-	-	>1.0	-
Lemon 3 x 8.97 kg a.s./ha	1	-	>1.2	>1.6	-
	7	<0.83	1.1	1.4	<0.83
	21	-	-	>1.2	-
Median	1	0.725	1.2	2.4	1.7
	7	0.83	2.3	1.6	1.9
	21	0.83	1.8	2.25	2.7
	Overall	**0.83**	**1.8**	**1.8**	**1.9**
Mean	*1*	*0.725*	*1.86*	*2.93*	*1.73*
	7	*0.79*	*2.17*	*2.28*	*1.61*
	21	*0.83*	*1.8*	*2.25*	*2.7*
	Overall	***0.78***	***1.94***	***2.53***	***1.82***

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.3/03
Report author	[redacted]
Report year	1988
Report title	Glyphosate residues in potatoes and processed fractions of potatoes after treatment with Roundup herbicide
Report No	MSL-7877
Document No	-
Guidelines followed in study	EPA Guideline 171-4: Magnitude of Residue-Crop Field Trials
Deviations from current test guideline	None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in potato (tuber) and processed fraction chips, wet peel (chips), flakes, wet peel (flakes), dry peel (flakes) and granules after one application of Roundup, an EC formulation.

The study included 2 processing trials in the USA. There was one soil treatment before emergence of the potatoes at rates of either 4.2, 8.4, 21 or 42 kg glyphosate per hectare. Potato samples for processing were collected at 97-104 days after the application. The residues of glyphosate in potato tubers were always below the LOQ of 0.05 mg/kg. The residues of AMPA in potato tubers were <0.05 mg/kg for the lower application rates of 4.2 and 8.4 kg a.s./ha and for the higher application rates of 21 and 42 kg a.s./ha between <0.05 and 0.23 mg/kg.

The mean processing factors for AMPA were 1.7 in chips, 0.45 in wet peel (from chips processing), 1.4 in flakes, 0.47 in wet peel (from flakes processing), 1.5 in dry peel (from flakes processing), and 1.7 in granules.

I. Materials and Methods

A. Materials

1. Test material

Description:	Roundup
Batch number:	LIRP-05098-X
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. analysed:	41.36 %
Formulation type:	EC

B. Methods

1. Field phase

Two residue trials were conducted on potatoes (*Solanum tuberosum*) during 1987 in the USA. There was one application of Roundup to the soil before emergence of the potatoes at rates of either 4.2, 8.4, 21 or 42 kg glyphosate per hectare. The volume of water used to prepare the spray solution was 374 L/ha.

Care was taken that the spray solution was properly homogenised by mixing before application. Ground spray applications were made via backpack sprayer with a boom equipped with flat fan nozzles.

2. Sampling

Specimens of potato were taken by hand from treated and untreated plots 97 or 104 days after the application.

Treated and untreated olive fruit specimens were maintained in a deep frozen condition and adequately separated during storage and shipment to the processing facility.

Table 6.5.3-6: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Quantity	Date of sampling
California, Arvin	Potato	Tuber Tuber for processing	97	4.5 kg 113-159 kg	23.06.1987
California, Lamont	Potato	Tuber Tuber for processing	104	4.5 kg 113-159 kg	30.06.1987

1 Days after last application

3. Processing

Processing was performed to obtain the processed fractions of chips, wet peel (from chips processing), flakes, wet and dry peel (from flakes processing) and granules. The technology used was a lab-scale process similar to the industrial process.

For potato chips, the raw potatoes were washed and destoned. After peeling and slicing, the starch on the surface of the slices was washed away. Then the slices were fried.
For potato flakes the raw potatoes were washed and destoned. After peeling the potatoes were sliced, washed, cooked and mashed, then crushed by rollers on the surface of a drum. The mashed potatoes were dried by heating to temperatures from 162 to 170°C, and then the layer of dried, mashed potatoes is scraped from the drum. The flakes were milled to the right size and frozen.
For potato granules the raw potatoes were washed and destoned. After peeling the potatoes were sliced, washed, cooked and mashed. Subsamples were frozen and afterwards after thawing and drying of one subsample this subsample was mixed with a freshly thawed subsample. This process was repeated six times. The final mixture was dried to a water content of 8-10 %.

4. Analytical phase

All samples were analysed using the analytical method XA001, which based on the well-established method DFG 405 (refer to CA 4.1.2). Treated and untreated olive samples were maintained deep frozen until analysis. Processed samples were stored cold (< 5°C) until analysis.

For the determination of glyphosate and AMPA the samples were extracted with water and dichloromethane and cleaned-up by elution through Chelex 100 resin followed by anion exchange chromatography. Glyphosate and AMPA were quantified by HPLC after post-column derivatisation with o-phthaldialdehyde with a fluorescence detector.
For glyphosate and AMPA in olives and olive oil the limit of quantitation (LOQ) was 0.05 mg/kg.

During analysis of olive specimens, fortification experiments with glyphosate and AMPA at fortification levels of 0.05 mg/kg (LOQ) and higher were performed. Some of the single recoveries are below 70 %. But since the calculated overall mean recoveries are in the range from 70 to 110 % the results can be regarded as valid. The results are summarised in the table below.

Table 6.5.3-7: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)[1]	Relative standard deviation (%)[1]	Number analyses (n)
Potato whole	Glyphosate	0.05	94-109	101	10.1	10.0	2
		0.1	99-101	100	1.6	1.6	2
		0.2	100	100	N/A	N/A	1
		0.5	97-104	100	3.8	3.8	3
		1.0	92-105	97	6.4	6.6	3
		2.0	103	103	N/A	N/A	1
		Overall	**92-109**	**100**	**4.7**	**4.8**	**12**
	AMPA	0.05	82-110	96	20.2	21.1	2
		0.1	81-96	89	10.6	12.0	2
		0.2	80	80	N/A	N/A	1
		0.5	82-91	88	5.5	6.3	3
		1.0	91-98	95	3.5	3.7	3
		2.0	91	91	N/A	N/A	1
		Overall	**80-110**	**91**	**8.8**	**9.7**	**12**
Potato chips	Glyphosate	0.05	67	67	N/A	N/A	1
		0.2	100	100	N/A	N/A	1
		0.5	102	102	N/A	N/A	1
		2.0	96	96	N/A	N/A	1
		Overall	**67-102**	**91**	**16.6**	**18.1**	**4**
	AMPA	0.05	135	135	N/A	N/A	1
		0.2	111	111	N/A	N/A	1
		0.5	102	102	N/A	N/A	1
		2.0	86	86	N/A	N/A	1
		Overall	**86-135**	**108**	**20.5**	**18.9**	**4**
Potato chips, stock feed	Glyphosate	0.05	96	96	N/A	N/A	1
		0.2	88	88	N/A	N/A	1
		0.5	92	92	N/A	N/A	1
		2.0	91	91	N/A	N/A	1
		Overall	**88-96**	**92**	**3.4**	**3.7**	**4**
	AMPA	0.05	103	103	N/A	N/A	1
		0.2	85	85	N/A	N/A	1
		0.5	91	91	N/A	N/A	1
		2.0	85	85	N/A	N/A	1
		Overall	**85-103**	**91**	**8.3**	**9.2**	**4**

Table 6.5.3-7: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery				
			Range (%)	Mean (%)	Standard deviation (%)[1]	Relative standard deviation (%)[1]	Number analyses (n)
Potato flakes	Glyphosate	0.05	80-91	86	7.9	9.2	2
		0.1	78	78	N/A	N/A	1
		0.2	85	85	N/A	N/A	1
		Overall	**78-91**	**83**	**5.8**	**7.0**	**4**
	AMPA	0.05	91-109	100	12.6	12.5	2
		0.1	74	74	N/A	N/A	1
		0.2	82	82	N/A	N/A	1
		Overall	**74-109**	**89**	**15.2**	**17.1**	**4**
Potato flakes raw stock	Glyphosate	0.1	85-92	89	4.8	5.4	2
		1.0	92-95	93	2.5	2.6	2
		Overall	**85-95**	**91**	**4.1**	**4.5**	**4**
	AMPA	0.1	94-92	88	5.3	6.0	2
		1.0	82-87	85	3.2	3.8	2
		Overall	**82-92**	**86**	**4.1**	**4.7**	**4**
Potato flakes, dry stock	Glyphosate	0.05	90-93	92	2.3	2.5	2
		0.1	64	64	N/A	N/A	1
		0.2	82-95	88	8.7	9.9	2
		Overall	**64-95**	**85**	**12.5**	**14.7**	**5**
	AMPA	0.05	60-87	78	14.9	19.2	3
		0.1	91	91	N/A	N/A	1
		0.2	70-78	74	5.4	7.4	2
		0.5	69	69	N/A	N/A	1
		Overall	**60-91**	**77**	**11.2**	**14.5**	**7**
Potato granules	Glyphosate	0.05	84-92	88	3.8	4.3	3
		0.2	80	80	N/A	N/A	1
		0.5	80-85	83	3.7	4.5	2
		Overall	**80-92**	**85**	**4.7**	**5.5**	**6**
	AMPA	0.05	72-94	83	10.8	13.0	3
		0.2	69	69	N/A	N/A	1
		0.5	68-71	69	2.1	3.1	2
		Overall	**68-94**	**76**	**10.4**	**13.6**	**6**

1 Calculations of mean, SDs, RSDs and overall values were performed using excel with individual recovery values as given in the report.

N/A Not applicable

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA in processed commodities after usage of Roundup when applied as per the study.

The residues of glyphosate in potato tubers were always below the LOQ of 0.05 mg/kg.
For chips, wet peel (chips), flakes and for granules the residues of glyphodate were always <0.05 mg/kg and no processing factor could be calculated. In one sample of wet peel (flakes) and for dry peel (flakes) residues of glyphosate 0.06 mg/kg and 0.28 mg/kg, respectively (processing factors 1.2 and 1.6, respectively). In all other samples of wet and dry peel (flakes) the residues of glyphosate were <0.05 mg/kg and no processing factors could be calculated.

The residues of AMPA in potato tubers were <0.05 mg/kg for the lower application rates of 4.2 and 8.4 kg a.s./ha and between <0.05 and 0.23 mg/kg for the higher application rates of 21 and 42 kg a.s./ha.
For chips the residues of AMPA ranged from 0.12 mg/kg to 0.38 mg/kg resulting in processing factors from 1.4 to >2.6, for wet peel (chips) from <0.05 to 0.051 mg/kg (processing factors 0.2-<0.8), for flakes from 0.08 to 0.36 mg/kg (processing factors 1.1-1.7), for wet peel (flakes) from <0.05 to 0.051 mg/kg (processing factors 0.3-<0.8), for dry peel (flakes) 0.12 to 0.25 mg/kg (processing factors 1.1 - >2.4) and for granules 0.13-0.48 mg/kg (processing factors 1.3 - >3.0).

The processing factors for glyphosate for wet peel (flakes) and dry peel (flakes) were 1.2 and 1.6, respectively.

The median processing factors for AMPA for chips, wet peel (chips), flakes, wet peel (flakes), dry peel (flakes) and granules were 1.8, 0.34, 1.6, 0.34, 1.8 and 2.0, respectively.

Table 6.5.3-8: Residues of glyphosate and AMPA in potato processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors	Residues found (mg/kg)	Processing factors[3]
California, Arvin/ USA / 1987	Potato / Kennebeck	1 x 4.2	97	Tuber	<0.05	-	<0.05	-
				Chips	N/A	-	N/A	-
				Wet peel (chips)	N/A	-	N/A	-
				Flakes	N/A	-	N/A	-
				Wet peel (flakes)	N/A	-	N/A	-
				Dry peel (flakes)	N/A	-	N/A	-
				Granules	N/A	-	N/A	-
		1 x 8.4	97	Tuber	<0.05	-	<0.05	-
				Chips	N/A	-	N/A	
				Wet peel (chips)	N/A	-	N/A	
				Flakes	N/A	-	N/A	
				Wet peel (flakes)	N/A	-	N/A	
				Dry peel (flakes)	N/A	-	N/A	
				Granules	N/A	-	N/A	
		1 x 21	97	Tuber	<0.05	-	0.062 0.065 Mean=0.063	-
				Chips	<0.05	-	0.120 0.124 Mean=0.122	1.9
				Wet peel (chips)	<0.05	-	<0.05	<0.8

Table 6.5.3-8: Residues of glyphosate and AMPA in potato processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors	Residues found (mg/kg)	Processing factors[3]
				Flakes	<0.05	-	0.107 0.107 Mean=0.107	1.7
				Wet peel (flakes)	<0.05	-	<0.05	<0.8
				Dry peel (flakes)	<0.05	-	0.141 0.157 Mean=0.149	2.3
				Granules	<0.05	-	0.121 0.140 Mean=0.131	2.1
		1 x 42	97	Tuber	<0.05	-	<0.05	-
				Chips	<0.05	-	0.129 0.133 Mean=0.131	>2.62
				Wet peel (chips)	<0.05	-	<0.05	-
				Flakes	<0.05	-	0.076 0.091 Mean=0.084	>1.68
				Wet peel (flakes)	0.06 0.06 Mean=0.06	-	<0.05	-
				Dry peel (flakes)	0.07 0.08 Mean=0.08	-	0.114 0.123 Mean=0.119	>2.38
				Granules	<0.05	-	0.145 0.152 Mean=0.149	>2.98
California, Lamont/ USA / 1987	Potato / Kennebeck	1 x 4.2	104	Tuber	<0.05	-	<0.05	-
				Chips	<0.05	-	N/A	-
				Wet peel (chips)	<0.05	-	N/A	-
				Flakes	<0.05	-	N/A	-
				Wet peel (flakes)	<0.05	-	N/A	-
				Dry peel (flakes)	<0.05	-	N/A	-
				Granules	<0.05	-	N/A	-
		1 x 8.4	104	Tuber	<0.05	-	<0.05	-
				Chips	<0.05	-	N/A	-
				Wet peel (chips)	<0.05	-	N/A	-
				Flakes	<0.05	-	N/A	-
				Wet peel (flakes)	<0.05	-	N/A	-
				Dry peel (flakes)	<0.05	-	N/A	-

Table 6.5.3-8: Residues of glyphosate and AMPA in potato processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found[2] (mg/kg)	Processing factors	Residues found (mg/kg)	Processing factors[3]
				Granules	<0.05	-	N/A	-
		1 x 21	104	Tuber	<0.05	-	0.147 0.150 Mean=0.149	-
				Chips	<0.05	-	0.205 0.218 Mean=0.212	1.4
				Wet peel (chips)	<0.05	-	<0.05	<0.34
				Flakes	<0.05	-	0.153 0.187 Mean=0.170	1.1
				Wet peel (flakes)	<0.05	-	<0.05	<0.34
				Dry peel (flakes)	<0.05	-	0.175 0.191 Mean=0.183	1.2
				Granules	<0.05	-	0.162 0.183 Mean=0.183	1.2
		1 x 42	104	Tuber	<0.05	-	0.233 0.235 Mean=0.234	
				Chips	<0.05	-	0.365 0.398 Mean=0.382	1.6
				Wet peel (chips)	<0.05	-	0.049 0.052 Mean=0.051	0.22
				Flakes	<0.05	-	0.299 0.422 Mean=0.361	1.5
				Wet peel (flakes)	<0.05	-	0.065 0.067 Mean=0.066	0.28
				Dry peel (flakes)	<0.05	-	0.242 0.259 Mean=0.251	1.1
				Granules	<0.05	-	0.365 0.529 Mean=0.447	1.9

1 Days after last application
2 In the case all replicates were <0.05 mg/kg only the mean value of < 0.05 mg/kg is given
3 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample
N/A Not analysed, due to residues <0.05 mg/kg in the potato tuber (RAC).

III. Conclusion

No residues above the LOQ of 0.05 mg/kg were found for glyphosate in potato tubers (RAC). Therefore, no processing factors could be derived for glyphosate. Only in sample of wet peel (flakes) and dry peel

(flakes) residues of glyphosate of 0.06 and 0.08 mg/kg, respectively were found. The processing factors were 1.2 and 1.6, respectively.
The median processing factors for AMPA were 1.8 in chips, 0.34 in wet peel (from chips processing), 1.6 in flakes, 0.34 in wet peel (from flakes processing), 1.8 in dry peel (from flakes processing), and 2.0 in granules.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. Processing factors could only be derived in the trials where the application rate excced by far the supported application rate in the EU. Nevertheless, the study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 508. It adequately supports the representative processing processes for glyphosate and AMPA in potato.

Assessment and conclusion by RMS:

Summary of potato processing factors

The processing factors of the above study are summarised in the table below.

Table 6.5.3-9: Overall mean processing factors for glyphosate residues in potato processed fractions

Source: DocID (trial reference)	Chips	Wet peel (chips)	Flakes	Wet peel (flakes)	Dry peel (flakes)	Granules
Glyphosate						
California, Arvin 42 kg a.s./ha	-	-	-	1.2	1.6	-
AMPA						
California, Arvin 21 kg a.s./ha	1.9	<0.79	1.7	<0.79	2.3	2.1
California, Arvin 42 kg a.s./ha	>2.62	-	>1.68	-	>2.38	>2.98
California, Lamont 21 kg a.s./ha	1.4	<0.34	1.1	<0.34	1.2	1.2
California, Lamont 42 kg a.s./ha	1.6	0.22	1.5	0.28	1.1	1.9
Median	**1.8**	**0.34**	**1.6**	**0.34**	**1.8**	**2.0**
Mean	**1.9**	**0.45**	**1.5**	**0.47**	**1.7**	**2.0**

Olives

For olives, the essential processing product is oil. In the following three studies on the processing of olives to olive oil are presented.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.3/04
Report author	[redacted]
Report year	1996
Report title	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with Roundup® herbicide. Spanish field trials, 1995
Report No	MLL 30469
Document No	95-GLY-20 Sp
Guidelines followed in study	OECD GLP FAO Guidelines
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 508: • Sample quantity and number of trees sampled were not provided • The oil samples were stored at temperatures <5 °C • The study was not conducted at exaggerated application rates
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in olive (fruit) and the processed fraction olive oil (raw and refined) after one application of Roundup, an SL formulation containing 360 g/L of glyphosate.

The study included 4 field trials with processing in the southern zone. There was one application to the soil under the olive trees at a target rate of 2.16 kg glyphosate per hectare either 28, 14, or 7 days before commercial harvest (each trial included 3 treated plots, one per pre-harvest interval). Olive samples for oil production were collected at commercial harvest from the soil (ground fallen). The residues of glyphosate in ground fallen olive harvested 7 or 14 days after application ranged from 0.1 mg/kg to 0.9 mg/kg. The residues of glyphosate in ground fallen olives harvested 28 days after application were below the limit of quantification (LOQ) of 0.05 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 7, 14, or 28 days after application.

The processing factors for glyphosate and AMPA in all trials were ≤ 1, indicating that there was no concentration of glyphosate or AMPA residue in raw or refined olive oil relative to the raw commodity, whole olive fruit collected from the ground.

I. Materials and Methods

A. Materials

1. Test material

Description:	Roundup
Batch number:	010395
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	31.2 %
Formulation type:	SL

B. Methods

1. Field phase

Four residue trials were conducted on olives (*Olea europaea*) during 1995 in Spain (AP/3065/ME/1, AP/3065/ME/2, AP/3065/ME/3, and AP/3065/ME/4). One application of Roundup (360 g/L glyphosate) was performed onto the soil under the olive trees (6-10 plants per plot) at 6.0 L product/ha (2.16 kg a.s./ha) either 28, 14, or 7 days before harvest. The volume of water used to prepare the spray solution was in the range of 381-440 L/ha. The main application parameters are outlined in the table below.

Table 6.5.3-10: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
AP/3065/ME/1	T3	7 days before harvest	2.141	396
	T2	14 days before harvest	2.188	405
	T1	28 days before harvest	2.147	398
AP/3065/ME/2	T3	7 days before harvest	2.341	433
	T2	14 days before harvest	2.374	440
	T1	28 days before harvest	2.160	400
AP/3065/ME/3	T3	7 days before harvest	2.281	422
	T2	14 days before harvest	2.143	397
	T1	28 days before harvest	2.056	381
AP/3065/ME/4	T3	7 days before harvest	2.279	422
	T2	14 days before harvest	2.132	395
	T1	28 days before harvest	2.151	398

Regions, varieties and cultivation were typical for the cultivation of olives.

Care was taken that the spray solution was properly homogenised by mixing before application. Ground spray applications were made via plot sprayer according to the label directions. The actual applied amount was calculated by measuring the remaining spray solution after application.

2. Sampling

Specimens of olive were taken by hand from treated and untreated plots on the day of application (growth stage approaching maturity) and at 7, 14, and 28 days after treatment (commercial harvest). Specimens were taken from random points within each plot. No specimens were taken from plot edges or from the

area of spray overlap. Control specimens were taken before treated specimens. Sampling equipment was cleaned before use.

For processing, specimens of olives were collected from the ground underneath the tree.

Treated and untreated olive fruit specimens were maintained in a deep frozen condition and adequately separated during storage and shipment to the processing facility.

Table 6.5.3-11: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Quantity	Date of sampling
AP/3065/ME/1	Olive	Fruit, from ground (for processing)	7 14 28	≥ 10.0 kg	05.12.1995
AP/3065/ME/2	Olive	Fruit, from ground (for processing)	7 14 28	≥ 10.0 kg	07.12.1995 (08.12.1995 – treatment 2)
AP/3065/ME/3	Olive	Fruit, from ground (for processing)	7 14 28	≥ 10.0 kg	04.12.1995
AP/3065/ME/4	Olive	Fruit, from ground (for processing)	7 14 28	≥ 10.0 kg	06.12.1995

1 Days after last application

3. Processing

Processing was performed to obtain the processed fractions of raw and refined olive oil. The technology used was a lab-scale process similar to the industrial process.

The olives were defrosted and placed in a shallow layer in a grinder. In cases where the olives were small or not ripe, they were initially crushed with an electric fruit crusher prior to grinding. Olive pulp was recovered and mixed at 25 to 30 °C. The pulp was recovered in nylon cloths, which were then pressed, and the vegetable water and oil collected. The water and oil mixture was heated at approx. 30 °C and the raw oil recovered. A 2.9 M NaOH solution was added to the raw oil and the mixture heated in an oven at 60 to 70 °C. Refined olive oil was decanted off and filtered prior to use. The raw and refined oil were stored at 20 °C until shipment to the analytical facility.

4. Analytical phase

Residue analysis was conducted according to Monsanto method XA001. The residues of glyphosate and AMPA were extracted from the samples by water/dichloromethane partitioning/extraction followed by Chelex 100 resin isolation and anion exchange chromatographic clean-up. Quantification was based on a HPLC post column O-phthalaldehyde reaction system and comparison of peak area/height with known standards.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 207 days. Samples were stored frozen prior to analysis. Raw and refined oil samples were stored in cold storage (< 5 °C) until analysis.

For glyphosate and AMPA in olives (fruit) and olive oil, the limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.02 mg/kg each.

During analysis of olive (fruit) specimens, fortification experiments were performed with glyphosate and AMPA at fortification levels of 0.05, 0.1, 0.5, and 1.0 mg/kg, with additional fortifications at 10 and 20 mg/kg for glyphosate alone. Concurrent recoveries for glyphosate and AMPA in olive oil were determined at fortification levels of 0.05 and 0.1 mg/kg. The results are summarised in the table below.

Table 6.5.3-12: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Olives, fruit	Glyphosate	0.05	63-110	86	21	24	5
		0.1	100-109	105	3.6	3.4	5
		0.5	94-100	98	2.3	2.3	5
		1.0	97-108	103	4.2	4.1	6
		10	79	-	-	-	1
		20	85	-	-	-	1
		Overall	**63–110**	**97**	**12**	**13**	**23**
	AMPA	0.05	67-90	74	8.2	11	7
		0.1	61-96	74	11	15	8
		0.5	77-80	79	1.7	2.2	4
		1.0	82	-	-	-	1
		Overall	**61–96**	**76**	**8.4**	**11**	**20**
Olive, oil	Glyphosate	0.05	71-91	81	7.2	8.9	5
		0.1	68-99	87	14	16	5
		Overall	**68-99**	**84**	**11**	**13**	**10**
	AMPA	0.05	68-101	83	14	17	5
		0.1	64-106	80	18	23	6
		Overall	**64-106**	**81**	**16**	**20**	**11**

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.02 mg/kg).
2 Mean and standard deviation values at each individual fortification level, as well as all relative standard deviation values, were calculated for this summary.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA in processed commodities after usage of Roundup when applied as per the study.

Residues of glyphosate in ground fallen olives harvested 7, 14 or 28 days after application ranged from 0.1 mg/kg to 0.9 mg/kg. Residues of glyphosate in ground fallen olives harvested 28 days after application were below the LOQ of 0.05 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 0, 7, 14, or 28 days after application. In raw and refined olive oil, no residues of glyphosate were found above the LOQ and no residues of AMPA were found above the LOD. Detailed residue levels are shown in the table below.

Table 6.5.3-13: Residues of glyphosate and AMPA in olive processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found (mg/kg)	Processing factors	Residues found (mg/kg)	Processing factors
AP/3065/ME/1 / 29250 Cartaojal, Malaga, Spain / SEU / 1995	Olive / Hojiblanca	2.141	7	Fruit	0.14		<0.02	-
				Oil, raw	<0.02	<0.14	<0.02	-
				Oil, refined	<0.02	<0.14	<0.02	-
		2.188	14	Fruit	0.12		<0.02	-
				Oil, raw	<0.02	<0.17	<0.02	-
				Oil, refined	<0.02	<0.17	<0.02	-
		2.147	28	Fruit	<0.02		<0.02	-
				Oil, raw	<0.02	-	<0.02	-
				Oil, refined	<0.02	-	<0.02	-
AP/3065/ME/2 / 14100 La Carlota, Cordoba, Spain / SEU /1995	Olive / Picual	2.341	7	Fruit	0.11	-	<0.02	-
				Oil, raw	<0.02	<0.18	<0.02	-
				Oil, refined	<0.02	<0.18	<0.02	-
		2.374	14	Fruit	0.11	-	<0.02	-
				Oil, raw	<0.02	<0.18	<0.02	-
				Oil, refined	<0.02	<0.18	<0.02	-
		2.160	28	Fruit	<0.02	-	<0.02	-
				Oil, raw	<0.02	-	<0.02	-
				Oil, refined	<0.02	-	<0.02	-
AP/3065/ME/3 / 14640 Villa del Rio, Cordoba, Spain / SEU /1995	Olive / Picual	2.281	7	Fruit	0.53	-	<0.02	-
				Oil, raw	<0.05	<0.09	<0.02	-
				Oil, refined	<0.02	<0.04	<0.02	-
		2.143	14	Fruit	0.13	-	<0.02	-
				Oil, raw	<0.02	<0.15	<0.02	-
				Oil, refined	<0.05	<0.38	<0.02	-
		2.056	28	Fruit	<0.05	-	<0.02	-
				Oil, raw	<0.02	-	<0.02	-
				Oil, refined	<0.05	-	<0.02	-
AP/3065/ME/4 / 23400 Ubeda, Jaen, Spain / SEU /1995	Olive / Picual	2.279	7	Fruit	0.93	-	<0.02	-
				Oil, raw	<0.02	<0.02	<0.02	-
				Oil, refined	<0.02	<0.02	<0.02	-
		2.132	14	Fruit	0.93	-	<0.02	-
				Oil, raw	<0.02	<0.02	<0.02	-
				Oil, refined	<0.05	<0.05	<0.02	-
		2.151	28	Fruit	<0.05	-	<0.02	-
				Oil, raw	<0.02	-	<0.02	-
				Oil, refined	<0.02	-	<0.02	-

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

III. Conclusion

The median calculated processing factors of glyphosate for raw oil and refined oil was <0.12,. Glyphosate does not concentrate in matrices destined for human consumption.
For AMPA no residues above the LOD were present in the raw agricultural commodity. Therefore, a calculation of processing factors was not possible.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. Even though the sample sizes and the number of sampled trees were not specified, the sample sizes for processing were above 10 kg per sample. The oil samples were stored at temperatures <5 °C. Nevertheless processing factors could be derived. These can be regarded as reliable as it is not expected that residues of glyphosate will be found in high amounts in the oil due to the low log P_{ow} (-2.47). The study was not conducted at exaggerated application rates but residues of glyphosate were found in the RAC 7 and 14 days after the application so that processing factors could be derived for olive oil. Therefore, the study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 508. It adequately supports the representative processing processes for glyphosate and AMPA in olives.

The use of glyphosate in olives for oil production with harvesting of olives from treated soil is not supported in the dossier. Therefore, the study is not directly relevant to the representative uses and is considered as supportive.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.3/005
Report author	[redacted]
Report year	1993
Report title	Residues of glyphosate and AMPA in olives and olive oil, following a soil treatment with MON 65040 herbicide. Italian field trials, 1993
Report No	MLL 30319
Document No	93-GLY-01
Guidelines followed in study	No test guidelines cited in the report
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 508: • The oil samples were stored at room temperatures • The study was not conducted at exaggerated application rates
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in olive (fruit) and processed fraction olive oil after one application of MON 65040, an SL formulation containing 360 g/L of glyphosate.

The study included 2 field trials in the southern zone. There was one application to the soil under the olive trees at a target rate of 1.44 kg glyphosate per hectare. Olive samples were collected 7 and 14 days after the application from the soil (ground fallen). Residues of glyphosate in ground fallen olives harvested 7 or 14 days after application ranged from <0.05 mg/kg to 0.4 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 7 or 14 days after application.

The processing factors for glyphosate and AMPA in all trials were ≤ 1, indicating that there was no concentration of glyphosate or AMPA residue in raw or refined olive oil relative to the raw commodity, whole olive fruit collected from the ground.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON 65040
Batch number:	5093015
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	360 g/L
Content of a.s. analysed:	Not provided
Formulation type:	SL

B. Methods

1. Field phase

Two residue trials were conducted on olives (outdoor) during the 1993 season in Italy (Francavilla, Puglia; San Casciano, Toscana). One application of MON 65040 (360 g/L glyphosate) was performed onto the soil under the olive trees (6-14 plants per plot) at 4.0 L product/ha (1.44 kg a.s./ha). The volume of water used to prepare the spray solution was in the range of 200-300 L/ha. The main application parameters are outlined in the table below.

Table 6.5.3-14: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
Francavilla, Puglia	T	6 days before harvest	1.44	200
San Casciano, Toscana	T	7 days before harvest	1.44	300

Regions, varieties and cultivation were typical for the cultivation of olives.

Care was taken that the spray solution was properly homogenised by mixing before application. Ground spray applications were made via knapsack sprayer with fan nozzles according to the label directions.

2. Sampling

Specimens of olive were taken by hand from treated and untreated plots on the day of application, at 6-7 and at 13-14 days after treatment. Specimens were collected from the ground underneath the tree.

All samples were frozen within 24 hours from collection. Large samples for oil extraction were collected in double polyethylene bags and maintained at room temperature.

Table 6.5.3-15: Crop sampling information

Trial	Crop	Commodity	DALA[1]	Quantity	Date of sampling
Francavilla, Puglia	Olive	Fruit, from ground (for processing)	6 13	2.0 kg+20 kg for processing	19.01.93 27.01.93
San Casciano, Toscana	Olive	Fruit, from ground (for processing)	7 14	2.0 kg+5.0 kg for processing	11.12.92 13.12.92

1 Days after last application

3. Processing

The olives were processed in raw olive oil. The technology used was a lab-scale process similar to the industrial process.

After washing and shaking to remove excess of water, olives were thoroughly mashed with a laboratory size olive grindstone mill. In one trial (Francavilla, Puglia) quantity of NaCl equivalent to about the 10 % of the milled mass was added and accurately mixed to facilitate separation of liquid fraction from solids. The liquid fraction, an instable emulsion of oil and fruit water, was centrifuged to separate the oil.

4. Analytical phase

Residue analysis was conducted according to Monsanto method XA001. The residues of glyphosate and AMPA were extracted from the samples by water/dichloromethane partitioning/extraction followed by Chelex 100 resin isolation and anion exchange chromatographic clean-up. Quantification was based on a HPLC post column O-phthalaldehyde reaction system and comparison of peak area/height with known standards.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 171 days. Samples were stored frozen prior to analysis. Raw oil samples were stored in cold storage (< 5 °C) until analysis.

For glyphosate and AMPA in olives (fruit) and olive oil, the limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.02 mg/kg each.

During analysis of olive (fruit) specimens, fortification experiments were performed with glyphosate and AMPA at fortification levels of 0.05, 0.1 and 0.5 mg/kg, with additional fortifications at 1.0 mg/kg for glyphosate alone. Concurrent recoveries for glyphosate and AMPA in olive oil were determined at fortification levels of 0.05 and 0.1 mg/kg. The results are summarised in the table below.

Table 6.5.3-16: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Olives, fruit	Glyphosate	0.05	68-84	76	-	-	2
		0.1	77-93	85	-	-	2
		0.5	79	-	-	-	1
		1	77	-	-	-	1
		Overall	**68-93**	**80**	**8**	**10**	**6**
	AMPA	0.05	54-58	74	-	-	2
		0.1	71	71	-	-	1
		0.2	53	53	-	-	1
		Overall	**53-71**	**76**	**8**	**14**	**4**
Olive, oil	Glyphosate	0.05	90	90	-	-	1
		0.1	95	95	-	-	1
		Overall	**90-95**	**93**	**-**	**-**	**2**
	AMPA	0.05	76	76	-	-	1
		0.1	80	80	-	-	1
		Overall	**76-80**	**78**	**-**	**-**	**2**

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.02 mg/kg).
2 Mean and standard deviation values at each individual fortification level, as well as all relative standard deviation values, were calculated for this summary.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA in processed commodities after usage of MON 65040 when applied as per the study.

Residues of glyphosate in ground fallen olives harvested 7 or 14 days after application ranged from <0.05 mg/kg to 0.6 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 7 or 14 days after application.
Residues of glyphosate and AMPA in the unrefined oil were always below the LOQ of 0.05 mg/kg.
Detailed residue levels are shown in the table below.

Table 6.5.3-17: Residues of glyphosate and AMPA in olive processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found (mg/kg)	Processing factor[2]	Residues found (mg/kg)	Processing factor[2]
Francavilla, Puglia, Italy / SEU / 1993	Olive / Nardo	1.44	6	Fruit	0.3		<0.05	-
				Oil, unrefined	<0.05	<0.17	<0.05	-
		1.44	13	Fruit	0.6		<0.05	-
				Oil, unrefined	<0.05	<0.08	<0.05	-

Table 6.5.3-17: Residues of glyphosate and AMPA in olive processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found (mg/kg)	Processing factor[2]	Residues found (mg/kg)	Processing factor[2]
San Casciano, Toscana, Italy / SEU /1993	Olive / Leccino	1.44	7	Fruit	<0.05	-	<0.05	-
				Oil, unrefined	<0.05	-	<0.05	-
		1.44	14	Fruit	<0.05	-	<0.05	-
				Oil, unrefined	<0.05	-	<0.05	-

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

III. Conclusion

The mean calculated processing factors of glyphosate for unrefined oil was <0.13. Glyphosate does not concentrate in matrices destined for human consumption.
For AMPA no residues above the LOD were present in the raw agricultural commodity. Therefore, a calculation of processing factors was not possible.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The oil samples were stored at room temperature. Nevertheless processing factors could be derived. These can be regarded as reliable as it is not expected that residues of glyphosate will be found in high amounts in the oil due to the low log P_{ow} (-2.47). The study was not conducted at exaggerated application rates but in one trial residues of glyphosate were found in the RAC 6 and 13 days after the application so that processing factors could be derived for olive oil. Therefore, the study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 508. It adequately supports the representative processing processes for glyphosate and AMPA in olives.

The use of glyphosate in olives for oil production with harvesting of olives from treated soil is not supported in the dossier. Therefore, the study is not directly relevant to the representative uses and is considered as supportive.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.5.3/06
Report author	[redacted]
Report year	1992
Report title	Residues of glyphosate/AMPA in olives and olive oil following use of Sting SE - Spanish field trials 1990/1992
Report No	MLL 30297
Document No	90-GLY-02/92-GLY-01 SP

Guidelines followed in study	No test guidelines cited in the report
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 508: • Sample quantity and number of trees sampled were not provided • The oil samples were stored at temperatures <5 °C • The study was not conducted at exaggerated application rates • The mean concurrent recovery for AMPA in olive fruit was below 70 %.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes,
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to determine the magnitude of the residues of glyphosate and AMPA in olive (fruit) and processed fraction olive oil after one application of Sting SE, also referred to as MON 20072 or MON 14477, a formulation containing 120 g/L of glyphosate.

The study included 6 field trials in the southern zone. There was one application to the soil under the olive trees at a target rate of 0.36 kg glyphosate per hectare. Olive samples were collected 1 and 7 days after the application from the soil (ground fallen) in two trials and 0 and 24-41 days after the application in the four remaining trials. Residues of glyphosate in ground fallen olives harvested 0-1 days after application ranged from 0.08 mg/kg to 1.8 mg/kg and harvested 7-41 days after the application from 0.4 mg/kg to 2.0 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 0-41 days after application.

The processing factors for glyphosate and AMPA in all trials were ≤ 1, indicating that there was no concentration of glyphosate or AMPA residue in raw or refined olive oil relative to the raw commodity, whole olive fruit collected from the ground.

I. Materials and Methods

A. Materials

1. Test material

Description:	Sting SE, also referred to as MON 20072 or MON 14477
Batch number:	Not provided
Active ingredient(s):	Glyphosate
CAS number:	1071-83-6
Content of a.s. nominal:	120 g/L
Content of a.s. analysed:	Not provided
Formulation type:	SL

B. Methods

1. Field phase

Six residue trials were conducted on olives (*Olea europaea*) during 1990-1992 in. One application of Sting SE (120 g/L glyphosate) was performed onto the soil under the olive trees at 3.0 L product/ha (0.36 kg a.s./ha). The volume of water used to prepare the spray solution was 200 L/ha. The main application parameters are outlined in the table below.

Table 6.5.3-18: Application information

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
ES- Pedrera, (Servilla)	T	01.02.90	0.36	200
ES- Puebla de Cazalla, (Servilla)	T	01.02.90	0.36	200
ES-Torreblascopedro, (Jaén)	T	13.12.91	0.36	200
ES-Sierra Yegua, (Málaga)	T	20.01.92	0.36	200
ES-Linares, (Jaén)	T	15.01.92	0.36	200
ES-Córdoba, (Córdoba)	T	15.01.92	0.36	200

Regions, varieties and cultivation were typical for the cultivation of olives.

Care was taken that the spray solution was properly homogenised by mixing before application. Ground spray applications were made via knapsack sprayer with fan nozzles according to the label directions.

2. Sampling

Specimens of olive were taken by hand from treated and untreated plots on the day of application and at 6-7 and 13-14 days after treatment. Specimens were collected from the ground underneath the tree.

All samples were frozen within 24 hours from collection. Large samples for oil extraction were collected in double polyethylene bags and maintained at room temperature.

Table 6.5.3-19: Crop sampling information

Trial	Crop	Commodity	DALA	Quantity	Date of sampling
ES- Pedrera, (Servilla)	Olive	Fruit, from ground (for processing)	1 7	Not provided	02.02.90 08.02.90
ES- Puebla de Cazalla, (Servilla)	Olive	Fruit, from ground (for processing)	1 7	Not provided	02.02.90 08.02.90
ES-Torreblascopedro, (Jaén)	Olive	Fruit, from ground (for processing)	0 32	Not provided	13.12.91 14.01.92
ES-Sierra Yegua, (Málaga)	Olive	Fruit, from ground (for processing)	0 24	Not provided	20.01.92 13.02.92
ES-Linares, (Jaén)	Olive	Fruit, from ground (for processing)	0 30	Not provided	15.01.92 14.02.92
ES-Córdoba, (Córdoba)	Olive	Fruit, from ground (for processing)	0 41	Not provided	15.01.92 25.02.92

T Days after last application

3. Processing

Processing was performed to obtain the processed fractions of raw olive oil. The technology used was a lab-scale process similar to the industrial process.

After washing olives are mixed with a blender for 2 minutes. The so obtained paste was stirred for 20 min in a water bath at 50° C, after adding water at 80°C. The stirring was continued for 10 min. The mixture is centrifuged for 10 min at 9000 rpm, to separate oil and water from solid residues.

4. Analytical phase

Residue analysis was conducted according to Monsanto method XA001. The residues of glyphosate and AMPA were extracted from the samples by water/dichloromethane partitioning extraction followed by Chelex 100 resin isolation and anion exchange chromatographic clean-up. Quantification was based on a HPLC post column O-phthalaldehyde reaction system and comparison of peak area/height with known standards.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from harvest to extraction was 171 days. Samples were stored frozen prior to analysis. Raw oil samples were stored in cold storage (< 5 °C) until analysis.

For glyphosate and AMPA in olives (fruit) and olive oil, the limit of quantitation (LOQ) was 0.05 mg/kg with a limit of detection (LOD) of 0.02 mg/kg each.

During analysis of olive (fruit) specimens, fortification experiments were performed with glyphosate and AMPA at fortification levels of 0.05, 0.1, 0.5, and 1.0 mg/kg, with additional fortifications at 5 mg/kg for glyphosate alone. Concurrent recoveries for glyphosate and AMPA in olive oil were determined at fortification levels of 0.05 and 0.1 mg/kg. The overall mean recovery value for each analyte and matrix was in the acceptable range of 70-110 % and RSDs were below 20 %. Only for AMPA in olive fruit the mean recovery was 61 %. The results are summarised in the table below.

Table 6.5.3-20: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
Olives, fruit	Glyphosate	0.05	63-87	74	11	15	5
		0.1	62-104	76	15	20	6
		0.2	71	71	-	-	1
		0.5	60-70	65	4.8	7.3	4
		1	61-98	83	16	20	4
		5	70	70	-	-	1
		Overall	**60-104**	**74**	**13**	**17**	**21**
	AMPA	0.05	53-70	60	8.7	14	3
		0.1	57-76	67	9.6	14	3
		0.2	55-61	58	3.2	5.5	4
		0.5	68	68	-	-	1
		1	53	53	-	-	1
		Overall	**53-76**	**61**	**7.6**	**12**	**12**
Olive, oil	Glyphosate	0.05	66-96	81	10	12	7

Table 6.5.3-20: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)[2]	Relative standard deviation (%)[2]	Number analyses (n)
		0.1	66-97	78	12	15	7
		Overall	**66-97**	**80**	**11**	**13**	**14**
	AMPA	0.05	57-95	72	12	16	7
		0.1	55-75	68	7.4	11	7
		Overall	**55-95**	**70**	**9.6**	**14**	**14**

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.02 mg/kg).
2 Mean and standard deviation values at each individual fortification level, as well as all relative standard deviation values, were calculated for this summary.

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA in processed commodities after usage of MON 65040 when applied as per the study.

Residues of glyphosate in ground fallen olives harvested 7 or 14 days after application ranged from <0.05 mg/kg to 0.6 mg/kg. No residues of AMPA above the LOQ were found in ground fallen olives harvested 7 or 14 days after application.
Residues of glyphosate and AMPA in the unrefined oil were always below the LOQ of 0.05 mg/kg.
Detailed residue levels are shown in the table below.

Table 6.5.3-21: Residues of glyphosate and AMPA in olive processed fractions

Trial No. / Location / EU zone / Year	Crop/ Variety	Application rate kg a.s./ha	DALA[1] (days)	Processed commodity	Glyphosate		AMPA	
					Residues found (mg/kg)	Processing factor[2]	Residues found (mg/kg)	Processing factor[2]
ES- Pedrera, (Servilla) / SEU /1990	Olive / Hojiblanca	0.36	1	Fruit	0.9	-	n.a.	-
				Oil, unrefined	<0.05	<0.06	n.a.	-
		0.36	7	Fruit	1.1	-	n.a.	-
				Oil, unrefined	<0.05	<0.05	n.a.	-
ES- Puebla de Cazalla, (Servilla) / SEU /1990	Olive / Lechin	0.36	1	Fruit	1.1	-	n.a.	-
				Oil, unrefined	<0.05	<0.05	n.a.	-
		0.36	7	Fruit	1.2	-	n.a.	-
				Oil, unrefined	<0.05	<0.04	n.a.	-
ES-Torreblascopedro, (Jaén) / SEU /1991-1992	Olive / Picual	0.36	0	Fruit	1.8	-	<0.05	-
				Oil, unrefined	<0.05	<0.03	<0.05	-
		0.36	32	Fruit	0.8	-	<0.05	-
				Oil, unrefined	<0.05	<0.06	<0.05	-
ES-Sierra Yegua, (Málaga) / SEU /1992	Olive / Hojiblanca	0.36	0	Fruit	0.3	-	<0.05	-
				Oil, unrefined	<0.05	<0.17	<0.05	-
		0.36	24	Fruit	0.4	-	<0.05	-
				Oil, unrefined	<0.05	<0.13	<0.05	-
ES-Linares, (Jaén) / SEU /1992	Olive / Picual	0.36	0	Fruit	0.2	-	<0.05	-
				Oil, unrefined	<0.05	<0.25	<0.05	-
		0.36	30	Fruit	2.0	-	<0.05	-
				Oil, unrefined	<0.05	<0.03	<0.05	-
ES-Córdoba, (Córdoba) / SEU /1993	Olive / Hojiblanca	0.36		Fruit	0.08	-	<0.05	-
				Oil, unrefined	<0.05	<0.63	<0.05	-
		0.36		Fruit	0.4	-	<0.05	-
				Oil, unrefined	<0.05	<0.13	<0.05	-

1 Days after last application
2 The processing factor is calculated by dividing the residue in the processed fraction by the residue in the RAC sample

III. Conclusion

The mean calculated processing factors of glyphosate for unrefined oil was <0.06. Glyphosate does not concentrate in matrices destined for human consumption.
For AMPA no residues above the LOD were present in the raw agricultural commodity. Therefore, a calculation of processing factors was not possible.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study was previously evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The oil samples were stored at room temperature. Nevertheless processing factors could be derived. These can be regarded as reliable as it is not expected that residues of glyphosate will be found in high amounts in the oil due to the low log P_{ow} (-2.47). The study was not conducted at exaggerated application rates but in one trial residues of glyphosate were found in the RAC 6 and 13 days after the application so that processing factors could be derived for olive oil. Therefore, the study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 508. It adequately supports the representative processing processes for glyphosate and AMPA in olives.

The use of glyphosate in olives for oil production with harvesting of olives from treated soil is not supported in the dossier. Therefore, the study is not directly relevant to the representative uses and is considered as supportive.

Assessment and conclusion by RMS:

Summary of olive processing factors

The processing factors of the three above studies are summarised in the table below.

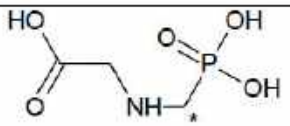
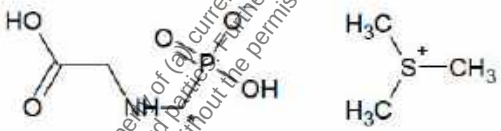
Table 6.5.3-22: Overview of processing factors for glyphosate residues in olive processed fractions

Source: DocID (trial reference)	Olive oil, unrefined	Olive oil, refined
MLL 30469 AP/3065/ME/1	<0.14 <0.17	<0.14 <0.17
MLL 30469 AP/3065/ME/2	<0.18 <0.18	<0.18 <0.18
MLL 30469 AP/3065/ME/3	<0.09 <0.15	<0.04 <0.38
MLL 30469 AP/3065/ME/4	<0.02 <0.02	<0.02 <0.05
MLL 30319 Francavilla, Puglia	<0.17 <0.08	-
MLL 30297 ES- Pedrera, (Servilla)	<0.06 <0.05	-
MLL 30297 ES- Puebla de Cazalla, (Servilla)	<0.05 <0.04	-
MLL 30297 ES-Torreblascopedro, (Jaén)	<0.03 <0.06	-
MLL 30297 ES-Sierra Yegua, (Málaga)	<0.17 <0.13	-
MLL 30297 ES-Linares, (Jaén)	<0.25 <0.03	-
MLL 30297 ES-Córdoba, (Córdoba)	<0.63 <0.13	-
Mean	**<0.13**	**<0.15**
Median	**<0.11**	**<0.15**

CA 6.6 Residues in Rotational Crops

CA 6.6.1 Metabolism in rotational crops

Four confined rotational crop studies were conducted with various rotational crops using *N*-(phosphono-^{14}C-methyl)glycine; two rotational crop study were conducted with various rotational crops using *N*-(phosphono-^{14}C-methyl)glycine trimesium salt:

Label	Structural formula (* indicates the label position)	Code Number (Synonyms) That indicated in bold was used in the summary dossier
Glyphosate CP 67573 *N*-(phosphono-methyl)glycine		
^{14}C-methane-label		• ***N*-(phosphono-^{14}C-methyl)glycine** • ^{14}C-methane-glyphosate
Available CRC studies	CA 6.6.1/001: [redacted] 1998 CA 6.6.1/003: [redacted] 1990 CA 6.6.1/005: [redacted] 1978 CA 6.6.1/006: [redacted] 1976	
Glyphosate-trimesium ICIA0224 Trimesium salt of glyphosate *N*-(phosphono-methyl)glycine trimesium salt		
PMG-label		• ***N*-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labelled glyphosate-trimesium)** • PMG • [^{14}C]-phosphonomethylene glyphosate trimesium • *N*-phosphono-methylglycine anion • [^{14}C-PMG]glyphosate-trimesium • ^{14}C-PMG-labeled glyphosate-trimesium
Available CRC studies	CA 6.6.1/002: [redacted] 1993 CA 6.6.1/004: [redacted] 1989	

Several metabolites were identified in the studies conducted with the different radiolabels of the active substance. The chemical structures and report names used in the summaries are provided in the List of Metabolites in Document N3.

In the following the different metabolism studies on rotational crops are summarised as full OECD summaries and are assessed again by the applicant.

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.6.1/001
Report author	[redacted]
Report year	1998
Report title	LX1146-02 (Glyphosate technical) Confined Rotational Crop Study on lettuce, radish, and wheat in California
Report No	1651-91-146-01-09B-17
Document No	459-GLY
Guidelines followed in study	Pesticide Assessment Guideline Subdivision N, Number 165-1
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 502: • TRR was only determined by combustion followed by LSC analysis but not determined after extraction of radioactive residues, but was for all samples ≥ 0.01 mg/kg and for all edible crops ≥ 0.05mg/kg; • The extractability of crop samples following extraction with chloroform and 0.1 M HCl was not determined and hence, majority of radioactivity (>99.9 %) is neither extracted, nor characterised, nor identified and no attempts to do so are reported. Peaks of metabolites other than AMPA and glyphosate were not integrated in the HPLC-chromatogram. Only glyphosate and AMPA are reported to be identified in very low amounts in all crop samples but the vast majority of remaining radioactivity is not further analysed. • Growth stage at sampling of immature crop samples is not given within the report • No flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed. • The limit of quantitation for HPLC analyses is due to the standards as high as 0.05 mg/kg • The storage duration of crop samples of the study was approx. 6 – 20 months, 2 – 16 months, and 7 – 10 months for the first, second and third rotation, respectively • The analytical residue method for analysis of glyphosate and AMPA was validated but mean validation recoveries of glyphosate and AMPA were between 72.3-101 % for all matrices except wheat chaff in which the mean recovery was 61.9 %
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes 1) There was a field protocol and an analytical protocol until an amendment was issued on February 10, 1992, attaching or appending the analytical protocol to the field protocol. 2) Characterisation of the test and reference substances was not performed under GLP Standard §160.105. 3) Weather data was not collected from weather stations maintained under GLP procedures.
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The metabolism of glyphosate was examined in rotational crops. The study was conducted to determine the amount of ^{14}C glyphosate and its major metabolite (AMPA) that are found in plants grown in pots of soil treated with ^{14}C glyphosate. The treated soil was aged for 30 days, simulating a crop failure, 120 days, simulating a second crop planting in the same year, and 365 days, simulating a yearly rotational planting. This aging period prior to planting simulated a rotational planting scheme. The test crops for this study were radish, lettuce, and wheat. All crops were harvested at an immature stage of development, as well as at maturity, for the purpose of residue analysis in these crops. Soil samples were also taken at strategic points throughout the study to determine the amount and nature of the radiolabelled residue in the soil. Samples were frozen and shipped to PHARMACY LSR for analysis.

Total radioactive residues (TRR) were determined by combustion followed by LSC for all matrices of the crops of all rotations. The rotational crops from the 30 DALT pots contained 0.24 – 2.0 mg/kg TRR in edible matrices and 1.3 – 4.8 mg/kg TRR in inedible matrices. Crops from the 120 DALT planting contained TRR of 0.15 – 0.7 mg/kg and 0.17 – 1.4 mg/kg for edible and inedible matrices, respectively. Crops from the 365 DALT planting contained TRRs of 0.02 – 0.16 mg/kg and 0.01 – 0.19 mg/kg for edible and inedible matrices, respectively.

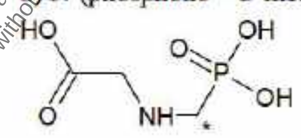
In mature, edible samples (lettuce leaves, wheat grain and radish root) of all three rotations, glyphosate was present only to amounts of < 0.05 mg/kg. In mature samples of wheat forage and chaff, glyphosate accounted for < 0.05 mg/kg, 0.3 – 0.4 mg/kg and < 0.05 – 0.06 mg/kg for the first, second and third rotation, respectively. AMPA residues were only seen at concentrations above the limit of quantification (0.05 ppm) in mature 30 and 120 day wheat forage, chaff, and seed, accounting for up to 0.4 mg/kg.

I. Materials and Methods

A. Materials

1. Test material N-(phosphonomethyl)glycine; mixture of
a) N-(phosphono-^{14}C-methyl)glycine (99 mg)
b) N-(phosphono-^{12}C-methyl)glycine (2825 mg)

Chemical structure:



* Position of radiolabel

Radiochemical purity: ≥ 99 % (determined by HPLC)

Chemical purity: ≥ 97 % (± 0.5 %)

Specific activity of the test substance applied: 11.03 MBq/mg (1.89 GBq/mmol or 51 mCi/mmol)

0.376 MBq/mg (22545 dpm/µg)

CAS No: 1071-83-6 (glyphosate acid)
1066-51-9 (AMPA)

Log $P_{o/w}$ for glyphosate: - 3.2, pH 7 at 25°C (glyphosate)

2. Test system

Soil:	Sandy loam (pH: 7.9-8.0; cation exchange capacity: 5.1-5.6 meq./100 g; sand: 62 – 64 %; silt: 29 - 31 %; clay: 7 %; textural class (USDA): sandy loam)
Crop:	Radish (variety Cherry Belle), Lettuce (variety Waldmann's Green Leaf), Wheat (variety Yecora Rojo)
Botanical name:	*Latuca sativa* *Triticum aestivum* *Raphanus sativus*
Crop part(s):	Radish roots and leaves, lettuce leaves, wheat forage, chaff, grain (seeds)

B. Study design

1. In-life phase

The test substance contained 99 mg of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) with a specific activity of 11.03 MBq/mg (51 mCi/mmol) and 2825 mg of N-(phosphonomethyl)glycine (^{12}C-glyphosate). Final specific activity of the test substance was 0.376 MBq/mg.
Labelled and unlabelled glyphosate was mixed in an aqueous solution to a concentration of total glyphosate of 1.19 mg/mL. ^{14}C-glyphosate was applied to the test plots at actual rates of 6.5 kg a.s./ha on bare soil. A pipette was used for the application of the aqueous solution to the soil surface in each of 61 treated pots. Additionally, untreated control pots were set up separate from the treated plots throughout the experiment.

All plants were grown outdoors in 30.5 cm diameter plastic pots which were moved into greenhouses when weather conditions became threatening or to maintain conditions conducive to plant growth. Pots were filled with sandy loam soil, levelled and watered. The seeds were then sown in four rows/pot. There were five treated and five non-treated pots for each planting interval for both the radish and lettuce. The wheat had nine treated and nine non-treated pots-for each planting interval. There were a total of 38 pots for planting all three crops at each planting interval (soil aged 30, 120, and 365 days). Eight pots (four treated and four non-treated) were used solely for soil sampling at intervals prior to plant harvest. The pots were maintained in accordance with normal agricultural practice until a conventional harvest of each of these rotational crops was completed.

2. Sampling

The rotational crops were grown in Porterville, California, USA. The rotational crops were planted at 30 DALT, 120 DALT, and 365 DALT. Treated and non-treated mature and immature (50 % mature) radish, lettuce, and wheat were harvested. At half maturity, wheat forage was collected and at maturity above-ground growth was collected. Radish and lettuce crop samples were rinsed free of adhering soil and weighed. Radishes were separated into leaf and root, and above-growth of wheat plants at maturity was separated into forage, chaff and grain at the appropriate intervals. The same number of pots were harvested at the same intervals for the non-treated plants.

The soil in which the crops were growing was analysed at individual time points to follow the degradation of glyphosate and to identify the metabolites to which the rotational crops were exposed. Samples were taken prior to and immediately after treatment, at each rotational crop planting and harvest. Cores were collected to a depth of ca. 15.2 cm and separated into 0-7.6 cm and 7.6-15.2 cm sections. For collecting the soil cores, tubes (30.5 cm) were pushed by hand in the soil to the bottom of the pot (for soil samples till the first post-application interval) or entire pots were excavated.

All samples were bagged and frozen as soon as possible (usually within 1-2.5 hours) after collection. All treated post-application cores were sectioned approximately 14 hours later. All other cores were sectioned

prior to freezing. Frozen samples were shipped to Pharmaco LSR. Samples were stored frozen below -20°C until preparation.

3. Analytical procedures

Total radioactive residues (TRR) in all plant samples were determined by liquid scintillation counting (LSC) following combustion. Moisture content of soil samples was determined at the time combustion aliquots were taken.
Crop samples were prepared by grinding the whole frozen sample in a Waring type blender with dry ice to a homogenous mixtures. Crop samples containing significant levels of total radioactivity (≥ 0.01 mg/kg), were analysed further utilizing an analytical residue method. Subsamples of each crop sample were homogenised with chloroform and 0.1 M HCl. Samples were centrifuged and the aqueous layer was decanted, filtered if required and made up to volume with water to reach a pH of approx. 2.0.
The sample was purified using a Chelax resin column. The glyphosate and AMPA fractions were eluted with 6 M HCl and concentrated HCl was added to the collected fractions. For further purification, the sample was loaded on an anion exchange column (AG-1-X8) and eluted with 6 M HCl. The eluted sample was concentrated and prepared for HPLC.

The concentrated samples were analysed by HPLC and a post column reaction method specific for primary amines. Glyphosate was oxidised with sodium hypochlorite. The product from the oxidation reaction coil (glycine) and the AMPA were each coupled with o-phtalaldehyde in the presence of mercaptoethanol to give detectable fluorophors. HPLC was performed using two Aminex A-9 cation exchange columns.

Identification of glyphosate and AMPA in the sample extracts was done by retention time comparison with authentic standards.

Homogenised soil samples were analysed by combustion to determine the amount of ^{14}C residue. Moisture content was determined at the time combustion aliquots were taken. A pooled soil sample (of triplicates) was prepared for each sampling point, prior to combustion followed by LSC analysis.

II. Results and Discussion

A. Total radioactive residues (TRRs)

Total radioactive residue (measured as 14C glyphosate equivalents) was highest in the leafy crops or crop parts and generally declined from the earliest harvest dates to the latest harvest dates.

A summary of crop analyses through harvest of 30-, 120- and 365-day plantings is shown in Table 6.6.1-1. After the first rotation, total radioactive residue (TRR) in edible commodities (lettuce leaves, radish roots and wheat grain) ranged from 0.24 to 2.0 mg/kg, and in non-edible commodities (radish leaves, wheat forage and wheat chaff) from 0.46 to 4.8 mg/kg. After the second rotation, TRR values in edible commodities (lettuce leaves, radish roots and wheat grain) ranged from 0.15 to 0.71 mg/kg, and in non-edible commodities (radish leaves, wheat forage and wheat chaff) from 0.17 to 1.4 mg/kg. After the third rotation, TRR values in edible commodities (lettuce leaves, radish roots and wheat grain) ranged from 0.02 to 0.16 mg/kg, and in non-edible commodities (radish leaves, wheat forage and wheat chaff) from 0.01 to 0.19 mg/kg.

Soil from untreated control and treated containers was assayed for total glyphosate equivalents. None of the soil or crop samples from control containers demonstrated radioactivity above background levels. A summary of soil analyses through harvest of 30-, 120- and 365-day plantings is shown in Table 6.6.1-2. Residue levels for dry soil were calculated from assays of soil as received from the field and based on the moisture content determined from loss of weight from drying of corresponding samples. Levels of glyphosate equivalents found immediately after treatment of soil (pre-planting application) were 2.6 – 6.4 mg/kg in the upper 7 cm of the wet soil and 3.2 – 7.9 mg/kg in the upper 7 cm of the dry soil. Concentrations of glyphosate equivalents in the upper approx. 7 cm of the wet soil decreased from

approximately 1.6 – 2.5 mg/kg at 55 – 120 DALT to approximately 0.6-1.1 mg/kg at 390 – 455 DALT. Concentrations of glyphosate equivalents in the upper approx. 7 cm of the dry soil decreased from approximately 1.8 – 3 mg/kg at 55 – 120 DALT to approximately 0.6-1.2 mg/kg at 390 – 455 DALT.

The soil characteristics indicated a sandy soil with a low organic matter content. After application to the surface, movement of glyphosate downward was minimal and residue levels in soil of ca. 7 – 15 cm depth accounted for most samples for ≤ 0.1 mg/kg and was for all samples in the range of 0.03 mg/kg to 1.0 mg/kg.

Table 6.6.1-1: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

Rotation	PBI (days)	Crop	Sampled commodity	Sampling (DALT)	TRR (mg/kg)
1st rotation	30	Lettuce	Leaves	55	0.46
				75	0.34
		Wheat	Forage	60	0.46
				120	1.3
			Chaff	120	1.6
			Grain	120	2.0
		Radish	Leaves	55	2.2
				75	4.8
			Root	55	0.38
				75	0.24
2nd rotation	120	Lettuce	Leaves	145	0.68
				165	0.25
		Wheat	Forage	150	0.45
				210	1.4
			Chaff	210	1.0
			Grain	210	0.7
		Radish	Leaves	145	0.33
				165	0.17
			Root	145	0.71
				165	0.15
3rd rotation	365	Lettuce	Leaves	390	0.02
				410	0.02
		Wheat	Forage	395	0.01
				455	0.08
			Chaff	455	0.19
			Grain	455	0.16
		Radish	Leaves	390	0.01
				410	0.02
			Root	390	0.06
				410	0.05

TRR – total radioactive residue, expressed as glyphosate equivalents
DALT – days after last treatment
PBI – plant back interval

Table 6.6.1-2: Total radioactive residues in soil after application of ^{14}C-glyphosate to bare soil

Rotation. PBI	DALT	TRR (mg/kg)			
		Soil wet		Soil dry	
		depth 0 – 3 inches (ca. 0 – 7 cm)	depth 0 – 6 inches (ca. 7 - 15 cm)	depth 0 – 3 inches (ca. 0 – 7 cm)	depth 0 – 6 inches (ca. 7 - 15 cm)
-	0 (post application)	2.6 6.4	--- 0.1	3.2 7.9	--- 0.1
1st rotation PBI 30 days	55	2.2	0.1	2.5	0.1
	75	2.1	0.04	2.3	0.04
	55	2.5	0.04	3	0.05
	75	2.5	0.02	2.8	0.02
	60	1.6	0.03	1.8	0.03
	120	2.2	0.1	2.6	0.1
2nd rotation PBI 120 days	145	1.0	0.9	1.1	1.0
	165	2.0	0.09	2.2	0.1
	145	3.2	0.05	3.6	0.06
	165	1.5	0.1	1.8	0.1
	150	1.8	0.2	2.4	0.3
	210	1.9	0.1	2.1	0.1
3rd rotation PBI 365 days	390	0.9	0.03	1.0	0.03
	410	0.7	0.08	0.8	0.09
	390	1.1	0.04	1.2	0.04
	410	0.7	0.06	0.8	0.07
	395	0.7	0.04	0.8	0.04
	455	0.6	0.09	0.6	0.1

TRR – total radioactive residue, expressed as glyphosate equivalents; calculated within the report based on soil dry or wet weight
DALT – days after last treatment
PBI – plant back interval

B. Extraction and characterisation of residues

Plant samples were extracted and analysed for glyphosate and AMPA by HPLC (Table 6.6.1-3). Validation results for recovery and determination of parent glyphosate and AMPA were obtained by analysing samples each previously fortified at the 0.1 mg/kg level. Results are shown in Table 6.6.1-4 for analysis of mature crops. Mean validation recoveries of glyphosate were between 74.3 % and 101 % for all matrices except wheat chaff in which the mean recovery was 61.9 %. Mean validation recoveries for AMPA ranged from 72.3 % to 94.3 % for all matrices.

Glyphosate and AMPA were not detected in any radish root or lettuce leaf samples harvested at maturity at any of the three planting intervals. Mature wheat forage samples showed <0.05 mg/kg glyphosate and 0.2 mg/kg AMPA at the first rotation, 0.4 mg/kg glyphosate and 0.1 mg/kg AMPA at the second rotation and <0.05 mg/kg for both glyphosate and AMPA at the third rotation. Wheat chaff exhibited <0.05 mg/kg and 0.4 mg/kg glyphosate and AMPA, respectively, at the first rotation, 0.3 and 0.2 mg/kg at the second rotation and 0.06 mg/kg and < 0.05 mg/kg at the third rotation. Wheat seed did not exhibit glyphosate at concentrations greater than 0.05 mg/kg at the first, second or third rotations whereas AMPA was detected

at 0.3 mg/kg in samples from the first rotation, 0.2 mg/kg in samples from the second rotation and at <0.05 mg/kg in samples from the third rotation in these samples.

For all untreated (control) crop samples, levels of glyphosate and AMPA were < 0.1 mg/kg.

Table 6.6.1-3: Radioactive residues of glyphosate and AMPA in lettuce leaves, wheat (forage, chaff and grain), and radish (leaves and roots) of rotational crop (first rotation, PBI 30 days; second rotation, PBI 120 days and third rotation, PBI 365 days) planted after application of glyphosate to bare soil

Rotation	PBI (days)	Crop	Sampled commodity	Sampling (DALT)	Glyphosate (mg/kg)	AMPA (mg/kg)
1st rotation	30	Lettuce	Leaves	75	< 0.05	< 0.05
		Wheat	Forage	120	< 0.05	0.2
			Chaff	120	< 0.05	0.4
			Grain	120	< 0.05	0.3
		Radish	Root	75	< 0.05	< 0.05
2nd rotation	120	Lettuce	Leaves	165	< 0.05	< 0.05
		Wheat	Forage	210	0.4	0.1
			Chaff	210	0.3	0.2
			Grain	210	< 0.05	0.2
		Radish	Root	165	< 0.05	< 0.05
3rd rotation	365	Lettuce	Leaves	410	< 0.05	< 0.05
		Wheat	Forage	455	< 0.05	< 0.05
			Chaff	455	0.06	< 0.05
			Grain	455	< 0.05	< 0.05
		Radish	Root	410	< 0.05	< 0.05

DALT – days after last treatment
PBI – plant back interval

Table 6.6.1-4: Validation of Analytical Residue Method for Analysis of glyphosate and AMPA in various crop matrices

	Matrix[1]	Sample	% Recovery[2]	
			Glyphosate	AMPA
Radish	Root	control; PBI 30	82.9	80.45
	Leaf	control; PBI 30	73.5	76.1
Lettuce	Leaf	control; PBI 365	74.3	72.3
Wheat	Forage	control; PBI 30	87.1	86.0
	Chaff	control; PBI 30	61.9	94.3
	Grain	control; PBI 30	101.0	77.4

PBI – Plant back interval
1 Duplicate samples analysed for each matrix except one analysis of wheat grain. Mean values were calculated upon dossier compilation.
2 Fortified with 0.1 mg/kg glyphosate and AMPA

C. Storage stability

All plant and soil samples were stored frozen below -20°C until preparation / LSC measurement. The storage period of the plant samples was approx. 6 – 20 months, 2 – 16 months, and 7 – 10 months for the first, second and third rotation, respectively. Radish samples were analysed 607, 162 and 259 days after sampling, for the first, second and third rotation, respectively. Lettuce samples were analysed 501, 50 and

286 days after sampling, for the first, second and third rotation, respectively. Wheat samples were analysed 186 -197, 485 and 202-203 days after sampling, for the first, second and third rotation, respectively.

D. Degradation pathway

Degradation pathway of glyphosate in rotational crops will be provided at the end of this chapter.

III. Conclusion

The rotational crops (lettuce, radish, and wheat) were planted into pots at 30, 120, and 365 days after herbicide treatment.

Total radioactive residues (TRR) were determined by combustion followed by LSC for all matrices of the crops of all rotations. The TRR detected in the various crops, in both mature and immature growth stages, was generally highest in the early planting and sampling intervals and lower in later intervals. The rotational crops from the 30 DALT planting contained 0.24 - 1.6 mg/kg TRR in edible matrices and up to 4.8 mg/kg TRR in inedible matrices. Crops from the 120 DALT planting contained TRR of 0.15 – 0.7 mg/kg and 0.17 – 1.4 mg/kg for edible and inedible matrices, respectively. Crops from the 360 DALT planting contained TRRs of 0.02 – 0.16 mg/kg and 0.01 – 0.19 mg/kg for edible and inedible matrices, respectively. Hence, there was a more significant decrease in total radioactive residues between the 120 and 365 day planting interval than between the 30 day and 120 day planting interval as expected.

Rotational crop samples were extracted with chloroform and 0.1 M HCl and analysed by HPLC for the abundance of glyphosate and aminomethylphosphonic acid (AMPA). Even though TRRs were as high as several mg/kg, parent glyphosate was only detected at concentrations of < 0.05 mg/kg for mature, edible samples (lettuce leaves, wheat grain and radish root) of all three rotations. In mature samples of wheat forage and chaff, glyphosate accounted for < 0.05 mg/kg, 0.3 – 0.4 mg/kg and < 0.05 – 0.06 mg/kg for the first, second and third rotation, respectively. AMPA residues were only seen at concentrations above the limit of quantification (0.05 ppm) in mature 30 and 120 day wheat forage, chaff, and seed, accounting for 0.1 – 0.4 mg/kg.
This indicates that glyphosate and AMPA do not accumulate in the rotational crops tested and that the majority of carbon which was initially part of the glyphosate molecules applied to the soil that is taken up by these plants becomes incorporated into plant components or is converted into compounds other than glyphosate and AMPA.

Residues of glyphosate equivalents in soil showed little downward (i.e., through the soil profile) movement throughout the duration of the study. Total 14C-glyphosate equivalents in soil declined steadily throughout the study period with little downward movement. Generally a correlation of levels in the crop compared to soil was not evident; however, rotational crops harvested at 390 – 455 days after soil treatment showed < 0.1 mg/kg glyphosate equivalents corresponding to lower levels found in soil during the same time period.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessing the level of total residues of radioactive glyphosate equivalents in rotational crops (lettuce, wheat and radish) has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with deficits:

TRR was only determined by combustion followed by LSC analysis but not determined after extraction of radioactive residues, but was for all samples ≥ 0.01 mg/kg and for all edible crops ≥ 0.05mg/kg; The extractability of crop samples following extraction with chloroform and 0.1 M HCl was not determined and hence, majority of radioactivity (>99.9 %) is neither extracted, nor characterised, nor identified and no attempts to do so are reported. Peaks of metabolites other than AMPA and glyphosate were not integrated in the HPLC-chromatogram. However, the scope of the study was not to elucidate the metabolic pathway of glyphosate in CRC, but to estimate the amounts of glyphosate and AMPA in the different crop matrices, which was successfully completed for glyphosate and AMPA; Only glyphosate and AMPA are reported to be identified in very low amounts in all crop samples but the vast majority of remaining radioactivity is not further analysed. Although glyphosate and AMPA were not identified in two dissimilar HPLC systems, co-chromatographies (by comparison of retention times with reference compounds) strongly suggest the occurrence of glyphosate and its metabolite in the designated samples; The analytical residue method for analysis of glyphosate and AMPA was validated but mean validation recoveries of glyphosate and AMPA were between 72.3-101 % for all matrices except wheat chaff in which the mean recovery was 61.9 %.; The limit of quantitation for HPLC analyses is due to the standards as high as 0.05 mg/kg and therefore above the limit suggested by the OECD guidelines; The storage duration of crop samples of the study was approx. 6 – 20 months, 2 – 16 months, and 7 – 10 months for the first, second and third rotation, respectively; Growth stage at sampling of immature crop samples is not given within the report, but they can be roughly estimated based on planting and sampling dates; No flow chart provided depicting the overall extraction and fractionation strategies employed for each sample matrix analysed.

As the scope of the study was only to estimate the amounts of glyphosate and AMPA in the different crop matrices, the study is considered supportive for the assessment of the metabolic behaviour of glyphosate in rotational crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.6.1/002
Report author	[redacted]
Report year	1993
Report title	[^{14}C-Anion] Glyphosate-Trimesium: Confined Accumulation Studies on Rotational Crops
Report No	RR92-096B
Document No	Not available
Guidelines followed in study	EPA Pesticide Registration Guideline Subdivision N, Number 165-1
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Metabolism in Rotational Crops, 502:

	• Developmental stages of the crops at application and harvesting are not reported, but could be roughly estimated based on planting and sampling dates. • Detailed information on sampling methods are not reported • Analysis of crop samples was not done within 6 months after sampling, but within 14 – 18 months. Storage stability data were generated to cover this period. • Extraction rates were low to moderate for crop samples (23.8 to 54.2 % of the TRR). Attempts (acid and/or basic hydrolysis) were made to characterise the non-extractable radioactivity, but final residues were between 13.1 to 59.4 % of the TRR, with absolute residues levels between 0.005 – 0.014 mg/kg.
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The uptake and metabolism of glyphosate was examined in rotational crops. *N*-(phosphono-methyl)glycine labelled in the methylene position (^{14}C-PMG-label) was applied as its trimesium salt to two plots at different application rates; three additional plots received a comparable treatment of unlabelled active substance as control plots. The test item ^{14}C-PMG labelled glyphosate-trimesium was applied at a rate of 5.617 kg a.s./ha (3.87 kg a.s./ha expressed as glyphosate equivalents, plot 1.0) and at a total rate of 9.51 kg a.s./ha (three monthly applications, 6.56 kg a.s./ha expressed as glyphosate equivalents, plot 5.0) to bare soil.

A primary crop of soybeans was planted prior to treatment in all plots containing sandy loam soil. After removal of the primary crop, the rotational crops lettuce, radish, and wheat were planted into the subplots at 35, 63, and 308 days after herbicide treatment (35, 63, and 308 plant-back intervals, PBI).

The soya cover crop was not analysed. For the characterisation and identification of residues in the rotated crops, samples were extracted using a mixture of 0.1N HCl and chloroform, followed by column fractioning using different solvents to separate residues and natural products. To characterise incorporation into natural products post-extraction solids were additionally hydrolysed under acid and basic conditions. The TRR levels in matrices obtained from rotational crops were relatively low, not exceeding 0.1 mg/kg, except for lettuce (0.127 mg/kg). The rotational crops from the 35 and 63 PBI plots contained TRR levels of 0.020 – 0.076 mg/kg and 0.021 – 0.127 mg/kg, respectively. Crops from the 308 PBI contained TRR levels of 0.010 – 0.038 mg/kg. All the residue levels were determined as N-phosphonomethylglycine (PMG) anion equivalents (mg ^{14}C-PMG anion equiv./kg, stated in the following only as mg/kg).

Analysis of rotational crop samples, after extraction with water and chloroform, revealed three residue components, PMG (glyphosate-anion), aminomethylphosphonic acid (AMPA) and a polar unknown metabolite (called metabolite 1 within the report). AMPA was found at levels of 8.7 - 34 % of the TRR. PMG was also detected in most samples, however its levels were <2.3 % of the TRR. Metabolite 1 was not identified as the amount was <0.01 mg/kg in all RAC's. Residues after extraction with water and chloroform were further investigated and were identified as being starch, lignins, amino acids and cellulose, as well as carbohydrates as glucose and fructose.

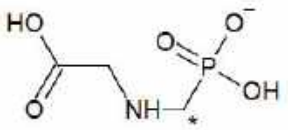
I. Materials and Methods

A. Materials

1. Test material

N-(phosphonomethyl)glycine trimesium salt; mixture of
a) *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt (82.3 mg, named ^{14}C-PMG labelled glyphosate as trimesium within this summary)
b) *N*-(phosphono-^{12}C-methyl)glycine as trimesium salt (928 mg)

Chemical structure:



* Position of radiolabel

Radiochemical purity:	>97 %
Chemical purity	>99 %
Specific activityof the test substance applied:	0.68 MBq/mg (4.506 mCi/mmol)
CAS No:	81591-81-3 (glyphosate trimesium)
Log $P_{o/w}$ for glyphosate:	- 2.9

2. Test system

Soil:	Sandy loam (pH: 8.1; cation exchange capacity: 9.1 meq./100 g; sand: 54.8 %; silt: 36.1 %; clay: 9.9 %; textural class (USDA): sandy loam)
Crop:	*Primary crop:* Soybean (variety Pioneer 9271) *Rotational crops:* Lettuce (variety Fanfare), Radish (variety Icicle Short Top Radish), Wheat (variety Common Wheat, Germain's, W-444)
Botanical name:	*Glycine max* *Latuca sativa* *Raphanus sativus* *Triticum aestivum*
Crop part(s):	Lettuce leaves, wheat grain, wheat straw, wheat hay, wheat forage, radish root, radish top

B. Study design

1. In-life phase

The test substance contained 82.3 mg of ^{14}C-PMG labelled glyphosate-trimesium with a specific activity of 8.34 MBq/mg (55.3 mCi/mmol) and 928 mg of ^{12}C-glyphosate-trimesium. Final specific activity of the 1 % test substance was 0.68 MBq/mg (4.506 mCi/mmol).
Additionally, a test substance containing 1006.6 mg of unlabelled glyphosate-trimesium (^{12}C test substance) was prepared for application to the control plot.
The test area was divided into five plots which included two ^{14}C treated plots (plots 1.0 and 5.0), and three "control" plots treated with the non-radiolabelled material (plots 2.0, 3.0 and 4.0). Each of these plots was further subdivided into N.1, N.2, and N.3 subplots for planting the rotational crops. The sizes of the plots were 0.5 x 1 m for plots 1.0, 2.0 and 3.0, 0.7 x 1 m for plot 3.0 and 0.75 x 1 m for plot 5.0. Each plot area was clearly separated from other plot areas to avoid runoff of radioactive soil and mixing of the

treated soils by aluminum or plastic lawn edgers and plexiglass. All plots were tilled and raked to depth of approximately 32 cm before each planting.
All applications were made to bare soil. The test item ^{14}C-PMG labelled glyphosate-trimesium was applied to test plots 1.0 and 5.0. Plot 1.0 was treated once at a rate of 5.617 kg a.s./ha and plot 5.0 was treated three times at monthly intervals at rates of 6.361 kg a.s./ha, 2.08 kg a.s./ha and 1.07 kg a.s./ha, respectively (in total 9.51 kg a.s./ha). The control plots 2.0 and 4.0 were treated with nonradioactive glyphosate-trimesium, and served as controls in this study. Plot 2.0 was treated once at a rate of 5.79 kg a.s./ha and plot 4.0 was treated three times at monthly intervals at rates of 6.43 kg a.s./ha, 1.74 kg a.s./ha and 1.69 kg a.s./ha, respectively (in total 9.86 kg a.s./ha). Plot 3.0 was treated at a target rate of 33.88 kg a.s./ha of non-radioactive glyphosate-trimesium and served as an exaggerated rate treatment. An overview is given within the Table 6.6.1-1.
All treatment solutions were applied using a repetitive stroke syringe fitted with a one-way valve and a manifold dispenser having 11 dispensing tips. The width of the manifold dispenser was 10 cm. The one-way valve was attached with tubing to a reservoir containing treatment solution, which was delivered using fairly rapid strokes on the syringe. Prior to application in the field, all manifold dispensers were calibrated. Treatment solutions were weighed before and after application to measure the exact amount of solution applied. At each date of application the first application was carried out on the non-radioactive plot, followed by application to the radioactive plot.

Table 6.6.1-5: Overview of the different application scenarios

Plot No.	Label	Application [1]		Primary crop for all plots	Rotational crop for all plots
		Single rate (kg a.s./ha)	Total rate (kg a.s./ha)		
1.0	^{14}C	5.617	5.617	Soybean	Lettuce
2.0	^{12}C	5.79	5.79		
3.0	^{12}C	33.88	33.88		
4.0 [2]	^{12}C	6.43	9.86		Radish
		1.74			
		1.69			
5.0 [2]	^{14}C	6.361	9.51		Wheat
		2.08			
		1.07			

1 Application rates were calculated within the repot based on actual applied volumes.
2 The applications were conducted on a monthly interval.

As plots 2.0, 3.0 and 4.0 were treated with non-radioactive test item on the day before treatment of the radiolabelled plots 1.0 and 5.0 the calculated PBIs were one day longer than for the plots treated with radiolabelled test item. Plots 1.0, 2.0 and 3.0 were planted with rotational crops 35 or 36 days after treatment to simulate crop failure. Plots 4.0 and 5.0 were planted 64 or 63 and 309 or 308 days after the last treatment (125 and 371 days after the first treatment) to simulate rotation after harvest. The application dates were selected so that all rotational crops were planted at the same time to ensure the best climatic conditions for growing. Therefore, the multiple treatment plots (4.0 and 5.0) were treated first and the single treatment plots (1.0, 2.0, and 3.0) were treated last.
A cover crop of soybeans was planted in all subplots immediately prior to application of glyphosate-trimesium. Before planting the seeds were treated with "Garden Seed Inoculant Ni-Tro-Gen", a rhizobium inoculant of nitrogen fixing bacteria. The soybean cover crop on plots 4.0 and 5.0 germinated normally, but the soybeans did not germinate in plots 1.0, 2.0, and 3.0 because of cold outdoor temperature.
The rotational crops used in this study were lettuce, wheat, and radish, representing leafy, small grain, and root crops, respectively. Prior to planting, all five plots were divided into three equal subplots (N.1, N.2, and N.3). Radish was planted into subplots N.1, wheat into subplots N.2 and lettuce into subplots N.3. Before the second planting both plots were turned over with a hoe to the depth of about 15 cm and

smoothed with a rake. Lettuce, wheat and radish crops were planted into subplots different from the first planting to simulate crop rotation. Lettuce was planted on N.2 subplots (planted on N.3 in the first rotation), radish was planted on N.3 subplots (planted on N.1 in the first rotation), and wheat was planted on N.1 subplots (planted on N.2 in the first rotation).

2. Sampling

Primary crop soybean (forage) was harvested from plots 4.0 and 5.0 at 35 DALT (days after last treatment) by cutting at ground level at an immature stage. Soybean from plots 1.0, 2.0 and 3.0 failed to sprout, due to cool weather.

Rotational crops were harvested at maturity. In addition, wheat forage was harvested when the grain was in the milk stage. Detailed information on growth stages at harvest is missing. Samples of non-radiolabelled plots were taken first before harvesting samples of the ^{14}C-radioactive plots.

Harvest of radish was done at 132 DALT (plot 1.1, PBI 35), at 160 DALT (plot 5.1, PBI 63) and at 350 DALT (plot 5.3, PBI 308); raw agricultural commodity was separated into tops and roots directly. Harvest of lettuce was done at 147 DALT (plot 1.3, PBI 35), at 175 DALT (plot 5.3, PBI 63) and at 366 DALT (plot 5.2, PBI 308). Immature wheat forage was collected at 147 DALT (plot 1.2, PBI 35), at 175 DALT (plot 5.2, PBI 63) and at 373 DALT (plot 5.1, PBI 308); the remaining wheat plants were left on the plots until maturity. Harvest of mature wheat was carried out at 187 DALT (plot 1.2, PBI 35), at 215 DALT (plot 5.2, PBI 63) and at 454 DALT (plot 5.1, PBI 308) without separating grain from the straw. Detailed description of sampling methods is not reported.

Harvested and bagged crops were placed inside an ice-chest with dry-ice or water ice as they were harvested. Following weighing, crops were moved within one hour after harvest into a walk-in freezer located at the field station. Within a month, samples were shipped frozen to the analytical test facility. Upon receiving the samples, crops were transferred into lab freezers or walk-in freezer and remained frozen until analysis at temperatures at - 20 °C.

Soil samples were taken prior to and immediately after treatment, at crop planting and harvest time. Soil samples were collected with a soil core sampling device consisting of two different diameter hollow stainless steel tubes. Cores were collected to a depth of ca 16 inches (ca. 40 cm) and separated into two sections: a 0 4 inches and 2 inches in diameter section and a 4 - 16 inches and 1 inch in diameter section. Three different soil samples were taken: depth 0 - 4 inches (ca. 0 - 10 cm), depth 4 – 10 inches (ca. 10 - 25 cm) and depth 10 - 16 inches (ca. 25 - 40 cm). The deeper core was smaller in diameter to decrease the possibility of contamination of the deep core with surface soil. Soil samples were stored frozen until preparation.

3. Analytical procedures

Total radioactive residues (TRR) in all plant and soil samples were determined by LSC following combustion, except for soybean samples which were not analysed. Liquid samples were prepared for counting by combining aliquots (0.001 to 1 mL) with 15 - 20 mL Packard Scint-A XF cocktail. Samples were counted for a minimum of 5 minutes. After harvest, crops were divided into raw agricultural commodities (RAC's), processed and analysed by combustion.
Samples of radish were separated into root and tops on the day of the harvest. The radish roots were ground to a powder with dry ice. Radish tops, lettuce and wheat forage were ground with to a powder dry-ice in a food chopper. Wheat grain was separated from straw and chaff by hand. Grain was ground in a coffee grinder or in a blender with dry-ice. Wheat straw was chopped with dry-ice using a meat cutter or a food processor.
Processed crops were left in open plastic bags in the freezers at -20 °C for a few days to allow the dry-ice to evaporate. When all of the CO_2 had evaporated, 5 subsamples of each crop matrix were weighed out for combustion.

The homogenised samples were extracted with an immiscible mixture of 0.1 M HCl and chloroform. The sample was blended for 15 minutes using a Tekmar tissue homogeniser and then centrifuged for 20 minutes. Aqueous and chloroform layers were pipetted off into separate plastic bottles. Extraction was repeated again and the nonextractable fraction (referred to as "pulp" within the report) was separated from the aqueous and chloroform layers by vacuum filtration. The aqueous and chloroform fractions were separately analysed by LSC to determine the amount of ^{14}C extractable residue. The non-extractable fraction was air-dried, weighed and combusted to determine the amount of ^{14}C non-extractable residue. Only pulp-fractions containing residues of more than 0.01 mg/kg were analysed further. The chloroform layer was not analysed further because the total ^{14}C residue in these fractions was insignificant (less than 0.01 mg/kg).

The aqueous (0.1 M HCl) extract was run through a Chelex® 100 column from which up to 7 fractions were collected. Fraction 1 containing carbohydrates was analysed by HPLC using an Aminex® carbohydrate analysis column and retention times of the radioactive peaks compared with sucrose, glucose, fructose and malic acid. The ^{14}C residues in fractions 2 (wash with deionised water) and 3 (elution with 0.2 M HCl) were negligible and not further analysed. Fraction 4 containing PMG and AMPA was eluted with 6 M HCl and cleaned up on an anion exchange column followed by HPLC analysis. Both compounds were characterised by comparing HPLC retention times with AMPA and PMG standards; TLC analysis was used for confirmation. Metabolite 1, a polar unknown metabolite, also appeared in the same fraction in all of the RAC's, but was not identified, as it was less than 0.01 mg/kg for all matrices. Fractions 5-7 were all eluted with 6 N HCl. As the ^{14}C residue was low in all three fractions no analysis was performed.

A subsample of the pulp fraction was air dried, weighed and combusted to determine the amount of ^{14}C non-extractable residue. This pulp fraction contained residues in excess of 0.01 mg/kg and was hydrolysed with 0.5 N HCl for 6-7 hours at reflux. After cooling to room temperature, the hydrolysis solution was filtered and aliquots analysed by LSC. The acid hydrolysate was passed through a Chelex® 100 column. Fraction 1 contained the majority of radioactivity, which was indicative of ^{14}C carbohydrates being released by acid hydrolysis. If the ^{14}C residue level was higher than 0.01 mg/kg, the fraction was analysed by HPLC for the presence of ^{14}C carbohydrates. To verify the presence of ^{14}C carbohydrate residue, glucosazone was also made from this fraction. The remaining hydrolysed pulp was combusted to determine again the amount of ^{14}C residue. The ^{14}C residue exceeded 0.01 mg/kg, and the pulp was hydrolysed with 20 % NaOH solution for 8 hours at reflux. The pulp and base hydrolysate were separated by filtering or centrifugation and aliquots from the base hydrolysate were taken for LSC analysis. The base hydrolysate was acidified and the resulting precipitate was removed by centrifugation. The precipitate contained the lignin fraction, and the soluble part contained the soluble amino acids fraction from base hydrolysis of proteins. The pulp remaining after acid and base hydrolysis consisted of cellulose, which was combusted to determine the amount of incorporated ^{14}C radioactivity.

As described above, in addition to the HPLC analysis, glucosazone was made from the carbohydrate fraction to verify the presence of ^{14}C glucose. For this purpose, milled wheat grain (35 and 63 PBI, respectively) was blended with DMSO and deionised water and then stirred overnight in a cold room. After centrifugation the supernatant was combined with ethanol to precipitate starch. The starch was washed with ethanol and dried. Isolated starch from 35 PBI wheat grain was hydrolysed with HC1 and the sample was refluxed with stirring for 7 hours. After cooling to room temperature the sample was divided in two. One half of the sample was neutralised with NaOH and sodium acetate and phenylhydrazine hydrochloride were added. The mixture was stirred and heated for 2 hours. The resulting product, a bright yellow solid, was filtered and washed with methanol. The glucosazone was recrystallised 8 times from methanol and hot water and out of each recrystallisation small subsample were weighed out for combustion. The glucosazone was analysed by MS and its structure was confirmed by comparison with glucosazone made directly from glucose. The other half of the hydrolysed starch was passed through a Chelex®100 column from which three fractions were collected. Fraction 1 contained 71.8 %, fraction 2 contained 1.7 %, and fraction 3 contained 26.5 % of the radioactivity applied to the column. Fraction 1 (not retained on Chelex®100) was reacted with phenylhydrazine and sodium acetate following neutralisation to pH 7. The overall yield of glucosazone was, which was recrystallised 4 times with methanol and hot water, and each subsample combusted. A sample was also submitted for MS

confirmation of glucosazone. Isolated starch from 63 PBI was treated similarly yielding glucosazone. The final sample from recrystallisation was analysed and its structure confirmed by NMR and MS.

Soil samples from plots 1.0 and 5.0 were analysed by combustion to determine the ^{14}C residue. Similarly, the control soil cores from plot 2.0, 3.0, and 4.0 were combusted to determine if any ^{14}C contamination occurred on these plots during the study. Soil samples were extracted with different solvents: NH_4OH, HCl, H_2O, and a phosphate/ammonium hydroxide buffer (PAC). Best extractability was obtained with HCl, but these extracts were difficult to analyse by HPLC. Although extractability was not as good with water as it was with HCl, the water extracts were easily analysed. Consequently, the water extracts were further analysed. Soil extracts were quantified by HPLC by collecting fractions and counting by LSC; TLC analysis was used for confirmation.

II. Results and Discussion

A. Total radioactive residues (TRRs)

In crops, the TRR in the RAC's (raw agricultural commodities) was very low considering the application rates of 3.87 and 6.56 kg/ha radiolabelled glyphosate equivalents (as the trimesium salt) to the confined plots. These low TRR also show that the soil residues of PMG and AMPA were not concentrated in the crop RAC's. Total PMG and AMPA soil residues were 1.02 and 0.68 mg/kg at the time of planting in plots 1.0 (35 PBI) and 5.0 (63 PBI), respectively.

No analysis was undertaken on the cover crop of soybeans.

After the first rotation, total radioactive residues (TRR) in edible commodities (lettuce leaves, wheat grain, as well as radish roots) ranged from 0.020 to 0.076 mg/kg, and in non-edible commodities (wheat forage, wheat straw, wheat hay and radish tops) from 0.020 to 0.059 mg/kg. After the second rotation, TRR values in edible commodities ranged from 0.022 to 0.127mg/kg, and in non-edible commodities from 0.021 to 0.073 mg/kg. After the third rotation, TRR values in edible commodities ranged from 0.010 to 0.038 mg/kg, and in non-edible commodities from 0.016 to 0.034 mg/kg. Detailed results are presented in the following table (Table 6.6.1-6). Control samples of plots 2.0 and 4.0 were also analysed for the TRR by combustion. TRR values were in the range of 0.00120 mg/kg (radish root, plot 2.1) and 0.0124 mg/kg (wheat straw, plot 4.2); plot 3.0 was not analysed.

Table 6.6.1-6: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

Rotation	PBI (days)	Plot ID	Subplot ID	Crop	Sampled commodity	Sampling (DALT)	TRR (mg/kg)
1st rotation	35	1.0	1.1	Radish	Tops	*132*	0.020
					Root	*132*	0.020
			1.2	Wheat	Forage	*147*	0.024
					Hay	*187*	0.059
					Straw	*187*	0.050
					Grain	*187*	0.076
			1.3	Lettuce	Leaves	*147*	0.073
2nd rotation	63	5.0	5.1	Radish	Tops	*160*	0.021
					Root	*160*	0.022
			5.2	Wheat	Forage	*175*	0.033
					Hay	*215*	0.073
					Straw	*215*	0.063
					Grain	*215*	0.092

Table 6.6.1-6: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

Rotation	PBI (days)	Plot ID	Subplot ID	Crop	Sampled commodity	Sampling (DALT)	TRR (mg/kg)
			5.3	Lettuce	Leaves	*175*	0.127
3rd rotation	308	5.0	5.1	Wheat	Forage	*373*	0.017
					Hay	*454*	0.034
					Straw	*454*	0.031
					Grain	*454*	0.038
			5.2	Lettuce	Leaves	*366*	0.017
			5.3	Radish	Tops	*350*	0.016
					Root	*350*	0.010

PBI Plant-back interval (meaning time between last treatment and planting of rotational crop)
DALT Days after last treatment
TRR Total radioactive residue, expressed as mg ^{14}C-PMG anion equiv./kg (by combustion)
1 The primary crop was not further analysed.
Values calculated upon dossier compilation are presented in italics.

In soil the combustion analysis showed a rapid decrease in radioactive residue, so that at the time of planting, 35 and 63 days after the last treatment, the total radioactive residue (TRR) had declined to 60 - 70 % of the applied radioactivity. Most of the radioactivity was in the top (0 - 10 cm) of the soil. The TRR in the 0 - 10 cm soil layer at the time of planting of the first rotation was 1.44 and 1.65 mg/kg in plot 1.0 and plot 5.0, respectively and at the second rotation 1.07 mg/kg in plot 5.0.

Table 6.6.1-7: Total radioactive residues in soil after application of ^{14}C-glyphosate to bare soil

Plot ID	Days after treatment [1]	TRR (mg/kg)		
		Soil depth 0 – 4 inches (ca 0 – 10 cm)	Soil depth 4 – 10 inches (ca 10 - 25 cm)	Soil depth 10 – 16 inches (ca 25 - 40 cm)
1.0	-1	<0.0005	<0.0005	<0.0005
	0	3.55	0.003	0.001
	34 #	**1.44**	**0.001**	**<0.0005**
	132	0.806	0.010	0.001
	148	0.710	0.012	<0.0005
	188	0.590	0.007	<0.0005
5.0	-1	<0.0005	<0.0005	<0.0005
	0	3.78	0.013	0.004
	34 [2]	1.48	0.014	0.002
	34 [3]	2.97	0.005	0.001
	62 [4] (-1)	1.87	0.004	0.003
	63 [5] (0)	2.60	0.006	0.001
	***125* (62) #**	**1.65**	**0.016**	**<0.0005**
	223 (160)	1.41	0.009	0.002
	239 (176)	1.52	0.014	0.001
	279 (216)	0.557	0.008	0.001

Table 6.6.1-7: Total radioactive residues in soil after application of ^{14}C-glyphosate to bare soil

Plot ID	Days after treatment [1]	TRR (mg/kg)		
		Soil depth 0 – 4 inches (ca 0 – 10 cm)	Soil depth 4 – 10 inches (ca 10 - 25 cm)	Soil depth 10 – 16 inches (ca 25 - 40 cm)
	***370* (307)** [#]	**1.07**	**0.015**	**<0.0005**
	413 (350)	0.744	0.041	0.001
	436 (373)	0.271	0.019	0.001
	517 (454)	0.345	0.019	0.001

TRR Total radioactive residue, expressed as mg ^{14}C-PMG anion equiv./kg (by combustion)
ND Not detected
1 For plot 1.0 values in this column refer to days after last treatment, for plot 5.0 values refer to days after first treatment (values in brackets refer to days after final treatment).
2 Before second treatment
3 After second treatment
4 Before third treatment
5 After third treatment
Indicating the day before planting of the rotational crops.
Values calculated upon dossier compilation are presented in italics.

B. Extraction and characterisation of residues

Plant matrices were extracted with a mixture of an aqueous and an organic solvent and the results are summarised in the tables below. In edible matrices portions of 44.7 – 54.2 % of the TRR were extractable with conventional extraction methods. For these samples, the major part of the residue was extracted with the aqueous solvent (40.6 – 51.4 % TRR). The remaining non-extractable residues (RRR) were further investigated with acidic and basic hydrolysis. (Calculation explanation: TRR within the following tables was calculated within the report as ERR + RRR. Total was calculated within the repot as sum of single analyte results.)

Lettuce leaf

For the first plant-back interval, 54.2 and 50.3 % of the TRR (0.039 and 0.064 mg/kg) in 35 and 63 PBI lettuce was found in the ERR, whereas 45.8 % and 49.7 % of the TRR (0.033 and 0.063 mg/kg) remained in the solids. The water phases of 35 and 63 PBI lettuce leaves contained 0.7 – 0.9 % of the TRR (0.001 mg/kg) PMG, 18.5 – 20.4 % of the TRR (0.015 – 0.024 mg/kg) AMPA, 5.1 – 6.5 % of the TRR (0.004 – 0.008 mg/kg) metabolite 1, 9.3 – 10.0 % of the TRR (0.007 – 0.012 mg/kg) glucose, 6.8 – 7.5 % of the TRR (0.005 – 0.009 mg/kg) fructose and 6.5 – 7.2 % of the TRR (0.005 – 0.008 mg/kg) malic acid. After acidic hydrolysis of the RRR 4.4 to 4.9 % of the TRR (0.003 and 0.006 mg/kg) PMG/AMPA were found in the acid hydrolysates. In 35 PBI lettuce 22.4 % (0.016 mg/kg) carbohydrates were solubilised and the acid hydrolysate of 63 PBI lettuce contained 16.7 % of the TRR (0.021 mg/kg) glucose and 7.0 % of the TRR (0.009 mg/kg) fructose. The precipitate remaining after HCl extraction was subjected to a basic hydrolysis solubilizing lignins (4.8 % of the TRR or 0.006 mg/kg), amino acids (10.2 % of the TRR or 0.013 mg/kg) and cellulose (6.0 % of the TRR or 0.008 mg/kg) in 63 PBI lettuce. For the 308 plant-back interval, 44.7 % of the TRR (0.008 mg/kg) in lettuce was found in the water phase. The TRR in the chloroform phase was below the detection limit. The remaining non-extractable residues (RRR) amounted to 55.3 % of the TRR (0.009 mg/kg). Results are presented in Table 6.6.1-8.

Wheat grain

For all plant-back intervals, 40.6 to 50.0 % of the TRR (0.019 to 0.037 mg/kg) in wheat grain were found in the water phase and 0.8 to 7.1 % of the TRR (<0.001 to 0.007 mg/kg) in the chloroform phase. The RRR amounted to 49.2 to 52.3 % of the TRR (0.019 to 0.048 mg/kg). The water phase of wheat grain from 35 and 63 PBI contained 34.0 and 25.8 % of the TRR (0.026 and 0.024 mg/kg) of AMPA, 3.0 and

2.5 % of the TRR (0.002 mg/kg) of metabolite 1 and 9.8 % of the TRR (0.007 and 0.009) of carbohydrates, respectively. PMG was only detected in 63 PBI wheat grain amounting to 2.3 % of the TRR (0.002 mg/kg). In the water phase of wheat grain from the second plant-back interval (308 PBI) only carbohydrates were found with an amount of 8.4 % of the TRR (0.003 mg/kg). The remaining dissolved radioactive substances were detected in the Chelex fractions (41.7 % of the TRR and 0.016 mg/kg). Acidic hydrolysis of the RRR from the first plant-back interval led to solubilisation of 3.7 and 2.3 % of the TRR (0.003 mg/kg) PMG/AMPA and 32.3 and 39.8 % of the TRR (0.025 and 0.037 mg/kg) glucose. For wheat grain from the second plant-back interval (308 PBI) 36.1 % of the TRR (0.014 mg/kg) were found in the acid hydrolysate. Results are presented in Table 6.6.1-9.

Wheat straw

In 35 and 63 PBI wheat straw 40.7 and 38.1 % of the TRR (0.021 and 0.025 mg/kg) and in 308 PBI 23.8 % of the TRR (0.0072 mg/kg) were found in the ERR, whereas only a minor part of the residues was extracted with chloroform (0.7 – 1.4 % TRR). The water phases of 35 and 63 PBI contained 0.3 – 0.4 % of the TRR (0.0002 mg/kg) PMG, 11.7 – 12.7 % of the TRR (0.006 – 0.008 mg/kg) AMPA, 4.1 – 4.2 % of the TRR (0.002 – 0.003 mg/kg) metabolite 1 and 0.1 % of the TRR (0.0001 mg/kg) other anions. Carbohydrates were also found in the water phases of all wheat straw extracts. Wheat straw of the 35 and 308 PBI contained carbohydrates with amounts of 22.9 and 15.0 % of the TRR (0.012 and 0.004 mg/kg) and the carbohydrates in the 65 PBI sample were identified as glucose (3.5 % TRR and 0.002 mg/kg), fructose (3.7 % TRR and 0.002 mg/kg), malic acid (4.6 % TRR and 0.003 mg/kg) and metabolite 2 (8.3 % TRR and 0.005 mg/kg). The remaining dissolved radioactive substances of 308 PBI wheat straw were detected in the Chelex fractions (8.5 % of the TRR and 0.003 mg/kg) and were not further investigated. After acidic hydrolysis of the RRR 16.0 and 19.1 % of the TRR (0.008 and 0.012 mg/kg) were found in the acid hydrolysates. The precipitate remaining after HCl extraction was subjected to a basic hydrolysis to solubilise amino acids (20.7 – 15.6 % or 0.010 mg/kg), cellulose (22.6 – 13.9 % of the TRR or 0.011 – 0.009 mg/kg) and in case of 63 PBI wheat straw also lignins (12.8 % of the TRR or 0.008 mg/kg). Results are presented in Table 6.6.1-10.

Wheat forage

For wheat forage of the 35 and 63 PBI, 46.2 and 47.3 % of the TRR (0.011 and 0.016 mg/kg) were found in the water phase and 3.1 and 2.0 % of the TRR (0.001 mg/kg) in the chloroform phase. The RRR amounted to 50.7 % of the TRR (0.012 and 0.016 mg/kg). The water phase contained 20.5 % of the TRR (0.005 and 0.007 mg/kg) of AMPA, 1.9 and 2.0 % of the TRR (0.0003 and 0.001 mg/kg) of metabolite 1, 2.2 and 3.3 % of the TRR (0.001 mg/kg) of other anions and 17.9 and 21.6 % of the TRR (0.004 and 0.007 mg/kg) of carbohydrates. Only in the water phase of wheat forage at the 35 PBI PMG was found with an amount of 0.5 % of the TRR (0.0001 mg/kg). Acidic hydrolysis of the RRR from 63 PBI wheat forage led to solubilisation of 23.1 % of the TRR (0.008 mg/kg) carbohydrates. The precipitate remaining after HCl extraction was subjected to a basic hydrolysis solubilizing lignins (4.4 % of the TRR or 0.001 mg/kg), amino acids (10.5 % of the TRR or 0.003 mg/kg) and cellulose (12.7 % of the TRR or 0.004 mg/kg) in 63 PBI wheat forage. Due to low TRR values (0.017 mg/kg) wheat forage from the plant-back interval 308 PBI was not further extracted and analysed. Results are presented in Table 6.6.1-11.

Radish root

In radish root of all plant-back intervals, 46.7 to 51.4 % of the TRR (0.010 to 0.005 mg/kg) were found in the ERR, whereas only a minor part of the residues was extracted with chloroform (0.4 – 1.4 % of the TRR). The water phases of radish root of 35 and 63 PBI contained 1.7 – 1.8 % of the TRR (0.0004 mg/kg) PMG, 8.7 – 11.0 % of the TRR (0.002 mg/kg) AMPA, 1.0 – 1.2 % of the TRR (0.0002 – 0.0003 mg/kg) metabolite 1, 1.6 -2.1 % of the TRR (0.0004 mg/kg) other anions and 31.8 – 32.7 % of the TRR (0.007 mg/kg) carbohydrates. In the water phase of radish root samples of the 308 PBI 43.2 % of the TRR (0.004 mg/kg) were characterised as carbohydrates. Due to low TRR values the remaining non-extractable residues (RRR) were not further investigated with acidic and basic hydrolysis. Results are presented in Table 6.6.1-12.

Radish top

In radish top of all plant-back intervals, 40.4 to 45.0 % of the TRR (0.007 to 0.009 mg/kg) were found in the ERR, whereas only a minor part of the residues was extracted with chloroform (3.1 – 5.5 % of the TRR). The water phases of radish top samples of the 35 and 63 PBI contained 0.9 – 1.1 % of the TRR (0.0002 mg/kg) PMG, 9.5 – 12.3 % of the TRR (0.002 mg/kg) AMPA, 1.0 – 1.4 % of the TRR (0.0002 – 0.0003 mg/kg) metabolite 1, 1.8 % of the TRR (0.0004 mg/kg) other anions and 21.0 – 25.7 % of the TRR (0.004 - 0.005 mg/kg) not further characterised carbohydrates. In the water phase of radish top samples of the 308 PBI 27.4 % of the TRR (0.004 mg/kg) were characterised as carbohydrates. Due to low TRR values the remaining non-extractable residues (RRR) were not further investigated with acidic and basic hydrolysis. Results are presented in Table 6.6.1-13.

Table 6.6.1-8: Extraction of the radioactive residues in lettuce leaves of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in lettuce leaves					
PBI	35		63		308	
Plot-ID	1.3		5.3		5.2	
DALT (sampling)	147		175		366	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.072	100	0.127	100	0.017	100
ERR	***0.039***	***54.2***	***0.064***	***50.3***	***0.009***	***44.7***
Chloroform phase	0.002	2.8	0.002	1.6	<0.001	ND
Water phase	0.037	51.4	0.062	48.7	0.008	44.7
Chelex fractions [1]	*0.020*	*26.8*	*0.033*	*26.1*	0.003	19.6
PMG	0.001	0.7	0.001	0.9	NP	NP
AMPA	0.015	20.4	0.024	18.5		
Metabolite 1	0.004	5.1	0.008	6.5		
Other anion	0.0004	0.6	0.0003	0.2		
Carbohydrates [2]	*0.017*	*24.7*	*0.029*	*22.6*	0.004	25.1
Glucose	0.007	10.0	0.012	9.3	NP	NP
Fructose	0.005	7.5	0.009	6.8		
Malic acid	0.005	7.2	0.008	6.5		
RRR (extracted pulp)	**0.033**	**45.8**	**0.063**	**49.6**	**0.009**	**55.3**
Acid hydrolysate [3]	*0.019*	*26.8*	*0.036*	*28.6*	NP	NP
PMG/AMPA	0.003	4.4	0.006	4.9	-	-
Carbohydrates [2]	0.016	22.4	*0.030*	*23.7*	-	-
Glucose	NP	NP	0.021	16.7	-	-
Fructose			0.009	7.0	-	-
Pulp 2 (acid hydrolysed pulp)	0.014	19.0	-	-	-	-
Base hydrolysate [4]	NP	NP	*0.027*	*21.0*	NP	NP
Lignins	-	-	0.006	4.8	-	-
Amino acids	-	-	0.013	10.2	-	-
Cellulose	-	-	0.008	6.0	-	-
Identified [5]	*0.036*	*50.2*	*0.090*	*70.6*	-	-
Characterised [6]	*0.022*	*30.9*	*0.037*	*29.3*	*0.008*	*44.7*
Final residue	**0.014**	**19.0**	**-**	**-**	**0.009**	**55.3**
Total	**0.071**	**100.1**	**0.127**	**99.9**	**0.016**	**100.0**
Recovered radioactivity	**83.7**		**90.5**		**91.2**	

Table 6.6.1-8: Extraction of the radioactive residues in lettuce leaves of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

DALT – days after last treatment PBI – plant-back interval TRR – Total radioactive residue
RRR – Residual radioactive residue ND – not detected NP – not performed
ERR – Extractable radioactive residue (calculated as sum of water phase and chloroform phase)
1 Chelex fractions were calculated as sum of PMG, AMPA, metabolite 1 and other anion.
2 Carbohydrates were calculated as sum of results of glucose, fructose and malic acid.
3 Acid hydrolysate was calculated as PMG/AMPA, carbohydrates (glucose, fructose).
4 Base hydrolysate was calculated as sum of lignins, amino acids and cellulose.
5 Identified was calculated as sum of PMG, AMPA, malic acid, PMG/AMPA, glucose and fructose.
6 Characterised was calculated as sum of chloroform phase, metabolite 1, other anion, carbohydrates, lignins, amino acids and cellulose.
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values given as <0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-9: Extraction of the radioactive residues in wheat grain of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in wheat grain					
PBI	35		63		308	
Plot-ID	1.3		5.3		5.2	
DALT (sampling)	187		215		454	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.076	100	0.092	100	0.038	100
ERR	***0.038***	***50.0***	***0.044***	***47.7***	***0.020***	***50.8***
Chloroform phase	0.002	3.1	0.007	7.1	<0.001	0.8
Water phase	0.036	46.9	0.037	40.6	0.019	50.0
Chelex fractions [1]	*0.030*	*37.0*	*0.029*	*30.6*	0.016	41.7
PMG	<0.001	ND	0.002	2.3	NP	NP
AMPA	0.026	34.0	0.024	25.8		
Metabolite 1	0.002	3.0	0.002	2.5		
Other anion	<0.001	ND	<0.001	ND		
Carbohydrates	0.007	9.8	0.009	9.8	0.003	8.4
RRR (extracted pulp)	**0.038**	**50.1**	**0.048**	***52.3*** [6]	**0.019**	**49.2**
Acid hydrolysate [2]	*0.029*	*36.0*	*0.041*	*42.1*	0.014	36.1
PMG/AMPA	0.003	3.7	0.003	2.3	NP	NP
Carbohydrates [3]	*0.026*	*32.3*	*0.038*	*39.8*		
Glucose	0.025	32.3	0.037	39.8		
Fructose	<0.001	ND	<0.001	ND		
Pulp 2 (acid hydrolysed pulp)	0.011	14.1	0.009	10.2	0.005	13.1
Identified [4]	*0.056*	*70.0*	*0.067*	*70.2*	-	-
Characterised [5]	*0.012*	*15.9*	*0.019*	*19.4*	*0.034*	*87.0*
Final residue	**0.011**	**14.1**	**0.009**	**10.2**	**0.005**	**13.1**
Total	**0.076**	**100.0**	**0.093**	**99.8**	**0.038**	**99.3**
Recovered radioactivity	**99.7**		**97.5**		**92.2**	

Table 6.6.1-9: Extraction of the radioactive residues in wheat grain of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

DALT – days after last treatment PBI – plant-back interval TRR – Total radioactive residue
RRR – Residual radioactive residue ND – not detected NP – not performed
ERR – Extractable radioactive residue (calculated as sum of water phase and chloroform phase)
1 Chelex fractions were calculated as sum of PMG, AMPA, metabolite 1 and other anion.
2 Acid hydrolysate was calculated as PMG/AMPA, carbohydrates (glucose, fructose).
3 Carbohydrates were calculated as sum of results of glucose, and fructose.
4 Identified was calculated as sum of PMG, AMPA, PMG/AMPA, glucose and fructose.
5 Characterised was calculated as sum of chloroform phase, metabolite 1, other anion, and carbohydrates.
6 This value was recalculated as the value given in the report (51.9 %) does not fit to the given single values.
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values given as <0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-10: Extraction of the radioactive residues in wheat straw of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in wheat straw					
PBI	35		63		308	
Plot-ID	1.3		5.3		5.2	
DALT (sampling)	187		175		366	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.050	100	0.063	100	0.031	100
ERR	***0.021***	***40.7***	***0.025***	***38.1***	***0.0072***	***23.8***
Chloroform phase	0.001	1.4	0.001	0.9	0.0002	0.7
Water phase	0.020	39.3	0.024	37.2	0.007	23.1
Chelex fractions [1]	*0.008*	*16.3*	*0.011*	*17.3*	0.003	8.5
PMG	0.0002	0.4	0.0002	0.3	NP	NP
AMPA	0.006	11.7	0.008	12.7		
Metabolite 1	0.002	4.1	0.003	4.2		
Other anion	0.0001	0.1	0.0001	0.1		
Carbohydrates [2]	0.012	22.9	*0.012*	*20.1*	0.004	15.0
Glucose	NP	NP	0.002	3.5	NP	NP
Fructose			0.002	3.7		
Malic acid			0.003	4.6		
Metabolite 2			0.005	8.3		
RRR (extracted pulp)	**0.030**	**59.3**	**0.039**	***61.9*** [6]	**0.024**	**76.1**
Acid hydrolysate	0.008	16.0	0.012	19.1	0.011	34.5
Pulp 2 (acid hydrolysed pulp)	-	-	-	-	0.013	41.7
Base hydrolysate [3]	*0.021*	*43.3*	*0.027*	*42.3*	NP	NP
Lignins	included with Cellulose		0.008	12.8	-	-
Amino acids	0.010	20.7	0.010	15.6		
Cellulose	0.011	22.6	0.009	13.9		
Identified [4]	*0.006*	*12.1*	*0.015*	*24.8*	-	-
Characterised [5]	*0.044*	*87.8*	*0.048*	*74.9*	*0.018*	*58.7*
Final residue	**-**	**-**	**-**	**-**	**0.013**	**41.7**
Total	**0.050**	**99.9**	**0.063**	**99.6**	**0.031**	**99.9**
Recovered radioactivity	**106.8**		**88.8**		**100.3**	

Table 6.6.1-10: Extraction of the radioactive residues in wheat straw of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in wheat straw		
PBI	35	63	308

DALT – days after last treatment PBI – plant-back interval TRR – Total radioactive residue
RRR – Residual radioactive residue ND – not detected NP – not performed
ERR – Extractable radioactive residue (calculated as sum of water phase and chloroform phase)
1 Chelex fractions were calculated as sum of PMG, AMPA, metabolite 1 and other anion.
2 Carbohydrates were calculated as sum of results of glucose, and fructose.
3 Base hydrolysate was calculated as sum of lignins, amino acids and cellulose.
4 Identified was calculated as sum of PMG, AMPA, PMG/AMPA, glucose, fructose and malic acid.
5 Characterised was calculated as sum of chloroform phase, metabolite 1+2, other anion, acid hydrolysate, lignins, amino acids and cellulose.
6 This value was recalculated as the value given in the report (61.5 %) does not fit to the given single values.
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values given as <0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-11: Extraction of the radioactive residues in wheat forage of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in wheat forage					
PBI	35		63		308	
Plot-ID	1.3		5.3		5.2	
DALT (sampling)	147		175		373	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.024	100.0	0.033	100.0	0.017	100.0
ERR	***0.012***	***49.3***	***0.017***	***49.3***	NP	NP
Chloroform phase	0.001	3.1	0.001	2.0		
Water phase	0.011	46.2	0.016	47.3		
Chelex fractions [1]	*0.0064*	*25.1*	*0.010*	*25.8*		
PMG	0.0001	0.5	<0.001	ND		
AMPA	0.005	20.5	0.007	20.5		
Metabolite 1	0.0003	1.9	0.001	2.0		
Other anion	0.001	2.2	0.001	3.3		
Carbohydrates [2]	0.004	17.9	0.007	21.6		
RRR (extracted pulp)	***0.012*** [6]	***50.7*** [6]	**0.016**	**50.7**		
Acid hydrolysate [3]	NP	NP	*0.008*	*23.1*		
PMG/AMPA			ND	ND		
Carbohydrates			0.008	23.1		
Pulp 2 (acid hydrolysed pulp)			-	-		
Base hydrolysate [3]			*0.008*	*27.6*		
Lignins			0.001	4.4		
Amino acids			0.003	10.5		
Cellulose			0.004	12.7		
Identified [4]	*0.005*	*21.0*	*0.007*	*20.5*		
Characterised [5]	*0.006*	*25.1*	*0.026*	*79.6*		
Final residue	**0.012**	**50.7**	**-**	**-**		
Total	**0.024**	**99.9**	**0.033**	**100.1**		

Table 6.6.1-11: Extraction of the radioactive residues in wheat forage of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in wheat forage			
PBI	35	63	308	
Recovered radioactivity	**89.8**	**91.7**		

DALT – days after last treatment PBI – plant-back interval TRR – Total radioactive residue
RRR – Residual radioactive residue ND – not detected NP – not performed
ERR – Extractable radioactive residue (calculated as sum of water phase and chloroform phase)
1 Chelex fractions were calculated as sum of PMG, AMPA, metabolite 1 and other anion.
2 Acid hydrolysate was calculated as PMG/AMPA, carbohydrates.
3 Base hydrolysate was calculated as sum of lignins, amino acids and cellulose.
4 Identified was calculated as sum of PMG, AMPA, PMG/AMPA.
5 Characterised was calculated as sum of chloroform phase, metabolite 1, other anion, carbohydrates, lignins, amino acids and cellulose.
6 These values were recalculated based on the assumption that ERR+RRR=TRR (values given in the report: 0.013 mg/kg, 53.8 %).
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values given as <0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-12: Extraction of the radioactive residues in radish root of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in radish root					
PBI	35		63		308	
Plot-ID	1.3		5.3		5.2	
DALT (sampling)	132		160		350	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.020	100.0	0.022	100.0	0.010	100.0
ERR	***0.010***	***46.7***	***0.010***	***48.5***	***0.005***	***51.4***
Chloroform phase	0.0001	0.4	0.0003	1.4	0.0001	0.8
Water phase	0.010	46.3	0.010	47.1	0.005	50.6
Chelex fractions [1]	*0.0030*	*13.6*	*0.0031*	*15.5*	0.001	7.4
PMG	0.0004	1.8	0.0004	1.7	NP	NP
AMPA	0.002	8.7	0.002	11.0		
Metabolite 1	0.0002	1.0	0.0003	1.2		
Other anion	0.0004	2.1	0.0004	1.6		
Carbohydrates	0.007	32.7	0.007	31.8	0.004	43.2
RRR (extracted pulp)	**0.011**	**53.3**	**0.011**	**51.5**	**0.005**	**48.6**
Identified [2]	*0.002*	*10.5*	*0.002*	*12.7*	-	-
Characterised [3]	*0.008*	*36.2*	*0.008*	*36.0*	*0.005*	*51.4*
Final residue	**0.011**	**53.3**	**0.011**	**51.5**	**0.005**	**48.6**
Total	**0.021**	**100.0**	**0.021**	**100.0**	**0.010**	**100.0**
Recovered radioactivity	**109.1**		**112.5**		**76.7**	

Table 6.6.1-12: Extraction of the radioactive residues in radish root of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

DALT – days after last treatment PBI – plant-back interval TRR – Total radioactive residue
RRR – Residual radioactive residue ND – not detected NP – not performed
ERR – Extractable radioactive residue (calculated as sum of water phase and chloroform phase)
1 Chelex fractions were calculated as sum of PMG, AMPA, metabolite 1 and other anion.
2 Carbohydrates were calculated as sum of results of glucose, fructose and malic acid.
3 Acid hydrolysate was calculated as PMG/AMPA, carbohydrates (glucose, fructose).
4 Base hydrolysate was calculated as sum of lignins, amino acids and cellulose.
5 Identified was calculated as sum of PMG, AMPA, malic acid, PMG/AMPA, glucose and fructose.
6 Characterised was calculated as sum of chloroform phase, metabolite 1, other anion, carbohydrates, lignins, amino acids and cellulose.
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values given as <0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-13: Extraction of the radioactive residues in radish top of rotational crop planted after application of ^{14}C-PMG labelled glyphosate to bare soil

	Residues in radish top					
PBI	35		63		308	
Plot-ID	1.3		5.3		5.2	
DALT (sampling)	132		160		350	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.020	100.0	0.021	100.0	0.016	100.0
ERR	***0.008***	***41.7***	***0.009***	***45.0***	***0.007***	***40.4***
Chloroform phase	0.001	4.8	0.001	5.5	0.001	3.1
Water phase	0.007	36.9	0.008	39.5	0.006	37.3
Chelex fractions [1]	*0.0028*	*16.0*	*0.0029*	*13.8*	0.002	10.0
PMG	0.0002	0.9	0.0002	1.1	NP	NP
AMPA	0.002	12.3	0.002	9.5		
Metabolite 1	0.0002	1.0	0.0003	1.4		
Other anion	0.0004	1.8	0.0004	1.8		
Carbohydrates	0.004	21.0	0.005	25.7	0.004	27.4
RRR (extracted pulp)	**0.012**	**58.2**	**0.012**	**54.9**	**0.010**	**59.4**
Identified [2]	*0.002*	*13.2*	*0.002*	*10.6*	-	-
Characterised [3]	*0.005*	*23.8*	*0.006*	*28.9*	*0.007*	*40.5*
Final residue	**0.012**	**58.2**	**0.012**	**54.9**	**0.010**	**59.4**
Total	**0.020**	**99.9**	**0.022**	**99.9**	**0.017**	**99.9**
Recovered radioactivity	**102.4**		**88.8**		**102.9**	

DALT – days after last treatment PBI – plant-back interval TRR – Total radioactive residue
RRR – Residual radioactive residue ND – not detected NP – not performed
ERR – Extractable radioactive residue (calculated as sum of water phase and chloroform phase)
1 Chelex fractions were calculated as sum of PMG, AMPA, metabolite 1 and other anion.
2 Identified was calculated as sum of PMG, and AMPA.
3 Characterised was calculated as sum of chloroform phase, metabolite 1, other anion, and carbohydrates (for PBI 308 chloroform phase, Chelex fraction, and carbohydrates).
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values given as <0.001 mg/kg were set as 0.001 mg/kg.

Soil

The extractability of soil samples analysed for plot 1.0 was between 58.4 to 70.6 % of the TRR and for plot 5.0 between 30.4 to 41.2 % of the TRR. The only components found in soil extracts were PMG and AMPA. The amount of PMG in soil was at the highest level directly after application and decreased over time in all the samples. In plot 1.0 the amount of PMG decreased from 59.5 % of the TRR (2.11 mg/kg) to 3.54 % of the TRR (0.021 mg/kg) and in plot 5.0 the amount decreased from 31.9 % of the TRR (1.21 mg/kg) to 2.98 % of the TRR (0.045 mg/kg) in the period from 0 to 176 DALT and then slightly increased to 8.85 % of the TRR (0.049 mg/kg) for 216 DALT. In plot 1.0 AMPA was not detected directly after application. Afterwards, the amount of AMPA increased over time to 58.6 % of the TRR (0.844 mg/kg) for sample 34 DALT and then slightly decreased to 54.9 % of the TRR (0.324 mg/kg) for sample 183 DALT. In plot 5.0 AMPA residues increased from 6.31 % of the TRR (0.239 mg/kg) to 27.5 % of the TRR (0.564 mg/kg) and then slightly decreased to 24.1 % of the TRR (0.134 mg/kg) for sample 216 DALT. Results are presented in the following tables (Table 6.6.1-14 and Table 6.6.1-15).

Table 6.6.1-14: PMG and AMPA residues determined by HPLC analysis in 0 – 10 cm soil cores collected after treatment of plot 1.0 with 3.87 kg/ha ^{14}C-PMG (5.617 kg/ha glyphosate-trimesium)

Plot 1.0	Residues in soil cores (0 - 10 cm)					
DALT	0		34		188	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**3.55**	**100**	**1.44**	**100**	**0.590**	**100**
ERR	2.11	59.5	1.02	70.6	0.345	58.4
PMG	2.11	59.5	0.172	12.0	0.021	3.54
AMPA	0.000	0.0	0.844	58.6	0.324	54.9
RRR	1.44 [1]	40.5 [1]	0.423 [2]	29.4 [2]	0.245 [2]	41.6 [2]
TRR	**3.55**	**100**	**1.44**	**100**	**0.590**	**100**

DALT days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
1 These values were determined by difference within the report.
2 These values were determined by combustion analysis.
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg

Table 6.6.1-15: PMG and AMPA residues determined by HPLC analysis in 0 – 10 cm soil cores collected after treatment of plot 5.0 with 6.56 kg/ha ^{14}C-PMG (9.51 kg/ha glyphosate-trimesium)

Plot 5.0	Residues in soil cores (0 - 10 cm)									
DALT	0		62 (125)		160 (223)		176 (239)		216 (279)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	**3.78**	**100**	**1.65**	**100**	**1.41**	**100**	**1.52**	**100**	**0.557**	**100**
ERR	**1.44**	**38.2**	**0.681**	**41.2**	**0.446**	**31.6**	**0.461**	**30.4**	**0.183**	**32.9**
PMG	1.21	31.9	0.117	7.10	0.058	4.09	0.045	2.98	0.049	8.85
AMPA	0.239	6.31	0.564	27.5	0.388	27.5	0.416	27.4	0.134	24.1
RRR [1]	2.34	61.8	0.973	58.8	0.693	68.4	1.06	69.6	0.374	67.1
Total (TRR)	**3.78**	**100**	**1.65**	**100**	**1.41**	**100**	**1.52**	**100**	**0.557**	**100**

Table 6.6.1-15: PMG and AMPA residues determined by HPLC analysis in 0 – 10 cm soil cores collected after treatment of plot 5.0 with 6.56 kg/ha ^{14}C-PMG (9.51 kg/ha glyphosate-trimesium)

Plot 5.0	Residues in soil cores (0 - 10 cm)				
DALT	0	62 (125)	160 (223)	176 (239)	216 (279)

DALT days after last treatment (meaning after the third treatment; values in parenthesis correspond to the days after first treatment)
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue
1 These values were determined by difference; selected cores were combusted for verification of this approach.
All residue data are expressed as mg ^{14}C-PMG anion equiv./kg

C. Storage stability

All of the crop samples were analysed by combustion within 3 to 83 days after sampling, except 308 PBI radish, which was combusted 12 months after sampling. All of the RAC's were extracted and analysed 14 - 18 months (406 – 552 days) after sampling. All of the soil cores were analysed by combustion within 3 to 135 days after sampling. All of the soil cores that were extracted and analysed by chromatography were done 4 - 14 months (114 – 426 days) after sampling. Throughout the study all samples, crops and soils and extracts, were stored in freezers usually at -20 °C.

Analysis of crop extracts showed that glyphosate and AMPA were the major residues beside natural products (see storage stability investigations depicted below). For the stability of glyphosate and AMPA, a high number of storage stability investigations are available in storage stability studies summarised under 6.1. No degradation of glyphosate and its metabolites was found in matrices with high water content (sugar beet leaves, soybean forage, and clover). Over an investigated storage duration of 18 to 31 months no degradation was observed ([redacted] 2010, CA 6.1/03 and [redacted] 1991, CA 6.1/13). No degradation of glyphosate and its metabolites was found in matrices with high starch content (sugar beet roots and wheat grain). Over an investigated storage duration of 18 to 24 months days no degradation was observed ([redacted] 2010, CA 6.1/03 and [redacted] 1989, CA 6.1/14). Therefore, residues of glyphosate and AMPA have been shown to be stable under conditions and periods of storage relevant to this study.
For the storage stability of natural products, two crop samples, 63 PBI wheat straw and 63 PBI lettuce leaf were used. These samples were removed from the freezer and extracted at 184 and 654 days (wheat straw) and 230 and 692 days (lettuce leaf) after harvest. These crops were extracted using a modified method, which fractionated the crop into its main natural products components. At first, the crop was extracted with cold water and acetonitrile and filtrated, so that a soluble and an insoluble fraction were generated. Then ethanol was added in excess to the soluble fraction to precipitate pectic substances, starch, gums, and fructans. The precipitate and the supernatant, containing monosaccharides and soluble acids, were separated by centrifugation. The insoluble fraction was extracted with NaOH solution and centrifuged or filtered. The precipitate remaining after base extraction contained alpha-cellulose. The supernatant after base extraction was acidified to precipitate high molecular weight hemicellulose, and centrifuged. The supernatant contained low molecular weight hemicellulose. All terminal fractions were analysed for ^{14}C residue by combustion (solid fractions) or by LSC (liquid fractions). This extraction scheme was repeated at a much later date and compared with the results from the first extraction for the two selected crops, lettuce leaf and wheat straw. It is evident that the distribution of residue in these natural product fractions were similar at the two extraction dates for the same crop. This shows that the components of the residue in straw and lettuce were stable under frozen storage for approximately two years (22 to 23 months). The results of these extractions are shown in Table 6.6.1-16.

Table 6.6.1-16: Extraction of the radioactive residues of glyphosate in wheat straw and lettuce leaf – storage stability assessment

	Wheat straw (PBI 63)		Lettuce leaf (PBI 63)	
	184 (6 months)	654 (22 months)	230 (8 months)	692 (23 months)
	% TRR	% TRR	% TRR	% TRR
TRR	100	100	100	100
ERR	***44***	***38***	***47***	***55***
Acetone Layer	2	3	3	-
Juice layer	42	35	44	55
Pectins	5	13	23	22
Soluble monosaccharides	36	21	21	33
RRR	**56**	**63**	**53**	**45**
Alpha cellulose	36	34	20	27
Hemicellulose (low molecular weight)	19	28	26	12
Hemicellulose (high molecular weight)	1	1	4	6
NaOH wash	-	-	3	-
Total	**100**	**100**	**100**	**100**

TRR Total radioactive residue (expressed as mg ^{14}C-PMG anion equiv./kg)
ERR Extractable radioactive residue (calculated as sum of acetone layer and juice layer)
RRR Residual radioactive residue
1 Storage intervals were calculated using date of harvest and date of HPLC analysis (the latest event, reflecting the longest storage duration as the most critical scenario)
2 Too dark to LSC. Recoveries determined from subsequent fractions.
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding.

D. Degradation pathway

Degradation pathway of glyphosate in rotational crops will be provided at the end of this chapter.

III. Conclusion

The uptake and metabolism of glyphosate was examined in rotational crops. *N*-(phosphono-methyl)glycine labelled in the methylene position (^{14}C-PMG-label) was applied as its trimesium salt to two plots at different application rates; three additional plots received a comparable treatment of unlabelled active substance as control plots. The test item ^{14}C-PMG labelled glyphosate-trimesium was applied at a rate of 5.617 kg a.s./ha (3.87 kg a.s./ha expressed as glyphosate equivalents, plot 1.0) and at a total rate of 9.51 kg a.s./ha (three monthly applications, 6.56 kg a.s./ha expressed as glyphosate equivalents, plot 5.0) to bare soil.

A primary crop, soybean, was planted prior to treatment in all plots containing sandy loam soil. After removal of the primary crop, the rotational crops lettuce, radish and wheat were planted into the subplots at 35, 63, and 308 days after herbicide treatment (35, 63, and 308 plant-back intervals, PBI).

The soya cover crop was not analysed. Residues samples from the rotated crops were extracted using a mixture of 0.1N HCl and chloroform, followed by column fractioning using different solvents to separate residues and natural products. To characterise incorporation into natural products, postextraction solids were additionally hydrolysed under acid and basic conditions. The TRR levels in matrices obtained from rotational crops were relatively low, not exceeding 0.1 mg/kg, except for lettuce (0.127 mg/kg). The rotational crops from the 35 and 63 PBI plots contained TRR levels of 0.020 – 0.076 mg/kg and 0.021 – 0.127 mg/kg, respectively. Crops from the 308 PBI contained TRR levels of 0.010 – 0.038 mg/kg. All the

residues were determined as *N*-phosphonomethylglycine (PMG) anion equivalents (mg ^{14}C-PMG anion equiv./kg, stated in the following only as mg/kg).

Analysis of rotational crop samples, after extraction with water and chloroform, revealed three residue components: PMG (glyphosate-anion), aminomethylphosphonic acid (AMPA) and a polar unknown metabolite (called metabolite 1 within the report). AMPA was found at levels of 8.7 - 34 % of the TRR. PMG was also detected in most samples, but its levels were <2.3 % of the TRR. Metabolite 1 was not identified as the amount was <0.01 mg/kg in all RAC's. Residues after extraction with water and chloroform were further investigated and were identified as being starch, lignins, amino acids and cellulose, as well as carbohydrates as glucose and fructose.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study assessing the metabolic behavior of ^{14}C-labelled glyphosate in rotational crops of lettuce, wheat and radish has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Metabolism in Rotational Crops, 502 with some deficits (minor deviations from the current guideline are listed above in section 1, Information on the study). No attempts were made to further solubilise the non-extractable radioactivity, so final residues were only analysed by combustion in some cases and were between 29.4 to 69.6 % of the TRR (0.245 – 2.34 mg/kg). Analysis of crop samples was not done within 6 months after sampling, but within 14 – 18 months. A storage stability investigation was conducted for natural products. Beside natural products only the test item PMG and AMPA were found in crop samples. For PMG and AMPA storage stability is covered by separate storage stability studies for up to 18 months ([redacted] 2010, CA 6.1/03, [redacted] 1991, CA 6.1/13 and [redacted] 1989, CA 6.1/14), so the storage periods in this study are adequately covered. Extraction rates were low to moderate for crop samples (23.8 to 54.2 % of the TRR), several attempts (acid and/or basic hydrolysis) were made to characterise the non-extractable radioactivity. However, final residues were between 13.1 to 59.4 % of the TRR, with absolute residues levels between 0.005 – 0.014 mg/kg.

The study is considered to be reliable for the assessment of the metabolic behaviour of glyphosate in rotational crops

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.6.1/003
Report author	[redacted]
Report year	1990
Report title	Confined Rotational Crop Study of Glyphosate. Part I: In-Field Portion. (Part II: MSL-9811)
Report No	MSL-9810
Document No	-
Guidelines followed in study	Pesticide Assessment Guideline Subdivision N, Number 165-1
Deviations from current test guideline	See the table below

Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

Data point:	CA 6.6.1/003
Report author	[redacted]
Report year	1990
Report title	Confined Rotational Crop Study of Glyphosate, Part II: Quantitation, Characterisation, and Identification of Glyphosate and Its Metabolites in Rotational Crops, (Part I: MSL-9810)
Report No	MSL-9811
Document No	-
Guidelines followed in study	Pesticide Assessment Guideline Subdivision N, Number 165-1
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Testing of Chemicals, 501: • Growth stage at sampling of immature crop samples is not given within the report; however could be roughly estimated based on planting and sampling dates. • The frozen samples were stored at -5°C or below and not at -18°C, however, storage stability at -5°C was demonstrated for the study duration. • For barley straw and carrot tops the initial extraction for storage analysis was performed 7 months after sampling and not within the 6 months, for which the frozen samples are assumed to be stable. • No date of analysis is given within the report. Less than 90 % of TRR was identified or characterised. Several attempts were made to characterise the bound radioactivity for selected matrices, but at least 20.7 – 28.7 % TRR (0.0278 – 0.0581 mg/kg) remained non-extracted.
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The metabolism of glyphosate was examined in rotational crops. ^{14}C-glyphosate formulated as Roundup® was applied to plots of bare sandy loam soil at a rate of 4.16 kg a.s./ha. A primary crop of soybeans was planted 7 days after application. The primary crop was harvested and the plots rototilled before planting rotational crops of lettuce, carrots, and barley into the subplots at 30, 119, and 364 days after herbicide treatment.

The primary and rotational crops were sampled for analysis. The rotational crops from the 30 DALT plots contained 0.037 – 0.188 mg/kg of glyphosate equivalent residues. Crops from the 119 DALT planting contained residues of 0.017 – 0.078 mg/kg. Carrots, barley, and lettuce from the 364 day planting contained residues of 0.0096 to 0.061 mg/kg. Analysis of rotational crop samples revealed two residue components, aminomethylphosphonic acid (AMPA) and a polar metabolite (called Metabolite 1 within the report) characterised as being a mixture of sugars, primarily glucose and fructose. Glyphosate was present only in lettuce, barley straw and grain of the first rotation 1.0 – 9.8 % (0.0018 – 0.0184 mg/kg) and in lettuce DALT 167 of the second rotation 1.6 % TRR (0.0009 mg/kg). AMPA ranged from 3.7 – 17.9, 1.1 – 14.2 and 7.7 – 20.0 % TRR (0.0007 – 0.0336, 0.0003 – 0.0111 and 0.0045 – 0.0093 mg/kg) in the matrices of the crops of the first, second and third rotation, respectively. Metabolite 1 amounted to 7.7 – 40.8, 6.3 – 24.9, 6.6 – 31.9 % TRR (0.0136 – 0.0327, 0.0039 – 0.0147 and 0.0031 – 0.0182 mg/kg) in the matrices of the crops of the first, second and third rotation, respectively.
Residues after extraction with water and chloroform were further investigated and were identified as being starch, lignin and cellulose, as well as biopolymers of glucose

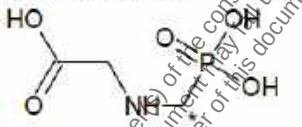
I. Materials and Methods

A. Materials

1. Test material

N-(phosphonomethyl)glycine; mixture of
a) N-(phosphono-^{14}C-methyl)glycine (227.3 mg)
b) N-(phosphono-^{12}C-methyl)glycine (3681.7 mg)

Chemical structure:



* Position of radiolabel

Radiochemical purity:	96.5 % (contained 2.4 % AMPA, 1 % nonionic component)
Chemical purity	>99 %
Specific activityof the test substance applied:	0.29 MBq/mg (1.31 mCi/mmol)
CAS No:	1071-83-6 (glyphosate acid) 38641-94-0 (glyphosate isopropylamine salt)
Log $P_{o/w}$ for glyphosate:	- 3.2

2. Test system

Soil:	Sandy loam (pH: 6.2-7.3; cation exchange capacity: 3.6-4.4 meq./100 g; sand: 62 – 70 %; silt: 23 - 31 %; clay: 9 %; textural class (USDA): sandy loam)
Crop:	Primary crop: Soybean (var. Williams) Rotational crops: Carrot (var. Goldmine), Lettuce (var. Waldmann's Green Leaf), Barley (variety Barley Blend BB88-2, 425:X1275)
Botanical name:	*Glycine max (L.) Merr.* *Daucus carota subsp. sativus.* Hoffm. *Latuca sativa* L. *Hordeum vulgare* L.
Crop part(s):	Carrot roots and tops, lettuce leaves, barley forage, straw, seeds

B. Study design

1. In-life phase

The in-life phase of the study was conducted outdoors in Madera, California.
The test substance contained 227.3 mg of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) with a specific activity of 4.93 MBq/mg (22.53 mCi/mmol) and 3681.7 mg of N-(phosphonomethyl)glycine (^{12}C-glyphosate). Final specific activity of the test substance was 0.29 MBq/mg (1.31 mCi/mmol).
Additionally, a test substance containing 3.909 g of unlabelled glyphosate (^{12}C test substance) was prepared for application to the control plot.
Glyphosate in both test substances was formulated as the isopropylamine salt, and a tallowamine surfactant was added. The relative proportions of glyphosate, isopropylamine, and surfactant in the test substance were prepared to be the same as in the formulation of Roundup®.
^{14}C-glyphosate was applied to the test plots at a target rate of 4.16 kg a.s./ha on bare soil. The control plots were treated with ^{12}C-glyphosate at the same rate. A CO_2 backpack sprayer was used for the application of the aqueous spray solutions.
Primary crop soybean was planted in all subplots 7 days after the application. For each subplot a single row of soybean seeds was hand planted to a depth of 2.5 cm. The plots were rototilled to a depth of 10 cm prior to planting.
To assess the results of crop failure, 30 days after treatment, the soybean crop foliage in one-third of the plot was collected and the plot then rototilled in both the control and ^{14}C treated plots. Each subplot was further divided into three equal mini-plots (0.61 x 1.69 m) which were planted with one of the rotational crops (carrots, lettuce, or barley).
A second subplot from each plot was prepared for planting 119 days after treatment. All soybean foliage within each of the 119 day subplots was collected. Three mini-plots (0.61 x 1.69 m) were each rototilled and planted with one of the rotational crops (carrots, lettuce, or barley).
The mature soybean crop was harvested from the final subplot and the plot prepared for planting 364 days after treatment. Three mini-plots (0.61 x 1.69 m) were each planted with one of the rotational crops (carrots, lettuce, or barley).
Each of the mini-plots was maintained in accordance with normal agricultural practice until a conventional harvest of each of these rotational crops was completed.

2. Sampling

The soybean foliage sample was harvested from the first subplot at 30 DALT. Soybean samples from the second subplot were harvested at 119 DALT, and mature soybeans from the third subplot were harvested at 182 DALT. The samples were divided in leaves, stems, pods and seeds. A soybean foliage sample from the second and third subplots was also harvested at 70 DALT. The rotational crops were planted at 30 DALT in the first subplot, at 119 DALT in the second subplot, and at 364 DALT in the third subplot. Lettuce was harvested at two intermediate times and at maturity in each subplot, while carrots were harvested only at maturity. Barley was harvested only at maturity in the 30 and 119 DALT subplots due to shortage of sample. In addition to the harvest at maturity, a barley forage sample was collected from the 365 DALT subplot. Carrots were separated into tops and root, and barley was separated into heads and straw. All samples were double-bagged with plastic and cloth bags and frozen immediately after collection. Frozen samples were placed in insulated containers with dry ice and shipped to the analytical facility via overnight delivery.
Samples were either immediately prepared for analysis or stored frozen below -5°C until preparation.
The soil in which the crops were growing was analysed at individual time points to follow the degradation of glyphosate and to identify the metabolites to which the rotational crops were exposed. Samples were taken prior to and immediately after treatment, at primary crop planting, and at each rotational crop planting and harvest. Soil samples were collected with a zero-contamination corer measuring one inch (ca 3 cm) in diameter. Cores were collected to a depth of ca 30 cm and separated into 0-15 cm and 15-30 cm sections. Soil samples were shipped frozen to the analytical facility. The samples were either immediately prepared for analysis or stored frozen below -5°C until sample preparation.

3. Analytical procedures

Total radioactive residues (TRR) in all plant samples were determined by liquid scintillation counting (LSC) following combustion. Moisture content was determined at the time combustion aliquots were taken.

Samples were prepared by grinding the whole frozen sample in a vertical cutter mixer with dry ice until the sample was a fine powder. Subsamples of each crop sample were homogenised first with water, than with chloroform. Samples were centrifuged and the aqueous layer was decanted and filtered. The aqueous extract was concentrated and an aliquot removed for LSC analysis. The concentrated samples were analysed by HPLC using an Aminex A-5 cation exchange column.

Unextractable residues were further analysed from a representative 105 DALT lettuce sample. This sample was chosen because of the quantity of sample available and the residue levels present. The solid plant tissue remaining after aqueous extraction was homogenised with 5.0 M NH_4OH. After centrifugation, the supernatant was decanted and concentrated. The concentrated extract was analysed by HPLC on an Aminex A-5 column. The solid tissue remaining from the ammonium hydroxide extraction was extracted with DMSO at 100°C for 16 hours to remove starch and lignin. After centrifugation, decantation and filtration, the supernatant was concentrated and an aliquot was taken for LSC analysis. The concentrated extract was lyophilised, and aliquots of the resultant solid were combusted. The solid residue from the lyophilisation of the DMSO extract was incubated with amyloglucosidase (from *Aspergillus niger*) in a sodium acetate buffer pH 4.5 at 55°C for 6 hours. This enzyme liberates glucose from starch. After centrifugation, the sample supernatant was concentrated and the concentrate applied to a Bio-Gel P-2 column. The column was eluted with water and 1.0 min fractions were collected and analysed by LSC. Fractions were also analysed for glucose using Chemstrips.

The 125 DALT barley grain and straw samples were analysed using the same procedure described for the 105 DALT lettuce sample. In addition, the straw tissue remaining after the DMSO extraction was washed with water to remove any residual DMSO. The sample was incubated with cellulase (from *Aspergillus niger*) in a 0.05 M sodium acetate buffer pH 5.0 at 37°C for 16 hours. This enzyme liberates glucose from cellulose. The mixture was centrifuged, the supernatant decanted, and concentrated. An aliquot of the concentrate was applied to a Bio-Gel P-2 column. The column was eluted with water and 1.0 min fractions were collected. The fractions were analysed for glucose using Chemstrips and by LSC.

The identity of the AMPA component was verified by co-elution with standards on two chromatography systems: Aminex A-5 cation exchange column and an anion exchange column packed with Dowex AG-1 in the chloride form. Metabolite 1 eluting near the void volume was isolated from the 105 DAT lettuce sample by making repeated injections on the HPLC, using the Aminex A-5 column, and collecting the effluent from 1 – 15 min. The effluent was concentrated and aliquots were analysed by anion exchange chromatography on a Spherisorb S-5 SAX column and by reverse phase chromatography on a Cl8 reverse phase column. The effluent was further purified by HPLC using the Cl8 reverse phase column. Aliquots of the combined and concentrated effluent were analysed by LSC, Chemstrips and HPLC (Bio-Rad HFX-87H column). Glucose, fructose and sucrose standards were also analysed by HPLC to compare retention times. Another aliquot, after additional purification (HPX-87H columns) was analysed by NMR. A mixture of glucose and fructose was also analysed by NMR for comparison. Another portion of the isolated metabolite matrix was analysed on a Bio-Gel P-2 sizing column (molecular weight range 100-1800). The sample was applied to the C18 column and eluted with water. Fractions were collected and analysed by LSC. Again, glucose, fructose, and sucrose standards were also analysed to compare retention times.

Homogenised soil samples were analysed by combustion to determine the amount of ^{14}C residue. Moisture content was determined at the time combustion aliquots were taken. A pooled soil sample was prepared for each sampling point. An aliquot was taken from each soil core of a given sampling time, and these aliquots were combined to generate the pooled sample. Each pooled sample was extracted twice with 0.5 M NH_4OH. Samples were centrifuged and the supernatant decanted. The supernatants were combined and an aliquot taken for LSC analysis. Concentrated extracts were analysed by LSC and HPLC using an Aminex A-9 cation exchange column.

Identification of glyphosate and AMPA was confirmed by co-elution with authentic standards under two separate chromatographic conditions (HPLC using an Aminex A-9 cation exchange column and column chromatography on Dowex AG-1 chloride form).

The metabolite eluting near the void volume (Metabolite A) was isolated from the 147 DAT soil sample by making repeated injections on the HPLC, using an Aminex A-9 column, and collecting the effluent from 1 – 8 min. The effluent was concentrated and an aliquot was reanalysed on an Aminex A-9 column. An aliquot of Metabolite A, isolated from the cation exchange column, was analysed by reverse phase chromatography on a C18 reverse phase column. Other aliquots of the metabolite were analysed by HPLC on a Bio-Rad HPX-87H organic acids column used for carbohydrate analysis and chromatographed on a Bio-Gel P-2 sizing column.

II. Results and Discussion

A. Total radioactive residues (TRRs)

After the first rotation, total radioactive residue (TRR) in edible commodities (lettuce leaves, carrot roots as well as barley grain) ranged from 0.048 to 0.188 mg/kg, and in non-edible commodities (carrot tops and barley straw) from 0.051 to 0.175 mg/kg. After the second rotation, TRR values in edible commodities (lettuce leaves, carrot roots as well as barley grain) ranged from 0.017 to 0.078 mg/kg, and in non-edible commodities (carrot tops and barley straw) from 0.028 to 0.056 mg/kg.
After the third rotation, TRR values in edible commodities (lettuce leaves, carrot roots as well as barley grain) ranged from 0.0096 to 0.057 mg/kg. In non-edible commodities (carrot tops, barley forage and straw) from 0.018 to 0.061 mg/kg.
Residue levels in soil of 0 – 15 cm depth decrease from approximately 0.74 mg/kg to approximately 0.18 mg/kg over the course of this study. The soil characteristics of the Madera soil indicated a sandy loam soil with a low organic matter content. The radioactivity found in the 15-30 cm cores may be due to contamination from the 0-15 cm level during sampling since the 0 DALT post application sample has the highest residue level of any 15-30 cm core.

Table 6.6.1-17: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

Rotation	PBI (days)	Crop	Sampled commodity	Sampling (DALT)	TRR (mg/kg)
Primary crop	-	Soybean	Foliage	30	0.4329
				70	0.3309
			Soybean	119	0.2327
			Leaves	177	0.0918
			Stems	182	0.0822
			Pods	182	0.2276
			Seeds	182	0.3185
1st rotation	30	Lettuce	Leaves	70	0.108
				90	0.048
				105	0.097
		Barley	Straw	125	0.175
			Grain	125	0.188
		Carrot	Tops	154	0.051
			Root	154	0.037
2nd rotation	119	Lettuce	Leaves	147	0.059
				167	0.055
				181	0.037
		Barley	Straw	314	0.056
			Grain	314	0.078

Table 6.6.1-17: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

Rotation	PBI (days)	Crop	Sampled commodity	Sampling (DALT)	TRR (mg/kg)
		Carrot	Tops	210	0.028
			Root	210	0.017
3rd rotation	364	Lettuce	Leaves	399	0.057
				425	0.043
				455	0.028
		Barley	Forage	412	0.056
			Straw	482	0.061
			Grain	482	0.047
		Carrot	Tops	482	0.018
			Root	482	0.0096

TRR – total radioactive residue, expressed as glyphosate, calculated within the report based on sample fresh weight
DALT – days after last treatment
PBI – plant back interval

Table 6.6.1-18: Total radioactive residues in soil after application of ^{14}C-glyphosate to bare soil

Rotation. PBI	DALT	TRR (mg/kg)	
		Soil depth 0 – 6 inches (ca 0 – 15 cm)	Soil depth 6 – 12 inches (ca 15 - 30 cm)
-	0 (post application)	0.711	0.0453
	7	0.738	0.0088
1st rotation, PBI 30 days	30	0.518	0.0017
	76	0.518	0.0036
	90	0.354	0.0016
	105	0.526	0.0021
	125	0.625	0.0009
	154	0.250	0.0010
2nd rotation, PBI 119 days	119	0.142	0.0011
	147	0.589	0.0001
	167	0.372	0.0006
	181	0.203	0.0009
	210	0.277	0.0006
	314	0.250	ND
3rd rotation, PBI 365 days	364	0.184	0.0009
	399	0.297	ND
	412	0.211	0.0032
	425	0.248	0.0011
	455	0.172	0.0021
	482	0.179	ND

TRR – total radioactive residue, expressed as glyphosate; calculated within the report based on soil dry weight
DALT – days after last treatment
ND – not detected

B. Extraction and characterisation of residues

Lettuce leaves

From the first rotation, 44.6 to 49.1 % of the TRR (0.0219 to 0.0530 mg/kg) in lettuce collected 70, 90 and 105 DALT was found in the water phase, whereas 48.9 % – 59.9 % of the TRR (0.0288 – 0.0551 mg/kg) remained in the solids. The water phase contained 8.1 – 14.6 % TRR (0.0039 – 0.0158 mg/kg) AMPA and 28.6 – 33.8 % TRR (0.0162 – 0.0327 mg/kg) Metabolite 1. Glyphosate was only found in lettuce leaves sampled 70 and 105 DALT and amounted to 2.9 to 3.8 % TRR (0.0028 – 0.0041 mg/kg) (Table 6.6.1-19).

The metabolite eluting near the void volume, Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.

Since the aqueous extraction removed less than 50 % of the total radioactive residue, the extraction was repeated and the residue was further examined for lettuce 105 DALT. 11.3 % TRR (0.0110 mg/kg) was additionally released by NH_4OH. The profiles of the NH_4OH extracts analysed by cation exchange chromatography were very similar to the profiles of the water extracts. The same components were extracted with water and ammonium hydroxide, although the ratios of the components were different. The residue remaining after the NH_4OH treatment was stirred with DMSO to solubilise starch and lignin. The solvent was removed from the solubilised material and the remaining solid was subjected to enzymatic degradation with amyloglucosidase. This enzyme liberates glucose from starch. Chromatography, on a Bio-Gel P-2 column, of the radioactivity in solution after degradation shows a broad peak which elutes in the region of glucose. Testing of the collected fractions with Chemstrips showed the co-elution of glucose with a significant fraction of the radioactivity. This result suggests that DMSO extracted starch, into which the label had been reincorporated. The material that was not digested by the enzyme could be lignin. 14.8 % TRR (0.0144 mg/kg) was released after DMSO treatment. The final residue amounted to 28.7 % of the TRR (0.0278 mg/kg) (Table 6.6.1-26).

From the second rotation, 34.3 to 40.1 % of the TRR (0.0148 to 0.0208 mg/kg) in lettuce collected 147, 167 and 181 DALT was found in the water phase. Non-extractable residues amounted to 57.0 to 70.3 % (0.0231 to 0.0415 mg/kg) of the TRR. The water phase contained 4.6 – 12.4 % TRR (0.0027 – 0.0050 mg/kg) AMPA and 15.1 – 24.9 % TRR (0.0083 – 0.0147 mg/kg) Metabolite 1. Glyphosate was found only in lettuce leaves sampled 167 DALT and amounted to 1.6 % TRR (0.0009 mg/kg) (Table 6.6.1-21).

From the third rotation, 37.2 to 56.4 % of the TRR (0.0158 to 0.0279 mg/kg) was found in the water phase. Non-extractable residues amounted to 45.3 to 62.2 % of the TRR (0.0127 to 0.0311 mg/kg). The water phase contained 10.5 – 20.0 % TRR (0.0045 – 0.0076 mg/kg) AMPA and 18.6 – 31.9 % TRR (0.0079 – 0.0182 mg/kg) Metabolite 1. Glyphosate was not detected (Table 6.6.1-23).

Barley grain

From the first, second and third rotations, 15.3, 25.2 and 24.8 % of the TRR (0.0288, 0.0197 and 0.0117 mg/kg) in barley grain was found, respectively, in the water phase. Non-extractable residues amounted to 82.8, 61.7 and 69.6 % of the TRR (0.1557, 0.1160 and 0.0327 mg/kg), respectively. The water phase of barley grain from the first, second and the third rotations contained 17.9, 14.2 and 15.7 % TRR (0.0336, 0.0111 and 0.0074 mg/kg) AMPA and 7.7, 6.3 and 6.6 % TRR (0.0144, 0.0049 and 0.0031) Metabolite 1, respectively. Glyphosate was only identified in grain of the first rotation at 9.8 % TRR (0.0184 mg/kg) (Table 6.6.1-20, Table 6.6.1-22 and Table 6.6.1-24).

For barley grain collected 125 DALT (first rotation) further treatment procedures were employed 22.5 % TRR (0.0423 mg/kg) was additionally released by NH_4OH. Similar to lettuce leaves the profiles of the NH_4OH extracts of barley grain analysed by cation exchange chromatography were very similar to the profiles of the water extracts. The same components were extracted with water and ammonium hydroxide, although the ratios of the components were different. The residue remaining after the NH4OH treatment was stirred with DMSO to solubilise starch and lignin. The solvent was removed from the solubilised material and the remaining solid was subjected to enzymatic degradation with

amyloglucosidase. Chromatography, on a Bio-Gel P-2 column, of the radioactivity in solution after degradation showed a broad peak which elutes in the region of glucose. Testing of the collected fractions with Chemstrips showed the co-elution of glucose with a significant fraction of the radioactivity. This result suggests that DMSO extracted starch, into which the label had been reincorporated. The material that was not digested by the enzyme could be lignin. 20.4 % TRR (0.0384 mg/kg) was released after treatment with DMSO. The final residue after solvent extraction and further treatments for solubilisation amounted to 30.9 % of the TRR (0.0581 mg/kg) (Table 6.6.1-26).

Carrot roots

From the first, second and third rotations, 54.4, 32.1 and 39.9 % of the TRR (0.0201, 0.0055 and 0.0038 mg/kg) in carrot roots was found in the water phase. Non-extractable residues amounted to 49.7, 61.9 and 64.3 % of the TRR (0.0184, 0.0105 and 0.0062 mg/kg), respectively. The water phase of carrot roots from the first and the second rotations contained 11.1 and 8.2 % TRR (0.0041 and 0.0014 mg/kg) AMPA and 40.8 and 22.9 % TRR (0.0151 and 0.0039) Metabolite 1, respectively. Glyphosate was not detected. (Table 6.6.1-20, Table 6.6.1-22 and Table 6.6.1-25).

For the carrot roots after the third rotation no metabolite elucidation was performed due to low TRRs found.

Barley forage

Barley forage was sampled only after the third rotation. A total of 31.1 % of the TRR (0.0174 mg/kg) in barley forage was found in the water phase. Non-extractable residues amounted to 67.3 % of the TRR (0.0377 mg/kg). The water phase of barley forage contained 16.6 % TRR (0.0093 mg/kg) AMPA and 14.6 % TRR (0.0082 mg/kg) Metabolite 1. Glyphosate was not detected (Table 6.6.1-24).

Barley straw

From the first, second and third rotation, 25.9, 19.2 and 22.7 % of the TRR (0.0453, 0.0108 and 0.0138 mg/kg) in barley straw was found, respectively, in the water phase. Non-extractable residues amounted to 74.3, 78.3 and 80.9 % of the TRR (0.1300, 0.0438 and 0.0493 mg/kg), respectively.

The water phase of barley straw from the first, second and the third rotations contained 3.7, 9.6 and 7.7 % TRR (0.0065, 0.0054 and 0.0047 mg/kg) AMPA and 16.8, 8.2 and 11.1 % TRR (0.0294, 0.0046 and 0.0068 mg/kg) Metabolite 1, respectively. Glyphosate was detected only in the straw of the first rotation at 1.0 % TRR (0.0018 mg/kg) (Table 6.6.1-20, Table 6.6.1-22 and Table 6.6.1-24).

For barley straw collected 125 DALT (first rotation) further treatment procedures were employed. 8.9 % TRR (0.0156 mg/kg) was additionally released by NH_4OH. The residue remaining after the NH_4OH treatment was stirred with DMSO to solubilize starch and lignin. Another 17.0 % TRR (0.0298 mg/kg) was released. NH_4OH and DMSO fractions from barley straw (125 DALT) were further analysed in the same way as the corresponding fractions from lettuce (105 DALT) and barley grain (125 DALT). The same findings were observed. Additionally, the unextracted barley straw tissue remaining after DMSO extraction was subjected to enzymatic digestion using a cellulase, which liberates glucose from cellulose. 36.0 % TRR (0.0630 mg/kg) was released. Chromatography on a BioGel P-2 sizing column again showed a broad peak which eluted in the region of glucose. Testing of the fractions with Chemstrips showed again that glucose co-eluted with a major portion of the sample radioactivity. The final residue amounted to 20.7 % of the TRR (0.0362 mg/kg) (Table 6.6.1-26).

Carrot tops

From the first, second and third rotation, 27.3, 24.5 and 22.7 % of the TRR (0.0139, 0.0069 and 0.0041 mg/kg) in carrot tops was found, respectively, in the water phase. Non-extractable residues amounted to 70.7, 83.2 and 67.0 % of the TRR (0.0361, 0.0233 and 0.0121 mg/kg), respectively.

The water phase of carrot tops from the first and the second rotations contained 1.4 and 1.1 % TRR (0.0007 and 0.0003 mg/kg) AMPA and 26.7 and 17.5 % TRR (0.0136 and 0.0049 mg/kg) Metabolite 1, respectively. Glyphosate was not detected.

For the carrot roots after the third rotation no metabolite elucidation was performed due to low TRRs found (Table 6.6.1-20, Table 6.6.1-22 and Table 6.6.1-25).

Table 6.6.1-19: Identification and characterisation of the radioactive residues of glyphosate in lettuce leaves of rotational crop (first rotation, PBI 30 days) planted after application of glyphosate to bare soil

	First rotation, PBI 30 days					
	Residues in lettuce leaves					
DALT	70		90		105[3]	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	0.108	100	0.048	100	0.097	100
Extraction with water and chloroform[1]						
Water phase	*0.0530*	49.1	*0.0219*	45.7	*0.0433*	44.6
Metabolite 1[2]	0.0309	*28.6*	0.0162	*33.8*	0.0327	*33.7*
Glyphosate	0.0041	*3.8*	ND	*ND*	0.0028	*2.9*
AMPA	0.0158	*14.6*	0.0039	*8.1*	0.0137	*14.1*
Identified	*0.0199*	*18.4*	*0.0039*	*8.1*	*0.0165*	*17.0*
Characterised	*0.0309*	*28.6*	*0.0162*	*33.8*	*0.0327*	*33.7*
ERR	*0.0530*	49.1	*0.0219*	45.7	*0.0433*	44.6
RRR	*0.0528*	48.9	*0.0288*	59.9	*0.0551*	*56.2*[4]
Total	0.1058	98.0	0.0507	105.6	0.0978	100.8

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
ND – not detected
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] only water phase was analysed. Chloroform phase was not analysed.
[2] Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.
[3] Amounts of glyphosate and its metabolites presented for lettuce leaves 105 DALT reflect the sum of the component levels of all extractions (see also Table 6.6.1-26)
[4] within the report erroneously reffered as 5.6 % TRR

Table 6.6.1-20: Identification and characterisation of the radioactive residues of glyphosate in barley and carrot commodities of rotational crop planted after application of glyphosate to bare soil

	First rotation, PBI 30 days							
	Barley straw		Barley grain		Carrot tops		Carrot roots	
DALT	125[3]				154			
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	0.175	100	0.188	100	0.051	100	0.037	100
Extraction with water and chloroform[1]								
Water phase	*0.0453*	25.9	*0.0288*	15.3	*0.0139*	27.3	*0.0201*	54.4
Metabolite 1[2]	0.0294	*16.8*	0.0144	*7.7*	0.0136	*26.7*	0.0151	*40.8*
Glyphosate	0.0018	*1.0*	0.0184	*9.8*	ND	*ND*	ND	*ND*

Table 6.6.1-20: Identification and characterisation of the radioactive residues of glyphosate in barley and carrot commodities of rotational crop planted after application of glyphosate to bare soil

	First rotation, PBI 30 days							
AMPA	0.0065	*3.7*	0.0336	*17.9*	0.0007	*1.4*	0.0041	*11.1*
Identified	*0.0083*	*4.7*	*0.0520*	*27.7*	*0.0007*	*1.4*	*0.0041*	*11.1*
Characterised	0.0294	*16.8*	0.0144	*7.7*	0.0136	*26.7*	0.0151	*40.8*
ERR	*0.0453*	25.9	*0.0288*	15.3	*0.0139*	27.3	*0.0201*	54.4
RRR	*0.1300*	74.3	*0.1557*	82.8	*0.0361*	70.7	*0.0184*	49.7
Total	*0.1754*	100.2	*0.1844*	98.1	*0.0481*	94.3	*0.0385*	104.1

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
ND – not detected
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] only water phase was analysed. Chloroform phase was not analysed.
[2] Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.
[3] Amounts of glyphosate and its metabolites presented for lettuce leaves 105 DALT reflect the sum of the component levels of all extractions (see also Table 6.6.1-26)

Table 6.6.1-21: Identification and characterisation of the radioactive residues of glyphosate in lettuce leaves of rotational crop (second rotation, PBI 119 days) planted after application of glyphosate to bare soil

	Second rotation, PBI 119 days					
	Lettuce leaves					
DALT	**147**		**167**		**181**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.059	100	0.055	100	0.037	100
Extraction with water and chloroform[1]						
Water phase	*0.0208*	35.3	*0.0189*	34.3	*0.0148*	40.1
Metabolite 1[2]	0.0147	*24.9*	0.0083	*15.1*	0.0087	*23.5*
Glyphosate	ND	*ND*	0.0009	*1.6*	ND	*ND*
AMPA	0.0027	*4.6*	0.0050	*9.1*	0.0046	*12.4*
Identified	0.0027	*4.6*	*0.0059*	*10.7*	0.0046	*12.4*
Characterised	0.0147	*24.9*	0.0083	*15.1*	0.0087	*23.5*
ERR	*0.0208*	35.3	*0.0189*	34.3	*0.0148*	40.1
RRR	*0.0415*	70.3	*0.0314*	57.0	*0.0231*	62.4
Total	*0.0623*	105.6	*0.0502*	91.3	*0.0379*	102.5

Table 6.6.1-21: Identification and characterisation of the radioactive residues of glyphosate in lettuce leaves of rotational crop (second rotation, PBI 119 days) planted after application of glyphosate to bare soil

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
ND – not detected
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] only water phase was analysed. Chloroform phase was not analysed.
[2] Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.

Table 6.6.1-22: Identification and characterisation of the radioactive residues of glyphosate in barley and carrot commodities of rotational crop (second rotation, PBI 119 days) planted after application of glyphosate to bare soil

	Second rotation, PBI 119 days							
	Barley straw		**Barley grain**		**Carrot tops**		**Carrot roots**	
DALT	**314**				**210**			
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.056	100	0.078	100	0.028	100	0.017	100
Extraction with water and chloroform[1]								
Water phase	*0.0108*	19.2	*0.0197*	25.2	*0.0069*	24.5	*0.0055*	32.1
Metabolite 1[2]	0.0046	*8.2*	0.0049	*6.3*	0.0049	*17.5*	0.0039	*22.9*
Glyphosate	ND	*ND*	ND	*ND*	ND	*ND*	ND	*ND*
AMPA	0.0054	*9.6*	0.0111	*14.2*	0.0003	*1.1*	0.0014	*8.2*
Identified	0.0054	*9.6*	0.0111	*14.2*	0.0003	*1.1*	0.0014	*8.2*
Characterised	0.0046	*8.2*	0.0049	*6.3*	0.0049	*17.5*	0.0039	*22.9*
ERR	*0.0108*	19.2	*0.0197*	25.2	*0.0069*	24.5	*0.0055*	32.1
RRR	*0.0438*	78.3	*0.1160*	61.7	*0.0233*	83.2	*0.0105*	61.9
Total	*0.0546*	97.5	*0.0679*	86.9	*0.0302*	107.7	*0.0160*	94.0

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
ND – not detected
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in italics
1 only water phase was analysed. Chloroform phase was not analysed.
2 Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.

Table 6.6.1-23: Identification and characterisation of the radioactive residues of glyphosate in lettuce leaves of rotational crop (third rotation, PBI 364 days) planted after application of glyphosate to bare soil

	Third rotation, PBI 364 days					
	Lettuce leaves					
DALT	**399**		**425**		**455**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.057	100	0.043	100	0.028	100
Extraction with water and chloroform[1]						
Water phase	*0.0279*	49.0	*0.0160*	37.2	*0.0158*	56.4
Metabolite 1[2]	0.0182	*31.9*	0.0080	*18.6*	0.0079	*28.2*
Glyphosate	ND	*ND*	ND	*ND*	ND	*ND*
AMPA	0.0076	*13.3*	0.0045	*10.5*	0.0056	*20.0*
Identified	0.0076	*13.3*	0.0045	*10.5*	0.0056	*20.0*
Characterised	0.0182	*31.9*	0.0080	*18.6*	0.0079	*28.2*
ERR	*0.0279*	49.0	*0.0160*	37.2	*0.0158*	56.4
RRR	*0.0311*	54.5	*0.0267*	62.2	*0.0127*	45.3
Total	*0.0590*	103.5	*0.0427*	99.4	*0.0285*	101.7

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
ND – not detected
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] only water phase was analysed. Chloroform phase was not analysed.
[2] Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.

Table 6.6.1-24: Identification and characterisation of the radioactive residues of glyphosate in barley commodities of rotational crop (third rotation, PBI 364 days) planted after application of glyphosate to bare soil

	Third rotation, PBI 364 days					
	Barley forage		**Barley straw**		**Barley grain**	
DALT	**412**		**482**		**482**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.056	100	0.061	100	0.047	100
Extraction with water and chloroform[1]						
Water phase	*0.0174*	31.1	*0.0138*	22.7	*0.0117*	24.8
Metabolite 1[2]	0.0082	*14.6*	0.0068	*11.1*	0.0031	*6.6*
Glyphosate	ND	ND	ND	ND	ND	ND
AMPA	0.0093	*16.6*	0.0047	*7.7*	0.0074	*15.7*
Identified	0.0093	*16.6*	0.0047	*7.7*	0.0074	*15.7*
Characterised	0.0082	*14.6*	0.0068	*11.1*	0.0031	*6.6*

Table 6.6.1-24: Identification and characterisation of the radioactive residues of glyphosate in barley commodities of rotational crop (third rotation, PBI 364 days) planted after application of glyphosate to bare soil

	Third rotation, PBI 364 days					
ERR	*0.0174*	31.1	*0.0138*	22.7	*0.0117*	24.8
RRR	*0.0377*	67.3	*0.0493*	80.9	*0.0327*	69.6
Total	*0.0551*	98.4	*0.0632*	103.6	*0.0444*	94.4

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
ND – not detected
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in italics
1 only water phase was analysed. Chloroform phase was not analysed.
2 Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.

Table 6.6.1-25: Identification and characterisation of the radioactive residues of glyphosate in carrot commodities of rotational crop (third rotation, PBI 364 days) planted after application of glyphosate to bare soil

	Third rotation, PBI 364 days			
	Carrot tops		**Carrot roots**	
DALT	**482**			
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
TRR	0.018	100	0.0096	100
Extraction with water and chloroform[1]				
Water phase	*0.0041*	22.7	*0.0038*	39.9
ERR	*0.0041*	22.7	*0.0038*	39.9
RRR	*0.0121*	67.0	*0.0062*	64.3
Total	*0.0161*	89.7	*0.0100*	104.2

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue
PBI – plant back interval
Total – sum of ERR and RRR
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] only water phase was analysed. Chloroform phase was not analysed.
2 Metabolite 1 was isolated from a 105 DALT lettuce sample for characterisation. An extensive analysis showed that that the major component of Metabolite 1 is glucose. Metabolite 1 also contains a closely related material of similar molecular weight which could be fructose.

Table 6.6.1-26: Additional treatments of the radioactive residues of glyphosate in lettuce leaves, barley straw and grain planted after application of glyphosate to bare soil

	First rotation, PBI 30 days					
	Lettuce leaves		Barley straw		Barley grain	
DALT	105		125		125	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
TRR	0.097	100	0.175	100	0.188	100
Extraction with water and chloroform[1]						
Water phase	*0.0438*	45.2	*0.0305*	17.4	*0.0493*	26.2
NH_4OH extract[2]	*0.0110*	11.3	*0.0156*	8.9	*0.0423*	22.5
DMSO[3]	*0.0144*	14.8	*0.0298*	17.0	*0.0384*	20.4
Extract after cellulase treatment	-	-	*0.0630*	36.0	-	-
Characterised	***0.0692***	**71.3**	***0.1388***	**79.3**	***0.1299***	**69.1**
Final residue	***0.0278***	***28.7***	***0.0362***	***20.7***	***0.0581***	***30.9***
Total	***0.097***	***100***	***0.175***	***100***	***0.188***	***100***

DALT – days after last treatment
TRR – total radioactive residue
ERR – extractable radioactive residue
RRR – residual radioactive residue, calculated assuming that there were no losses during extraction and purification
ND – not detected
All residue data are expressed as mg/kg glyphosate equivalents.
Values calculated upon dossier compilation are presented in *italics*
1 only water phase was analysed. Chloroform phase was not analysed.
2 The profiles of the NH_4OH extracts analysed by cation exchange chromatography were very similar to the profiles of the water extracts. The same components were extracted with water and ammonium hydroxide, although the ratios of the components were different (see also Table 6.6.1-19 and Table 6.6.1-20).
3 The solvent was removed from the solubilised material and the remaining solid was subjected to enzymatic degradation with amyloglucosidase. This enzyme liberates glucose from starch. Chromatography, on a Bio-Gel P-2 column, of the radioactivity in solution after degradation shows a broad peak which elutes in the region of glucose. Testing of the collected fractions with Chemstrips showed the coelution of glucose with a significant fraction of the radioactivity. This result suggests that DMSO extracted starch, into which the label had been reincorporated.

Soil

The extractability of soil samples varied with time: initially, at early sampling times, a very high percentage of the radioactivity from the soil was extracted (98 %). Over time the extractability decreased and then levelled off to the range of 45 - 76 % TRR. The only components found in soil extracts were non-metabolised glyphosate, AMPA, and an early eluting component referred to as Metabolite A. The amount of glyphosate in soil was the highest directly after application (90.5 % TRR, 0.6431 mg/kg) and decreased over time in the samples from each subplot. Thus, in the first, second and third rotation the glyphosate decreased from 28.1 (0.1453 mg/kg) to 3.2 % TRR (0.0081 mg/kg), 31.6 (0.0449 mg/kg) to 1.0 % TRR (0.026 mg/kg) and from 17.8 % TRR (0.0327 mg/kg) to not detected, respectively.
As for AMPA, only 4.7 % TRR (0.0334 mg/kg) could be detected directly after the application. Afterwards, the amount of AMPA slightly increased over time in the samples of each subplot. Thus, in the first, second and third rotation the AMPA residue increased from 39.0 % (0.2020 mg/kg) to 46.8 % TRR (0.1170 mg/kg), 27.4 % (0.0389 mg/kg) to 47.8 % TRR (0.1194 mg/kg) and from 45.6 % (0.0839 mg/kg) to 56.5 % TRR (0.1012 mg/kg), respectively. The maximum amount of AMPA detected in 30, 119 and 365 PBI subplots was 51.7, 56.5 and 58.4 % TRR (0.3014, 0.2287 and 0.1449 mg/kg). Thus, the absolute amount of AMPA (expressed as mg/kg TRR) slightly decreased over the time of study conduction.
The metabolite eluting near the void volume, Metabolite A, was isolated from a 147 DALT soil sample for characterisation. After isolation by cation exchange chromatography, the metabolite was analysed by reverse phase chromatography on a C18 column. The radioactivity eluted near the void volume,

suggesting that this material was very polar. It was also observed that the metabolite fraction contained two components. Another portion of the isolated metabolite was analysed by HPLC chromatography on a HPX-87H carbohydrate column. Again two major components were observed one of which elutes in the same region as glucose. Another portion of the isolated metabolite was analysed by chromatography on a Bio-Gel P-2 (weight range 100-1800). Unlabelled glucose was added to the sample applied to the column to determine where glucose elutes from the column. A single major peak was observed with approximately half of the activity eluting in the same region as glucose. The results of these analyses show that Metabolite A is made up of two components, one of which seems to be glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative. It ranged from 2.5 to 9.0 % TRR (0.0087 – 0.0465 mg/kg), from 2.2 to 8.2 % TRR (0.0080 – 0.0483 mg/kg) and from 3.3 to 7.1 % TRR (0.0057 – 0.0141 mg/kg) in the first, second and third rotation, respectively. Thus, the absolute amount of Metabolite A (expressed as mg/kg TRR) decreased over the time of study conduction.

Table 6.6.1-27: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	-		First rotation, PBI 30 days			
DALT	**0**		**30**		**76**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.711	100	0.518	100	0.518	100
Extraction with 0.5 M NH_4OH						
Aqueous extract	*0.6968*	98	*0.3937*	76	*0.3937*	76
Metabolite A[1]	0.2020	*28.4*	0.0315	*6.1*	0.0465	*9.0*
Glyphosate	0.6431	*90.5*	0.1453	*28.1*	0.1189	*23.0*
AMPA	0.0334	*4.7*	0.2020	*39.0*	0.2224	*42.9*
Identified	*0.6765*	*95.1*	*0.3473*	*67.0*	*0.3413*	*65.9*
Characterised	0.2020	*28.4*	0.0315	*6.1*	0.0465	*9.0*
ERR	*0.6968*	98	*0.3937*	76	*0.3937*	76
RRR	*0.0142*	*2*	*0.1243*	*24*	*0.1243*	*24*
Total	0.711	100	0.518	100	0.518	100

DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue
RRR – Residual radioactive residue, calculated assuming that there were no losses during extraction and purification
PBI – plant-back interval
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] Metabolite A is made up of two components, one of is glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative

Table 6.6.1-28: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	First rotation, PBI 30 days							
DALT	**90**		**105**		**125**		**154**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.354	100	0.526	100	0.625	100	0.250	100
Extraction with 0.5 M NH_4OH								
Aqueous extract	*0.2230*	63	*0.3103*	59	*0.3500*	56	*0.1375*	55
Metabolite A[1]	0.0087	*2.5*	0.0196	*3.7*	0.0210	*3.4*	0.0111	*4.4*

Table 6.6.1-28: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	First rotation, PBI 30 days							
DALT	**90**		**105**		**125**		**154**	
Glyphosate	0.0268	*7.6*	0.0456	*8.7*	0.0266	*4.3*	0.0081	*3.2*
AMPA	0.1831	*51.7*	0.2442	*46.4*	0.3014	*48.2*	0.1170	*46.8*
Identified	*0.2099*	*59.3*	0.2898	*55.1*	*0.3280*	*52.5*	*0.1251*	*50.0*
Characterised	0.0087	*2.5*	0.0196	*3.7*	0.0210	*3.4*	0.0111	*4.4*
ERR	*0.2230*	63	*0.3103*	59	*0.3500*	56	*0.1375*	55
RRR	*0.1310*	*37*	*0.2157*	*41*	*0.2750*	*44*	*0.1125*	*45*
Total	0.354	100	0.526	100	0.625	100	0.250	100

DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue
RRR – Residual radioactive residue, calculated assuming that there were no losses during extraction and purification
PBI – plant-back interval
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in italics
1 Metabolite A is made up of two components, one of is glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative

Table 6.6.1-29: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	Second rotation, PBI 119 days					
DALT	**119**		**147**		**167**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.142	100	0.589	100	0.372	100
Extraction with 0.5 M NH_4OH						
Aqueous extract	*0.0951*	67	*0.3181*	54	*0.1674*	45
Metabolite A[1]	0.0066	*4.6*	0.0483	*8.2*	0.0080	*2.2*
Glyphosate	0.0449	*31.6*	0.0083	*1.4*	ND	*ND*
AMPA	0.0389	*27.4*	0.2287	*38.8*	0.1590	*42.7*
Identified	*0.0838*	*59.0*	*0.237*	*40.2*	*0.1590*	*42.7*
Characterised	0.0066	*4.6*	0.0483	*8.2*	0.0080	*2.2*
ERR	*0.0951*	67	*0.3181*	54	*0.1674*	45
RRR	*0.0469*	*33*	*0.2709*	*46*	*0.2046*	*55*
Total	0.142	100	0.589	100	0.372	100

DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue
RRR – Residual radioactive residue, calculated assuming that there were no losses during extraction and purification
PBI – plant-back interval
ND – not detected
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] Metabolite A is made up of two components, one of is glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative

Table 6.6.1-30: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	Second rotation, PBI 119 days					
DALT	**181**		**210**		**314**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.203	100	0.277	100	0.250	100
Extraction with 0.5 M NH_4OH						
Aqueous extract	*0.1137*	56	*0.1911*	69	*0.1425*	57
Metabolite A[1]	0.0107	*5.3*	0.0176	*6.4*	0.0171	*6.8*
Glyphosate	0.0017	*0.8*	0.0029	*1.0*	0.0026	*1.0*
AMPA	0.0983	*48.4*	0.1565	*56.5*	0.1194	*47.8*
Identified	*0.1000*	*49.3*	*0.1594*	*57.5*	*0.1220*	*48.8*
Characterised	0.0107	*5.3*	0.0176	*6.4*	0.0171	*6.8*
ERR	*0.1137*	56	*0.1911*	69	*0.1425*	57
RRR	*0.0893*	*44*	*0.0859*	*31*	*0.1075*	*43*
Total	0.203	100	0.277	100	0.250	100

DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue
RRR – Residual radioactive residue, calculated assuming that there were no losses during extraction and purification
PBI – plant-back interval
ND – not detected
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] Metabolite A is made up of two components, one of is glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative

Table 6.6.1-31: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	Third rotation, PBI 365 days					
DALT	**364**		**399**		**412**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.184	100	0.297	100	0.211	100
Extraction with 0.5 M NH_4OH						
Aqueous extract	*0.1325*	72	*0.1455*	49	*0.1329*	63
Metabolite A[1]	0.0111	*6.0*	0.0141	*4.7*	0.0122	*5.8*
Glyphosate	0.0327	*17.8*	0.0413	*13.9*	0.0072	*3.4*
AMPA	0.0839	*45.6*	0.0853	*28.7*	0.0957	*45.4*
Identified	*0.1166*	*63.4*	*0.1266*	*42.6*	*0.1029*	*48.8*
Characterised	0.0111	*6.0*	0.0141	*4.7*	0.0122	*5.8*
ERR	*0.1325*	72	*0.1455*	49	*0.1329*	63
RRR	*0.0515*	*28*	*0.151*	*51*	*0.0781*	*37*
Total	0.184	100	0.297	100	0.211	100

Table 6.6.1-31: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue
RRR – Residual radioactive residue, calculated assuming that there were no losses during extraction and purification
PBI – plant-back interval
ND – not detected
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] Metabolite A is made up of two components, one of is glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative

Table 6.6.1-32: Identification and characterisation of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil

	Third rotation, PBI 365 days					
DALT	**425**		**455**		**482**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.248	100	0.172	100	0.179	100
Extraction with 0.5 M NH_4OH						
Aqueous extract	*0.1587*	64	*0.1015*	59	*0.1128*	63
Metabolite A[1]	0.0124	*5.0*	0.0057	*3.3*	0.0127	*7.1*
Glyphosate	ND	*ND*	0.0010	*0.6*	ND	*ND*
AMPA	0.1449	*58.4*	0.0757	*44.0*	0.1012	*56.5*
Identified	*0.1449*	*58.4*	*0.0767*	*44.6*	*0.1012*	*56.5*
Characterised	0.0124	*5.0*	0.0057	*3.3*	0.0127	*7.1*
ERR	*0.1587*	64	*0.1015*	59	*0.1128*	63
RRR	*0.0893*	*36*	*0.0705*	*41*	*0.0662*	*37*
Total	0.248	100	0.172	100	0.179	100

DALT – days after last treatment
TRR – Total radioactive residue
ERR – Extractable radioactive residue
RRR – Residual radioactive residue, calculated assuming that there were no losses during extraction and purification
PBI – plant-back interval
ND – not detected
All residue data are expressed as mg/kg glyphosate equivalents
Values calculated upon dossier compilation are presented in *italics*
[1] Metabolite A is made up of two components, one of is glucose and the other a closely related material of similar molecular weight, possibly a glucose derivative

C. Storage stability

Three crop samples, 105 DALT lettuce, 125 DALT barley straw, and 154 DALT carrot tops were used for storage stability testing in crops. These samples were removed from the freezer (<-5°C) and extracted at 5 to 7 months and again at 12 to 15 months after receipt. The extracts of each sample were analysed by HPLC and no significant difference between the respective profiles was detected. The combustion analysis and extractabilities of the respective samples were nearly identical as well. These results indicate that the crop metabolites are stable in the freezer over an extended period of time.

The storage stability test for soil was done in a similar manner. 76 DALT soil was used as the test sample. This sample was extracted with 0.5 M NH4OH and analysed by HPLC within 7 months of receipt. After storage below -5°C for 17 months, a fresh aliquot was extracted and analysed as before. Residue levels and the amount of radioactivity extracted again were compared as were the HPLC profiles of the original

extraction. No significant difference between the respective profiles was detected. The combustion analysis and extractabilities of the respective samples were similar.
The dates of analysis are not indicated within the report, therefore the storage period was calculated from the date of sampling to the date of report finalisation, as the worst case. In this case, the storage duration for crops from the third rotation was 11 – 14 months and is covered by storage stability analysis conducted within the study. For the second rotation and first rotation the storage duration is 17 – 22 and 22 – 25 months, respectively. Although in some cases the maximum storage periods are longer than are covered by the available storage stability data, because there was no visible change in profile after 12 months (lettuce leaves) and 15 months (barley straw and carrot tops) storage, it is reasonable to suppose that the residues remained stable throughout the duration of the study.

Table 6.6.1-33: Extraction of the radioactive residues of glyphosate in lettuce leaves, barley straw and carrot tops – storage stability assessment

	Lettuce leaves 105 DALT		Barley straw 125 DALT		Carrot tops 154 DALT	
Storage interval (months)	**5**	**12**	**7**	**15**	**7**	**15**
	% TRR	**% TRR**	**% TRR**	**% TRR**	**% TRR**	**% TRR**
TRR	100	100	100	100	100	100
ERR	45.2	45.2	25.9	27.6	32.4	34.6
RRR	*54.8*	*54.8*	*74.1*	*72.4*	*67.6*	*65.4*
Total	**100**	**100**	**100**	**100**	**100**	**100**

TRR Total radioactive residue (expressed as glyphosate equivalents)
ERR Extractable radioactive residue
RRR Residual radioactive residue, calculated based on the assumption that there were no losses during extraction.

Table 6.6.1-34: Extraction of the radioactive residues of glyphosate in soil – storage stability assessment

	Soil 76 DALT	
Storage interval (months)	**7**	**17**
	% TRR	**% TRR**
TRR	100	100
ERR	75.9	76.7
RRR	*24.1*	*23.3*
Total	**100**	**100**

TRR Total radioactive residue (expressed as glyphosate equivalents)
ERR Extractable radioactive residue
RRR Residual radioactive residue

D. Degradation pathway

Degradation pathway of glyphosate in rotational crops will be provided at the end of this chapter.

III. Conclusion

The metabolism of glyphosate was examined in rotational crops. ^{14}C-glyphosate formulated as Roundup® was applied to plots of bare sandy loam soil at a rate of 4.16 kg a.s./ha. A primary crop of soybeans was planted 7 days after application. The primary crop was harvested and the plots rototilled before planting rotational crops of lettuce, carrots, and barley into the subplots at 30, 119, and 364 days after herbicide treatment.
The primary and rotational crops were sampled for analysis. The rotational crops from the 30 DALT plots contained 0.037 – 0.188 mg/kg of glyphosate equivalent residues. Crops from the 119 DALT planting contained residues of 0.017 – 0.078 mg/kg. Carrots, barley, and lettuce from the 364 day planting

contained residues of 0.0096 to 0.061 mg/kg. Analysis of rotational crop samples revealed two residue components, aminomethylphosphonic acid (AMPA) and a polar metabolite (called Metabolite 1 within the report) characterised as being a mixture of sugars, primarily glucose and fructose. Glyphosate was present only in lettuce, barley straw and grain of the first rotation 1.0 – 9.8 % (0.0018 – 0.0184 mg/kg) and in lettuce DALT 167 of the second rotation 1.6 % TRR (0.0009 mg/kg). AMPA ranged from 3.7 – 17.9, 1.1 – 14.2 and 7.7 – 20.0 % TRR (0.0007 – 0.0336, 0.0003 – 0.0111 and 0.0045 – 0.0093 mg/kg) in the matrices of the crops of the first, second and third rotation, respectively. Metabolite 1 amounted to 7.7 – 40.8, 6.3 – 24.9, 6.6 – 31.9 % TRR (0.0136 – 0.0327, 0.0039 – 0.0147 and 0.0031 – 0.0182 mg/kg) in the matrices of the crops of the first, second and third rotation, respectively.
Residues after extraction with water and chloroform were further investigated and were identified as being starch lignin and cellulose, as well as biopolymers of glucose.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study assessing the metabolic behaviour of glyphosate in rotational crops lettuce, barley and carrot has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with some deficits: the frozen samples were stored at -5°C and not at -20°C, however, storage stability at -5°C or below was demonstrated within the study for at least 12 to 15 months, for barley straw and carrot tops (as well as for soil) the initial extraction for storage analysis was performed 7 months after storage and not within the 6 months, for which the frozen samples are assumed to be stable and no dates of analysis are given within the report.
Less than 90 % of TRR was identified or characterised and relatively large amounts of radioactivity remained unextracted. Several attempts were made to characterise the residual radioactive residue after extraction with water for lettuce leaves, barley straw and barley grain. After additional solubilisation procedures, 20.7– 30.9 % TRR (0.0278 – 0.0581 mg/kg) remained unextracted. Significant attempts to characterize non-extracted residues were performed with sequential treatments with NH_4OH, DMSO, amyloglucosidase and cellulase. The DMSO and enzyme treatments released up to 14.8, 20.4 % and 36.0 % of TRR (0.0144, 0.0384 and 0.0630 mg/kg) in lettuce leaves, barley grain and straw, respectively. It seems likely that a significant part of the non-extracted radioactivity could be attributed to natural plant constituents.

Storage stability
No date of analyses is stated within the report. However, since the storage stability was tested within the study, it is reasonable to assume according to the study design that at the time the storage stability was tested the final analyses have already been performed. For purposes of storage stability testing the initial extraction of barley straw and carrot tops was conducted after a storage period of 7 months. Assuming the worst case, the storage period can be calculated from the date of sampling to the date of report finalisation. In this case, the storage duration for crops from the third rotation is 11 – 14 months and is covered by storage stability analysis conducted within the study. For the second rotation and first rotation the storage duration is 17 – 22 and 22 – 25 months, respectively which is longer than the tested period.
In all of the plant matrices from all rotations, the same analytes were found: glyphosate, AMPA and Metabolite 1. Glyphosate and its primary metabolite AMPA are likely to be stable, as shown in storage stability studies. Metabolite 1, which was characterised as being a mixture of sugars, primarily glucose and fructose, is formed through plant anabolism and is unlikely to be a degradation product. Therefore, in total for all plant matrices from all rotations it is likely that the stored samples were stable from sampling to analysis.
The study is considered reliable for the assessment of the metabolic behaviour of glyphosate in rotational crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.6.1/004
Report author	[redacted]
Report year	1989
Report title	[^{14}C-Anion]ICIA0224 - Confined Accumulation Studies on Rotational Crops
Report No	WRC 89-25
Document No	VV-320956
Guidelines followed in study	Pesticide Assessment Guideline Subdivision N, Number 165-1
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Metabolism in Rotational Crops, 502: • A crop representing leafy vegetables was not included in the study. • Growth stage of crops at planting is not given. • The study was intended only to study uptake of radio-labelled residues and no attempt was made to characterise the residues. • The frozen samples were stored at -10°C and not at -18°C. Storage stability was not investigated, although the samples were kept frozen longer than 6 months. • No dates of analyses are given within the report. • Residue levels of the control samples are not included in the report. • The radiochemical purity of the application solution was <95 %; no specifications of the impurities were given.
Previous evaluation	No
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Supportive
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

A rotational crop uptake study was conducted to measure the uptake of glyphosate from soil by rotational crops, namely wheat and turnips, and to determine the identity of these residues. In this study only two crops were planted - wheat and turnips, representing a root crop and a small grain cereal.

For the investigations, a loamy sand soil was treated with *N*-(phosphono-^{14}C-methyl)glycine as its trimesium salt (^{14}C-glyphosate-trimesium). Treatment was performed at a nominal rate of 6 mg/kg ^{14}C-glyphosate-trimesium (corresponding to 4 mg/kg of ^{14}C-glyphosate).

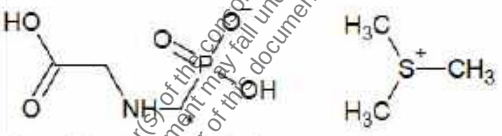
Wheat and turnips were planted 35, 95 and 370 days after treatment. At each interval, soil core samples (0 - 15 cm) were taken from the pots. The average radioactive residue in soil declined from 1.58 mg/kg (day 0) to 0.803 mg/kg (day 370).

The plants were harvested at maturity and the radioactive residues were determined in commodities of wheat (seed, chaff and stalks/leaves) and of turnips (leaves and bulbs).

Total glyphosate equivalent residues in wheat seeds, chaff and stalks/leaves were 0.25, 0.29 and 0.46 mg/kg grown on soil aged for 35 days, 0.28, 0.25 and 0.51 mg/kg (on soil aged for 95 days) and 0.06, 0.1 and 0.11 mg/kg (on soil aged for 370 days).
In turnip leaves and bulbs the radioactive residues amounted to 0.02 mg/kg for both commodities of turnips grown on soil aged for 35 days, to 0.09 and 0.03 mg/kg (on soil aged for 95 days) and were detected at 0.03 and 0.02 mg/kg (on soil aged for 370 days).
The radioactive residues in the plant matrices were not extracted and investigated for their identity, since the residue levels were considered to be too low for reasonable analyses

I. Materials and Methods

A. Materials

1. Test material	**Glyphosate-trimesium** • N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labelled glyphosate-trimesium) Mixture of a) N-(phosphono-^{14}C-methyl)glycine trimesium salt (5.7 mg) b) N-(phosphono-^{12}C-methyl)glycine trimesium salt (138.5 mg)
Chemical structure:	 * Position of radiolabel
Radiochemical purity:	a) 94 % (by TLC) radiochemical purity of applied solution is not given
Chemical purity	a) not given b) 95.7 %
Specific activity:	Applied solution: 0.53 MBq/mg (32000 dpm/µg)
CAS No:	(glyphosate) 81591-81-3 (glyphosate trimesium)
Log $P_{o/w}$ for glyphosate:	- 2.9

2. Test material	
Soil:	Loamy sand (pH: 6.9; cation exchange capacity: 12.2 meq/100 g; OM: 0.6 %, sand: 79.90 %; silt: 13.90 %; clay: 6.20 %; textural class (USDA): not given)
Crop:	*Primary crop*: No primary crops were planted *Rotational crops*: Wheat (variety Anza) Turnips (variety Purple-top White Globe)
Botanical name:	*Brassica rapa* *Triticum aestivum*
Crop part(s):	Wheat (chaff, seeds, stalks/leaves), turnips (leaves, bulbs)

B. Study design

1. In-life phase

Application solutions

Two application solutions were prepared, one containing a mixture of radiolabelled and unlabelled test item and another containing only unlabelled test item (used for control soil).

For preparation of the application solution containing ^{14}C-PMG-labelled glyphosate-trimesium, 5.7 mg of the radiolabelled test item with a specific activity of 4.53 MBq/mg (30 mCi/mmol) and 138.5 mg of unlabelled glyphosate-trimesium (equivalent to about 79.5 mg pure test item) were mixed in 100 mL distilled water. After assaying, the specific activity was calculated to be 0.37 MBq/mg (21911 dpm/µg) for glyphosate-trimesium or 0.53 MBq/mg (32000 dpm/µg) for ^{14}C-glyphosate.

For preparation of the application solution for the control pots, unlabelled glyphosate-trimesium (130.5 mg, equivalent to about 74.9 mg pure test item) were dissolved in 100 mL distilled water.

Soil preparation, treatment, aging and planting

Fifteen pots, each with a hole in the bottom for drainage were used. A total of 59 kg soil was mixed with 185 g of "17-17-17" fertilizer in a mixing drum, sifted through a 2 mm screen, and divided into the fifteen pots. Glass tubes were positioned down the centre of each of the pots and were secured in the bottom hole with packed cotton while the soil was added. These tubes served to drain any water in excess of 1 cm deep from the top of the soil, thereby avoiding unrealistic flooded conditions. Plastic buckets into which the pots were placed caught this drainage water as well as a leachate.

The top 7.5 cm of soil was removed from twelve of the pots and weighed. This was done in order to calculate the amount of glyphosate-trimesium needed to add to the soil to achieve a 6 mg/kg concentration to approximate the intended rate of 6 kg/ha glyphosate-trimesium, equivalent to 4.12 kg glyphosate/ha.

Radiolabelled glyphosate-trimesium was incorporated into half the removed soil (enough for 6 pots) using a twin shell blender. Unlabelled glyphosate-trimesium was incorporated into the other half. After mixing, the treated soil samples were subsampled for combustion analysis and placed back into the original twelve pots to give 6 radiolabelled and 6 "cold" treated pots of soil.

The actual treatment rate was calculated to be 5.8 mg/kg ^{14}C-glyphosate-trimesium or equivalent to 4.0 mg/kg glyphosate. The actual treatment rate of unlabelled glyphosate-trimesium was calculated to be 5.8 mg/kg glyphosate-trimesium or equivalent to 4.0 mg/kg glyphosate.

The pots of soil were aged outdoors for three intervals: 35, 95 or 370 days. Four pots of soil, two treated with ^{14}C-PMG-labelled glyphosate-trimesium and two with non-radioactive test item, were sampled and planted with crops at each interval.

After each aging period, a soil core sample was taken for analysis. All of the remaining soil was then taken out of each pot, mixed separately in a blender, sampled and returned to the pot. Two pots of soil were planted with wheat, one treated with ^{14}C- glyphosate-trimesium and the other with non-radioactive glyphosate-trimesium. Similarly, two pots of soil were planted with turnips. Plants were grown outside and were watered and thinned as needed until they reached maturity and were harvested. The plants were also treated with allethrin for insect control. Plant thinnings and leachates (from rain or irrigation) were collected and analysed during the growing period.

2. Sampling

Samples were collected from mature wheat plants by clipping the heads off the stalks and then clipping the stalks off at soil level. The heads were threshed, and the chaff, seeds, and stalks (including leaves) were weighed separately. These three crop parts were then ground separately in a lab mill and subsamples were taken for combustion.

The turnips were harvested when the roots were full and mature. Turnip leaves were clipped off the roots, weighed, frozen with liquid nitrogen, homogenised with a mortar and pestle, then subsampled for combustion analysis. The roots were rinsed free of dirt with distilled water, patted dry, weighed and ground using a food processor.

The processed plant materials were put in separate plastic bags, one bag for each plant-back interval: 35-day, 95-day, 370-day and controls. All samples were stored at <-10°°C.

The soil in which the crops were growing was analysed to follow the degradation of glyphosate-trimesium and to identify the metabolites to which the rotational crops were exposed.
After each aging period, a soil core sample (1 cm diameter x 15 cm deep) was taken from each of the four pots (two pots containing soil treated with ^{14}C- glyphosate-trimesium and two with non-radioactive test item). The 0 - 7.5 cm and the 7.5 – 15 cm segments were separated, placed into separate bags, subsampled for future combustion, and frozen at <-10°°C until analysed. After harvesting the plants, the soil was removed from the pots, weighed, sub-sampled for combustion analysis and stored at <-10°°C.

3. Analytical procedures

Two or more aliquots of all soil and plant samples were combined with an approximately equal volume of cellulose in combustion thimbles and combusted. The $^{14}CO_2$ generated was trapped with Carbosorb, then mixed with Permafluor scintillation cocktail. Liquid samples, including those resulting from combustion, were assayed by liquid scintillation counting (LSC).

The extractability of the ^{14}C-glyphosate from soil was tested by separately extracting 2 g subsamples from the 0-time soil with each of the following solvents: H_2O, 1 M HCl, 1 M NH_4Cl, 0.5 M NaOH, 0.5 M NaOH in methanol and 0.5 M HCl in methanol.

Each soil sample was weighed into a centrifuge tube, combined with an adequate volume of solvent, and extracted by shaking. The tubes were centrifuged and the solvent separated from the soil by decantation. Three successive extractions were done in this way using fresh solvent each time. Soils were combusted and analysed as previously described while soil extracts were analysed by LSC.

Large scale extractions (20 - 200 g soil) were done in a similar manner using a volume of solvent (in mL) equal to 5 times the weight of the soil (in g). 1 M HCl was determined to be the most suitable solvent for these extractions.
Zero-day, 30-day, 95-day, and 365-day soils were sampled just prior to planting with wheat and turnips. Extracts and soils were analysed by LSC. The extracts were concentrated by rotary vacuum evaporation (RVE) and reassayed by LSC.

The extracts were passed through individual, plastic disposable columns containing C-18 bonded silica, which had been pre-rinsed with methanol and 1 M HCl. Radioactivity was eluted with 1 M HCl and concentrated by RVE. The concentrate was diluted with distilled water and mixed with Dowex AG-50-WX8 (hydrogen form) and swirled at 25°°C for 2 hours. The resin was filtered, dried, and combusted to determine residual radioactivity. The filtrate was concentrated by RVE for TLC analysis.

Thin-layer chromatography (TLC) was performed on 250 µ Merck silica gel plates utilizing the following solvent systems developed both one- and two-dimensionally:
N-propanol/diethylamine/water (5/2/3) and N-propanol/triethylamine/water (5/2/3).

II. Results and Discussion

A. Total radioactive residues (TRRs)

The radioactive residues detected in commodities of wheat and turnips after three rotations are shown in Table 6.6.1-35.
For the commodities sampled from wheat (chaff, seeds and stalks/leaves), the total radioactive residues were comparable for samples taken from the first and the second rotation. The residues in seeds, chaff and stalks/leaves amounted to 0.25, 0.29 and 0.46 mg/kg (harvest 133 DALT) and to 0.28, 0.25 and 0.51 mg/kg (harvest 195 DALT). In samples from the one-year plant-back, harvested at 469 DALT lower residue levels were detected, amounting to 0.06, 0.1 and 0.11 mg/kg for seeds, chaff and stalks/leaves, respectively.

Turnips were harvested 195, 257 and 469 days after treatment. The radioactive residues in turnip leaves and bulbs were comparable (0.02 - 0.03 mg/kg for leaves and 0.02 - 0.03 mg/kg for bulbs) for samples of all three rotations, except for leaves of the second rotation, where radioactive residues amounted to 0.09 mg/kg.

Table 6.6.1-35: Total radioactive residues in rotational crops planted after application of 14C-PMG labelled glyphosate-trimesium to bare soil

PBI (days)	Crop	Sampled commodity	Sampling (DALT)	TRR (mg/kg)	% AR
35	Wheat	Seeds	133	0.25	0.020
		Chaff		0.29	0.008
		Stalks/leaves		0.46	0.064
	Turnip	Leaves	257	0.02	0.011
		Bulbs		0.02	0.016
95	Wheat	Seeds	195	0.28	0.013
		Chaff		0.25	0.004
		Stalks/leaves		0.51	0.025
	Turnip	Leaves	195	0.09	0.023
		Bulbs		0.03	0.015
370	Wheat	Seeds	469	0.06	0.005
		Chaff		0.1	0.003
		Stalks/leaves		0.11	0.017
	Turnip	Leaves	469	0.03	0.064
		Bulbs		0.02	0.026

PBI Plant back interval in days (time interval between treatment and planting)
DALT Days after treatment
AR Applied radioactivity
TRR Total radioactive residue

The radioactive residues detected in soil at the time points of planting and harvest are shown in Table 6.6.1-36.

At zero-time, all of the radioactive residue was confined to the top 7.5 cm of soil. The amount of material present in this top 7.5 cm was equal to 4.59 mg/kg glyphosate-trimesium or 3.15 mg/kg glyphosate. Averaged through the whole pot of soil (0-15 cm depth) the 0-day residue was 1.58 mg/kg glyphosate equivalents.

The soil residue declined from 1.58 to 0.68 mg/kg, representing a decline of 57 % over the course of the study (496 days). This is probably due to soil microbial degradation of glyphosate-trimesium.

Dissipation by leaching was not a significant mode for loss of the radiolabel from the soil. Analysis of the soil cores (0 - 7.5 cm and 7.5 – 15 cm depth) at each aging interval revealed, that most of the residues remained in the treated layer (0 - 7.5 cm) at all sampling intervals. However, significant residues were found in the lower layer (7.5 – 15 cm). These were probably not due to leaching, but rather to contamination from the treated layer because it was impossible to totally separate the two layers.

The amount of radioactivity found in the leachate was insignificant, indicating that movement of the chemical through the soil did not occur.

Table 6.6.1-36: Total radioactive residues in soil after application of 14C-PMG labelled glyphosate-trimesium to bare soil

Rotation, PBI	DALT	Pot [1]	TRR, mg/kg		
			Soil core (0.0 - 7.5 cm)	Soil core (7.5 - 15 cm)	Mixed soil
Before application	**0**		**3.15**	n.a.	**1.58**
1st rotation, PBI 35 days	**35**	1	2.04	0.45	1.43
		2	2.76	0.566	1.23
		Mean	**2.4**	**0.508**	**1.33**
2nd rotation, PBI 95 days	**95**	1	1.91	1.73	1.34
		2	1.84	0.534	1.25
		Mean	**1.89**	**1.13**	**1.30**
harvest of wheat **(1st rotation)**	133	1	n.a.	n.a.	0.96
		2	n.a.	n.a.	n.a.
		Mean	n.a.	n.a.	--
harvest of wheat and turnips **(2nd rotation)**	195	1	n.a.	n.a.	1.1
		2	n.a.	n.a.	0.64
		Mean	n.a.	n.a.	**0.87**
harvest of turnips **(1st rotation)**	257	1	n.a.	n.a.	n.a.
		2	n.a.	n.a.	0.52
		Mean	n.a.	n.a.	**--**
3rd rotation, PBI 370 days	**370**	1	1.43	0.23	0.878
		2	1.46	0.091	0.727
		Mean	**1.45**	**0.161**	**0.803**
harvest of wheat and turnips **(3rd rotation)**	469	1	n.a.	n.a.	0.64
		2	n.a.	n.a.	0.71
		Mean	n.a.	n.a.	**0.68**

PBI Plant back interval in days (time interval between treatment and planting)
DALT Days after treatment
TRR Total radioactive residue, expressed in glyphosate equivalents
n.a. Not analysed The pots of soil were aged outdoors for three intervals of 35, 95 or 370 days
[1] Pot 1: wheat, pot 2: turnips

B. Extraction and characterisation of residues

Determination of the extractability of radioactive residues from soil with different solvents resulted in an extractability of >95 % with 1 M HCl, <5 % with distilled water, <5 % with 1 M NH_4Cl and >90 % with 0.5 M NaOH. Therefore, radioactive residues were extracted with 1 M HCl.
Soil samples, taken prior to planting of the crops from all six pots treated with radiolabelled test item, were separately extracted. The extracts contained large quantities of substances, presumably dissolved soil organic matter, which interfered with TLC analysis. Attempts were made to purify the extracts using first a C-18 column and then a cation-exchange batch method. The efforts to purify the material resulted in losses which left about 25 % of the original extracted radioactivity. TLC analysis of one of the resulting "purified" extracts was not successful in characterizing soil metabolites because almost all of the radioactivity remained at the origin of the plate. In many other cases that are not shown in the report, streaking over the length of the plate occurred and it was assumed, that co-extractants interfered with this chromatography.
AMPA and the ^{14}C-glyphosate chromatographed as expected when they were run alone in the TLC systems. However, when AMPA or glyphosate standards were used to spike the purified soil extracts, the

standards did not move from the origin. Therefore the authors concluded, that the soil extracts contained some components which complexed with glyphosate-trimesium and its metabolites and prevented analysis by TLC. No further attempts for analyses of components in soil were performed.

Because of the comparatively low level of radioactivity in plant samples of wheat and turnips, and previous experience with the difficulty of purifying the ^{14}C-glyphosate and its metabolites from soil the authors stated that no characterisation of the residue in these samples was going to be successful. No attempts at analysis of the crop samples was made.

C. Storage stability

Throughout the study all samples of crops and soils, were stored in freezers usually at -10°C. No storage stability investigations were performed. The storage duration is not indicated in the report. Harvest of crop samples was between 24.03.1983 and 23.02.1984. The study was completed on 08.10.1985 and finalised on 27.07.1989. Thus the maximum storage time was 929 days.

III. Conclusion

N-(phosphono-^{14}C-methyl)glycine as its trimesium salt (^{14}C-glyphosate-trimesium) was incorporated into a loamy sand soil at a concentration of 4.6 mg/kg glyphosate-trimesium or 3.15 mg/kg of glyphosate. The ^{14}C-glyphosate concentration declined gradually to leave a ^{14}C residue of 1.45 mg/kg over a 370 day interval determined in the soil layer of 0 - 7.5 cm.

Wheat and turnips were planted in the treated soil at intervals of 35, 95, and 370 days after treatment (plant back intervals (PBI): 35, 95 and 370 days) and harvested at maturity. Although considerable residues of ^{14}C-glyphosate remained in the soil at these planting times, uptake of radioactivity by these crops was comparatively low. For the commodities sampled from wheat (chaff, seeds and stalks/leaves), the total radioactive residues were comparable for samples taken from the first and the second rotation. The residues in seeds, chaff and stalks/leaves amounted to 0.25, 0.29 and 0.46 mg/kg (PBI 35 days) and to 0.28, 0.25 and 0.51 mg/kg (PBI 95 days). In samples from the third rotation (PBI 370 days), lower residue levels were detected, amounting to 0.06, 0.1 and 0.11 mg/kg for seeds, chaff and stalks/leaves, respectively. The radioactive residues in turnip leaves and bulbs were comparable (0.02 - 0.03 mg/kg for leaves and 0.02 - 0.03 mg/kg for bulbs) for samples of all three rotations, except for leaves of the second rotation (PBI 95 days), where radioactive residues amounted to 0.09 mg/kg.
The radioactive residues in the plant matrices were not extracted, so no characterisation or identification of radioactive residues was performed.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The study assessed the uptake of glyphosate in rotational crops of turnips and wheat. It was performed under GLP. The study has significant deviations when compared with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Metabolism in Rotational Crops, 502:
No representative leafy vegetable crop was included in the study.
The radiochemical purity of the application solution was <95 %; no specifications of the impurities were given.
Developmental stages of the crops at planting and harvesting are not reported, though they could be roughly estimated based on sampling dates. No information on storage duration of plant samples and dates of analyses is given. The storage stability is not covered.
No attempts were made to identify or characterise the radioactive residues in the plant matrices. Especially in wheat matrices (seed, chaff and stalks/leaves), comparatively high residue levels were detected amounting to up to 0.28 mg/ kg in wheat seeds, up to 0.29 mg/kg in wheat chaff and up to 0.51 mg/kg in wheat stalks/leaves. In turnip leaves residues were between 0.02 and 0.06 mg/kg.
The analysis of the control experiments was not reported.

The study is considered to provide only supporting uptake data for the assessment of the metabolic behavior of glyphosate in rotational crops. The study assessing the level of total residues of radioactive glyphosate equivalents in rotational crops (lettuce, wheat and radish) has been previously evaluated at EU level. It was performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Testing of Chemicals, 501 with deficits:

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.6.1/005
Report author	[redacted]
Report year	1978
Report title	Uptake and metabolism of Glyphosate in root, leaf and cereal type rotation crops
Report No	MSL-0882
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	• A review of this study indicates the following deviations from OECD Guideline for the Metabolism in Rotational Crops, 502: • The radiochemical purity of the test item(s) is not clearly specified. • Details on application (formulation on test item) are missing within the report. • Information about timing of the second treatment (emergency crop) is missing. • Developmental stages of the crop at harvesting are not reported. • Harvest samples of wheat were not separated into grain and straw, no intermediate samples (green material) were collected; only results for the whole plant at harvest are available.

	• Details about sampling of cabbage is missing, it is assumed that the whole plant was sampled. • No information on the storage stability for all major components of the total radioactive residues. Storage conditions and duration of plant samples is not given. • Date of analysis is missing within the report. • Extraction rates are 32 – 76 %. The water extracts were only analysed by ion-exchange chromatography. No attempts were made to characterise the bound radioactivity. • The water extracts of the primary crop soybean were not further analysed by ion-exchange chromatography. • Identification of glyphosate and AMPA was done by comparison with elution volumes of respective standards; no additional analytic method was established. • Unextracted radioactive residues for each sample not precisely quantified (not analysed by combustion/further extraction, only calculated from TRR - ERR). • No quantification of the residues as concentration (mg/kg, as active ingredient equivalents) in the original sample matrix analysed (re-calculation possible) • No flow chart depicting the overall extraction and fractionation strategies employed for each sample matrix analysed.
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Invalid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The uptake and metabolism of glyphosate was examined in rotational crops. Glyphosate radio-labelled with ^{14}C in the methyl position [*N*-(phosphono-^{14}C-methyl)glycine, called ^{14}C-glyphosate in this summary] was applied to sandy loam soil in pots at a rate of 4.48 kg a.s./ha. Primary crops of soybean, cabbage, wheat and beet, were planted 3 days after application. In parallel, unlabelled glyphosate was applied to identical pots for control purposes and kept in the same greenhouse to account for $^{14}CO_2$ fixation from degradation in soil. After harvesting the primary crop, different succeeding crops were planted at plant-back intervals of 30, 120 or 365 days. To simulate crop failure, some of the pots containing the primary crop received a second treatment of 4.48 kg a.s./ha and were replanted with the same crops as before (except for soybean where beet was replanted).

Table 6.6.1-37: Overview of the different scenarios of crop rotation

Scenario type	Primary crop	Emergency crop (PBI 30 [1])	Four months rotation (PBI 120 [1])	One year rotation (PBI 365 [1])
A	Soybean	Beet	Beet	Cabbage
B	Cabbage	Cabbage	Wheat	Beet
C	Wheat	Wheat	Beet	Cabbage
D	Beet	Beet	Cabbage	Wheat

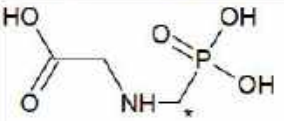
PBI Plant back interval in days (time interval between treatment and planting)
1 Days after soil treatment

Radioactive residues were extracted with water from respective plant materials. The extractability varied from 32 % TRR (beet, foliage, first rotation) to 76 % TRR (cabbage, third rotation). The extracts were analysed by ion-exchange column chromatography. Further ^{14}C-activity remained bound to the column. No further attempts were performed to resolublise these bound residues. In addition to the ^{14}C-activity that does not elute, many ^{14}C-products with elution patterns similar to glyphosate and AMPA were observed. No further investigations were conducted to identify those other ^{14}C-products.

Concerning radioactive residues found there was a reduction in the amount of uptake of ^{14}C-activity with time. There was no increase in the uptake of ^{14}C-activity in any of the rotation crops with the exception for emergency crops beet and cabbage, where a slight increase in TRRs was determined. The rotational crops from the 30 PBI scenario contained 0.002 – 0.018 mg/kg of glyphosate and 0.003 – 0.041 mg/kg AMPA, only for wheat residues were higher (0.046 mg/kg for glyphosate and 0.128 mg/kg for AMPA). Residues from respective plant materials for the 120 PBI decreased to <0.001 – 0.014 mg/kg for glyphosate and 0.001 – 0.010 mg/kg for AMPA. Residues from respective plant materials for the 365 PBI further decreased to <0.001 – 0.004 mg/kg for glyphosate and <0.001 – 0.004 mg/kg for AMPA.

I. Materials and Methods

A. Materials

1. Test material	N-(phosphono-^{14}C-methyl)glycine (namely ^{14}C-glyphosate within this summary)
Chemical structure:	 * Position of radiolabel
Radiochemical purity:	No further information within the report
Chemical purity	98 – 99 %
Specific activity of the test substance applied:	0.42 MBq/mg (1.9 mCi/mmol)
CAS No:	1071-83-6 (glyphosate acid)
Log P_{ow} for glyphosate:	- 3.2

2. Test material	
Soil:	Litonia sandy loam (pH: 6.5; 0.6 % organic matter, 86.0 % sand, 11.0 % silt, and 1.8 % clay)
Crop:	Primary crop: Soybean, beet, wheat and cabbage (no information on variety) Rotational crops: Beet, cabbage and wheat (no information on variety)
Botanical name:	*Glycine max* *Beta vulgaris* var. *vulgaris* *Triticum aestivum* *Brassica oleracea* var. *capitata*
Crop part(s):	Soybean (foliage, pod), cabbage (whole plant), wheat (whole plant), beet (foliage, root)

B. Study design

1. In-life phase

The test substance contained ^{14}C-glyphosate with a specific activity of 0.42 MBq/mg (1.9 mCi/mmol). Detailed information on formulation of the test item is missing. ^{14}C-glyphosate was applied to the test pots (diameter of ~20 cm) at target rates of 4.48 kg a.s./ha on bare soil. The remaining pots were treated with an equivalent amount of unlabelled herbicide (^{12}C-glyphosate) to serve as controls. These control pots were maintained in the same greenhouse in order to differentiate between radioactivity taken up by the roots of the plants from the soil and photosynthetic fixation of $^{14}CO_2$ liberated by soil degradation of ^{14}C-glyphosate.

Three days after treatment, ^{14}C-treated and control pots were planted with wheat, beet, soybeans, or transplanted cabbage plants. When growth was assured, the wheat was thinned to 15 plants, cabbage to one, and beet and soybeans to two plants per pot. The primary crop plants were grown in the greenhouse, watered as needed from the bottom, and fertilised monthly (15 mL of a solution of 24.0 g of Rapid Grow in 4.5 L of water). After the harvest of the primary crops, the soil surface of the pots was lightly tilled by hand and planted with the rotational crops, beet replacing soybeans and wheat, cabbage replacing beet, and wheat replacing cabbage. One year after treatment, the soil surface was lightly tilled by hand and replanted with cabbage, beet, and wheat replacing the primary crops of soybean, wheat, cabbage, and beet, respectively. In the interim time between the plant back interval (PBI) 120 and PBI 365 rotation crops, the pots of soil were kept moist by watering and fertilising as needed.

For emergency crops a second treatment as described for the primary crops was conducted. Further information on timing of second application and formulation of test item for application is missing. Primary crops were harvested after 30 days and the pots replanted with emergency crops cabbage, beet and wheat. These crops are representative of the crops that would be planted after the failure of the initial crop. The crops grown for the first 30 days were not analysed.

Another scenario was established to expose wheat to $^{14}CO_2$. $^{14}CO_2$ was released from 3 mCi of ^{14}C-$NaCO_3$ contained in a vial in the glove bag by addition of an excess of H_3PO_4.

2. Sampling

The primary crops were harvested 90 days after planting, except soybean which was harvested 112 days after planting. Crops of the 2nd and 3rd rotation were harvested 120 days after planting with the exception of the 1 year rotational cabbage crop which were harvested 97 days after planting. Emergency crops were harvested 90 days after planting. Samples of soybean were separated into foliage and pod; samples of beet were separated into foliage and root. Details on sampling of cabbage and wheat are missing; it is assumed that the whole plants were sampled. The crop samples were rinsed to remove all adhering soil, weighed,

frozen, lyophilised, weighed, and ground to 40 mesh in a Wiley mill. Information on storage of crop samples until analysis is missing.

3. Analytical procedures

Total radioactive residues (TRR) in all plant samples were determined by liquid scintillation counting (LSC) following combustion. Plant samples were combusted to $^{14}CO_2$ and trapped with phenethylamine based counting cocktail. The plants were lyophilised and ground to 40 mesh; 80 to 120 mg of plant material was placed directly into a gelatine capsule for combustion by Peterson Automatic Combustion Apparatus (PACA).

The lyophilised, ground, plant samples from each treatment were pooled and aliquots were extracted with of deionised water for 2 hours for each crop sample. The extracts were centrifuged, decanted, measured, and aliquoted for LSC. The extracts were analysed by chromatography on a column of AG 1-X8 (200 – 400 mesh) resin in the bicarbonate form prepared by washing the corresponding chloride form of resin with a solution of NH_4HCO_3 (1 M) followed by deionised water to give a neutral eluent. The entire water extract was applied to the column and eluted with solution of NH_4HCO_3 (0.2 M). For LSC analysis 5 mL fractions were collected.

Characterisation of glyphosate and AMPA in the water extracts was done by comparing of the elution volumes with a glyphosate and AMPA mixture and extracts of untreated crops spiked with glyphosate and AMPA (see elution volumes in the following table). An estimation of levels of glyphosate and AMPA was made on the basis of the ^{14}C-activity eluting in those areas where glyphosate and AMPA were shown to elute. The chromatograms of the standards showed the presence of approximately 1.0 % of methylphosphonic acid, a known impurity of the ^{14}C-glyphosate preparation.

Table 6.6.1-38: Comparison of column elution volumes of AMPA and glyphosate

Aqueous plant extract	Elution volume (mL)	
	AMPA	Glyphosate
Solvent	185 – 204	224 – 270
Soybean	l32 – 198	233 – 320
Wheat	122 – 176	215 – 303
Beet foliage	173 – 240	240 – 302
Beet root	170 – 240	240 – 300
Cabbage	139 – 192	192 – 259

II. Results and Discussion

A. Total radioactive residues (TRRs)

There was a reduction in the uptake of ^{14}C-activity with time. In no case was there an increase in the uptake of ^{14}C in any of the rotation crops with the exception of emergency crops of beet and cabbage, where a slight increase in TRRs was determined. TRRs in the primary crops ranged from 0.03 to 0.26 mg/kg for the control and from 0.13 to 3.65 mg/kg for the treated plant samples. These TRRs decreased after the first rotation to 0.05 to 0.23 mg/kg for the control and to 0.18 to 1.31 mg/kg for the treated plant samples. After the second rotation the TRRs further decreased to 0.01 to 0.27 mg/kg for the control samples and to 0.05 to 1.12 mg/kg for the treated plant samples. After the third rotation values for control samples were found to be 0.01 to 0.05 mg/kg and for treated plant samples to be 0.03 to 0.19 mg/kg.

Within the report, values for control samples were subtracted from corresponding treated values. These corrected values can be found within the following table (last column). For further calculation purposes

within this summary, uncorrected values for treated samples were used without subtraction of corresponding control sample values.

Uptake by the treated wheat plants was exceptionally high. A possible explanation is that the pots contained no supports to keep the wheat plants from touching the treated soil. By allowing the wheat foliage to rest on the surface of the treated soil, some of the glyphosate or AMPA migth enter the wheat plants through the foliage.

For unknown reasons crop growth was very poor during this study, resulting in a low wet weight. Watering by sub-irrigation with city water was believed to have caused a salt concentration on the surface of the pots causing toxic conditions for the plants. Watering from the top of the pots in the usual manner could have caused a dilution of pesticide and could have resulted in a lower uptake of ^{14}C.

Table 6.6.1-39: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

Rotation	PBI (days)	Scenario type	Crop	Sampled commodity	Sampling (days after planting)	Sampling (DALT)	TRR (mg/kg)		
							control [1]	treated [1]	treated corrected for control [2]
Primary crop	- [3]	A	Soybean	Foliage	112	*115*	*0.15*	*0.21*	0.06
				Pod	112	*115*	*0.10*	*0.14*	0.04
		B	Cabbage	Whole plant	90	*93*	*0.03*	*0.13*	0.10
		C	Wheat	Whole plant	90	*93*	*0.26*	*3.65*	3.39
		D	Beet	Foliage	90	*93*	*0.10*	*0.46*	0.36
				Root	90	*93*	*0.06*	*0.31*	0.24
1st rotation (emergency crop) [4]	30	A	Beet	Foliage	90	*123* [5]	*0.05*	*0.32*	0.27
				Root	90	*123* [5]	*0.08*	*0.49*	0.41
		B	Cabbage	Whole plant	90	*123* [5]	*0.07*	*0.18*	0.11
		C	Wheat	Whole plant	90	*123* [5]	*0.23*	*1.31*	1.08
		D	Beet	Foliage	90	*123* [5]	*0.05*	*0.20*	0.15
				Root	90	*123* [5]	*0.07*	*0.37*	*0.29* [6]
2nd rotation (4 months rotation)	120	A	Beet	Foliage	120	*240*	*0.02*	*0.05*	0.03
				Root	120	*240*	*0.02*	*0.08*	*0.06* [6]
		B	Wheat	Whole plant	120	*240*	*0.27*	*1.12*	0.86
		C	Beet	Foliage	120	*240*	*0.03*	*0.08*	0.05
				Root	120	*240*	*0.02*	*0.10*	0.08
		D	Cabbage	Whole plant	120	*240*	*0.01*	*0.08*	0.07
3rd rotation (1 year rotation)	365	A	Cabbage	Whole plant	97	*462*	*0.01*	*0.03*	0.02
		B	Beet	Foliage	120	*485*	*0.01*	*0.05*	0.04
				Root	120	*485*	*0.01*	*0.05*	0.04
		C	Cabbage	Whole plant	97	*462*	*0.02*	*0.06*	0.04
		D	Wheat	Whole plant	120	*485*	*0.05*	*0.19*	0.14

Table 6.6.1-39: Total radioactive residues in rotational crops planted after application of ^{14}C-glyphosate to bare soil

PBI Plant back interval (time between application onto bare soil and planting of crop)
DALT Days after last treatment (calculated as sum of days (given for PBI) + days (given for sampling days after planting) + 3 days (only for primary and emergency crop))
TRR Total radioactive residue
1 Calculated mean value of 2 replicates (4 replicates for first rotation)
2 Calculated within the report by subtraction of the control value from the respective treated value.
3 Primary crops were planted three days after treatment of test item onto bare soil.
4 Emergency crop after additional application of 4.48 kg a.s./ha. Information on exact time schedule is missing. After harvest of primary crop, primary plant parts were discarded without further analysis.
5 A second treatment was done to the pots before planting the emergency crop. The calculated DALT refers to the first treatment as information on timing of the second treatment is missing.
6 These values were recalculated as the mean values given in the report did not fit to the single values.
Italic figures were not part of the report, but correspond to values calculated upon figures given in the report.

B. Extraction and characterisation of residues

Plants containing ^{14}C-activity resulting from the soil treatment were analysed for extractability using water as the solvent for all matrices. The extractability for the treated samples varied from 32 % (beet, foliage, first rotation) to 76 % TRR (cabbage, third rotation, see Table 6.6.1-40 - Table 6.6.1-44). The extractability for the control samples varied from 29 % (wheat, first rotation) to 79 % TRR (cabbage, third rotation).
The extract was separated by an ion-exchange column (AG 1-X8 resin, HCO_3- form) into fractions representing glyphosate and AMPA and analysed by LSC.
For the control and treated samples, the recoveries of radioactivity after chromatography were between 14 and 88 %; further ^{14}C activity remained bound to the column. No further attempts were made to resolubilise these bound residues. The chromatograms of the control crops served as comparison and showed the presence of ^{14}C-products resulting from $^{14}CO_2$ fixation which have an elution pattern similar to AMPA and glyphosate. No further information on these ^{14}C-products is given within the report.

Soybean, foliage and pods
For soybean, foliage and pods a TRR of 0.21 mg/kg and 0.14 mg/kg was found, respectively. After extraction with water 33 % and 37 % of the TRR were found in the water extracts. The chromatograms of the extracts of the control and treated soybean crops, showed ^{14}C-activity eluting in the glyphosate and AMPA regions. Detailed information on chromatographic results on these sample materials are not reported.

Cabbage
For cabbage a TRR of 0.13 mg/kg was found in the primary crop. The TRR increased slightly in the emergency crop (0.18 mg/kg) and then decreases to 0.08 mg/kg for PBI 120 days and finally decreased to 0.03 – 0.06 mg/kg for the PBI 365 days. After extraction with water between 48 % and 76 % of the TRR were found in the water extracts. The water extract contained 3.9 – 10.0 % (0.002 – 0.008 mg/kg) glyphosate and 1.7 – 6.7 % (0.002 – 0.005 mg/kg) AMPA. The chromatograms of the control rotational crops (PBI 120 and 365) showed the presence of ^{14}C-products resulting from $^{14}CO_2$ fixation which have an elution pattern similar to AMPA and glyphosate. No further information on these ^{14}C-products is given within the report.

Wheat
For wheat, whole plant a TRR of 3.65 mg/kg was found in the primary crop. The TRR decreased in the emergency crop (1.31 mg/kg) and then decreases to 1.12 mg/kg for PBI 120 days and finally decreased to 0.19 mg/kg for the PBI 365 days. After extraction with water between 48 % and 64 % of the TRR were found in the water extracts. The water extracts contained 0.5 – 9.9 % TRR (or <0.001 – 0.362 mg/kg) glyphosate and 0.3 – 9.8 % TRR (or <0.001 – 0.128 mg/kg) AMPA. The chromatograms displayed a broad range of ^{14}C-natural products, some of which eluted in the same fraction as AMPA or glyphosate. The found levels for AMPA or glyphosate were much higher than those seen in cabbage and beet. In this

study it was difficult to grow wheat and to keep the plants from bending over and touching the soil. Harvested wheat was very dry resulting in a disproportionately high mg/kg value. The uptake of glyphosate was greater than AMPA with the exception of the emergency crop in which the uptake of AMPA was approximately three times the uptake of glyphosate. Comparison of the chromatographic traces shown indicates there were no unusual residues resulting from crop rotation.

Beet, foliage

For beet foliage a TRR of 0.46 mg/kg was found in the primary crop. The TRR decreased in the emergency crop (0.20 – 0.32 mg/kg) and then decreases to 0.05 – 0.08 mg/kg for PBI 120 days and finally decreased to 0.05 mg/kg for the PBI 365 days. After extraction with water between 32 % and 52 % of the TRR were found in the water extracts. The water extracts contained 1.0 – 2.0 % TRR (or <0.001 – 0.008 mg/kg) glyphosate and 1.3 – 4.6 % TRR (0.001 – 0.021 mg/kg) AMPA. The chromatographic analysis indicated there was a broad range of natural products arising from $^{14}CO_2$ fixation. The chromatograms of the emergency replant showed a narrow ^{14}C-peak eluting earlier than AMPA, and the corresponding control samples also showed ^{14}C-activity eluting in the same area. In all cases this unidentified peak corresponded to less than 7 % of the plant contained activity.

Beet, roots

For beet root a TRR of 0.31 mg/kg was found in the primary crop. The TRR increased in the emergency crop (0.37 – 0.49 mg/kg) and then decreases to 0.08 – 0.10 mg/kg for PBI 120 days and finally decreased to 0.05 mg/kg for the PBI 365 days. After extraction with water between 41 % and 58 % of the TRR were found in the water extracts. The water extracts contained 2.0 – 7.1 % TRR (or 0.002 – 0.022 mg/kg) glyphosate and 8.0 – 12.5 % TRR (or 0.004 – 0.041 mg/kg) AMPA. Within the chromatograms of the control samples there was a wide range of natural products containing ^{14}C-activity with the bulk of this activity eluting prior to the AMPA and glyphosate. The chromatographic patterns of the extracts of the treated samples were similar over the complete spectrum of different rotation scenarios. All of the extracts of the treated beet roots contained more activity in the region where AMPA eluted-than in the region where glyphosate eluted. The maximum level of glyphosate was 0.022 mg/kg, while the highest AMPA level seen was 0.041 mg/kg. There was no evidence of the occurrence of the formation of different degradation products as a function of time or the rotation of crops.

Table 6.6.1-40: Extraction of the radioactive residues of glyphosate in soybean (foliage and pods) as rotational crop planted after application of glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha

Experiment	**Soybean, foliage**		**Soybean, pods**	
PBI	Primary crop		Primary crop	
Sampling (DALT)	115		115	
Scenario type	A		A	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
TRR	0.21	100	0.14	100
Extraction with water				
Water extract	*0.069*	33	*0.053*	38
Characterised	*0.069*	33	*0.053*	38
ERR	*0.069*	33	*0.053*	38
RRR (unextracted)	*0.141*	*67*	*0.087*	*62*
Total	0.21	100	0.14	100

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
n.a. Not analysed
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding.

Table 6.6.1-41: Extraction of the radioactive residues of glyphosate in cabbage as rotational crop planted after application of glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha (two applications for emergency crops, final dose rate 8.96 kg/ha)

Experiment	Cabbage									
PBI	Primary crop		30 days		120 days		365 days		365 days	
Sampling (DALT)	93		123		240		462		462	
Scenario type	B		B		D		A		C	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.13	100	0.18	100	0.08	100	0.03	100	0.06	100
Extraction with water										
Water extract	*0.066*	51	*0.086*	48	*0.046*	58	*0.015*	57	*0.046*	76
Glyphosate	0.008	*6.2*	0.007	*3.9*	0.008	*10.0*	0.002	*6.7*	0.004	*6.7*
AMPA	0.005	*3.8*	0.003	*1.7*	0.005	*6.3*	0.002	*6.7*	0.002	*3.3*
Identified	*0.013*	*10.0*	*0.010*	*5.6*	*0.013*	*16.3*	*0.004*	*13.3*	*0.006*	*10.0*
Characterised	*0.053*	*41.0*	*0.076*	*42.4*	*0.033*	*41.8*	*0.013*	*43.7*	*0.040*	*66.0*
ERR	*0.066*	51	*0.086*	48	*0.046*	58	*0.017*	57	*0.046*	76
RRR (unextracted)	*0.064*	*49*	*0.094*	*52*	*0.034*	*42*	*0.013*	*43*	*0.014*	*24*
Total	0.13	100	0.18	100	0.08	100	0.03	100	0.06	100

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (mg/kg values were calculated as sum of identified and characterised)
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
Identified was calculated as sum of glyphosate and AMPA; Characterised was calculated as water extract – identified.
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding.

Table 6.6.1-42: Extraction of the radioactive residues of glyphosate in wheat as rotational crop planted after application of glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha (two applications for emergency crops, final dose rate 8.96 kg/ha)

Experiment	Wheat							
PBI	Primary crop		30 days		120 days		365 days	
Sampling (DALT)	93		123		240		485	
Scenario type	C		C		B		D	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	3.65	100	1.31	100	1.12	100	0.19	100
Extraction with water								
Water phase	*2.081*	57	*0.629*	48	*0.717*	64	*0.078*	41
Glyphosate	0.362	*9.9*	0.046	*3.5*	0.014	*1.3*	<0.001	*0.5*
AMPA	0.116	*3.2*	0.128	*9.8*	0.003	*0.3*	<0.001	*0.5*
Identified	*0.478*	*13.1*	*0.174*	*13.3*	*0.017*	*1.5*	*0.002*	*1.1*
Characterised	*1.603*	*43.9*	*0.455*	*34.7*	*0.700*	*62.5*	*0.076*	*39.9*
ERR	*2.081*	57	*0.629*	48	*0.717*	64	*0.078*	41
RRR (unextracted)	*1.570*	*43*	*0.681*	*52*	*0.403*	*36*	*0.112*	*59*
Total	3.65	100	1.31	100	1.12	100	0.19	100

Table 6.6.1-42: Extraction of the radioactive residues of glyphosate in wheat as rotational crop planted after application of glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha (two applications for emergency crops, final dose rate 8.96 kg/ha)

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (mg/kg values were calculated as sum of identified and characterised)
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
Identified was calculated as sum of glyphosate and AMPA. Characterised was calculated as water extract – identified.
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values below 0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-43: Extraction of the radioactive residues of glyphosate in beet, foliage as rotational crop planted after application of glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha (two applications for emergency crops, final dose rate 8.96 kg/ha)

Experiment	**Beet, foliage**											
PBI	Primary crop		30 days		30 days		120 days		120 days		365 days	
Sampling (DALT)	93		123		123		240		240		485	
Scenario type	D		A		D		A		C		B	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.46	100	0.32	100	0.20	100	0.05	100	0.08	100	0.05	100
Extraction with water												
Water extract	*0.216*	47	*0.102*	32	*0.082*	41	*0.019*	38	*0.041*	51	*0.026*	52
Glyphosate	0.008	*1.7*	0.005	*1.6*	0.002	*1.0*	<0.001	*2.0*	<0.001	*1.3*	<0.001	*2.0*
AMPA	0.021	*4.6*	0.006	*1.9*	0.004	*2.0*	0.001	*2.0*	0.001	*1.3*	0.002	*4.0*
Identified	*0.029*	*6.3*	*0.011*	*3.4*	*0.006*	*3.0*	*0.002*	*4.0*	*0.002*	*2.5*	*0.003*	*6.0*
Characterised	*0.187*	*40.7*	*0.091*	*28.6*	*0.076*	*38.0*	*0.017*	*34.0*	*0.039*	*48.5*	*0.023*	*46.0*
ERR	*0.216*	47	*0.102*	32	*0.082*	41	*0.019*	38	*0.041*	51	*0.026*	52
RRR (unextracted)	*0.244*	*53*	*0.218*	*68*	*0.118*	*59*	*0.031*	*62*	*0.039*	*49*	*0.024*	*48*
Total	0.46	100	0.32	100	0.2	100	0.05	100	0.08	100	0.05	100

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (mg/kg values were calculated as sum of identified and characterised)
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
Identified was calculated as sum of glyphosate and AMPA. Characterised was calculated as water extract – identified.
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values below 0.001 mg/kg were set as 0.001 mg/kg.

Table 6.6.1-44: Extraction of the radioactive residues of glyphosate in beet, root as rotational crop planted after application of glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha (two applications for emergency crops, final dose rate 8.96 kg/ha)

Experiment	Beet, root											
PBI	Primary crop		30 days		30 days		120 days		120 days		365 days	
Sampling (DALT)	93		123		123		240		240		485	
Scenario type	D		A		D		A		C		B	
Fraction	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
TRR	0.31	100	0.49	100	0.37	100	0.08	100	0.10	100	0.05	100
Extraction with water												
Water extract	*0.174*	56	*0.265*	54	*0.170*	46	*0.032*	40	*0.041*	41	*0.029*	58
Glyphosate	0.022	*7.1*	0.018	*3.7*	0.012	*3.2*	0.002	*2.5*	0.002	*2.0*	0.002	*4.0*
AMPA	0.030	*9.7*	0.041	*8.4*	0.036	*9.7*	0.010	*12.5*	0.008	*8.0*	0.004	*8.0*
Identified	*0.052*	*16.8*	*0.059*	*12.0*	*0.048*	*13.0*	*0.012*	*15.0*	*0.010*	*10.0*	*0.006*	*12.0*
Characterised	*0.122*	*39.2*	*0.206*	*42.0*	*0.122*	*33.0*	*0.030*	*25.0*	*0.031*	*31.0*	*0.023*	*46.0*
ERR	*0.174*	56	*0.265*	54	*0.170*	46	*0.032*	40	*0.041*	41	*0.029*	58
RRR (unextracted)	*0.136*	*44*	*0.225*	*46*	*0.200*	*54*	*0.048*	*60*	*0.059*	*59*	*0.021*	*42*
Total	0.31	100	0.49	100	0.37	100	0.08	100	0.1	100	0.05	100

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue (mg/kg values were calculated as sum of identified and characterised)
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
Identified was calculated as sum of glyphosate and AMPA. Characterised was calculated as water extract – identified.
Values calculated upon dossier compilation are presented in italics. Minor deviations may occur due to rounding. Values below 0.001 mg/kg were set as 0.001 mg/kg.

C. Storage stability

Within the report information on storage duration and conditions is missing. The date of analysis is not indicated within the report.

D. Degradation pathway

Degradation pathway of glyphosate in rotational crops will be provided at the end of this chapter.

III. Conclusion

The metabolism of glyphosate was examined in rotational crops. Glyphosate radio-labelled with ^{14}C in the methyl position [N-(phosphono-^{14}C-methyl)glycine, called ^{14}C-glyphosate in this summary] was applied to sandy loam soil in pots at a rate of 4.48 kg a.s./ha. Primary crops of soybean, cabbage, wheat and beet, were planted 3 days after application. In parallel, unlabelled glyphosate was applied to identical pots for control purposes and kept in the same greenhouse to account for $^{14}CO_2$ fixation from degradation in soil. After harvesting the primary crop, different succeeding crops (cabbage, wheat and beet) were planted at plant back intervals of 30, 120 or 365 days. To simulate crop failure, some of the pots containing the primary crop received a second treatment of 4.48 kg a.s./ha after 30 days and were replanted with the same crops as before (except for soybeans where beet were replanted). Extraction of sample materials with water released 32 – 76 % TRR; no further attempts were performed to resolublise the bound residues. The low recoveries of radioactivity after chromatography may be reflected by the fact that applied radioactivity had been incorporated via $^{14}CO_2$ fixation into a variety of natural products which remained bound to the column. In addition to the uneluted ^{14}C-activity, many ^{14}C-products with elution patterns similar to glyphosate and AMPA were observed. No further investigations were conducted to identify those ^{14}C-products. Concerning radioactive residues found there was a reduction in

the uptake of ^{14}C-activity with time. There was no increase in the uptake of ^{14}C in any of the rotation crops with the exception for emergency crops beet and cabbage, where a slight increase in TRRs was determined. The rotational crops from the 30 PBI scenario contained 0.002 – 0.046 mg/kg of glyphosate and 0.003 – 0.128 mg/kg AMPA. Residues from respective plant materials for the 120 PBI and 365 PBI decreased to <0.001 – 0.004 mg/kg for glyphosate and 0.001 – 0.004 mg/kg for AMPA.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study assessing the metabolic behaviour of glyphosate in rotational crops cabbage, wheat and beet has been previously evaluated at EU level. It was not performed under GLP. The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Metabolism in Rotational Crops, 502 with major deficits: The radiochemical purity of the test item is not clearly specified and details on applications (formulation on test item and timing) are missing within the report. Information about timing of the second treatment (emergency crop) is missing. Developmental stages of the crop at harvesting are not reported. Harvest samples of wheat were not separated into grain and straw, no intermediate samples (green material) were collected; only results for the whole plant at harvest are available. Details about sampling of cabbage is missing, it is assumed that the whole plant was sampled. No information is provided on the storage stability for all major components of the total radioactive residues. Storage conditions and duration of plant samples is not given. Date of analysis is missing within the report. Extraction rates were low (32 – 76 %). The water extracts were only analysed by ion-exchange chromatography. No attempts were made to characterise the bound radioactivity. Analysis of primary crop soybean for glyphosate and AMPA was not conducted. Identification of glyphosate and AMPA was done by comparison with elution volumes of respective standards; no additional analytic method was established. Unextracted radioactive residues for each sample were not precisely quantified (not analysed by combustion/further extraction, only calculated from TRR - ERR).
The study is considered as not reliable for the assessment of the metabolic behaviour of glyphosate in rotational crops.

Assessment and conclusion by RMS:

Study previously submitted to the EU

1. Information on the study

Data point:	CA 6.6.1/006
Report author	[redacted]
Report year	1976
Report title	Uptake and Metabolism of CP 67573 in representative vegetables and rotation crops
Report No	406
Document No	
Guidelines followed in study	Not specified
Deviations from current test guideline	A review of this study indicates the following deviations from OECD Guideline for the Metabolism in Rotational Crops, 502: • Not a typical rotational crop study regarding number of rotations and rotational intervals, only one rotation was conducted (PHI 29-101). • Information about application method and formulation of test

	item is missing. • Developmental stages of the crops at application and harvesting are not reported, but could be roughly estimated based on planting and sampling dates. • Physical facility and environmental conditions not described • No information on the storage stability. • No description of conditions and length of storage of samples. • Relevant amounts of residues remained in the extracts (>0.01 mg/kg, >10 % TRR) that were not further investigated. • Relevant amounts of non-extractable residues were not characterised / not investigated.
Previous evaluation	Yes, evaluated and accepted in the RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 2a

2. Full summary of the study according to OECD format

Executive Summary

The metabolism of glyphosate was examined in rotational crops. The uptake of glyphosate and/or its metabolites from soils into representative vegetables, carrots (root type), cabbage (leafy type), string beans and peas (both legumes) was investigated. In addition to the vegetables mentioned previously, sweet corn was used as one of the rotation crops. Plants were grown on two different soils, a sandy loam (Norfolk soil) and a silt loam (Ray soil). At the maximum plant growth of the primary crops, N-(phosphono-^{14}C-methyl)glycine (namely ^{14}C-glyphosate within this following summary) was applied to the bare soil at a rate of 4.48 kg a.s./ha. The primary crops were sampled 4 - 11 weeks after treatment. Rotational crops (same as primary plants plus sweet corn) were planted within a 1 - 23 day interval after harvest of the primary crops (PBI 29 - 79 / 101 days) and harvested 6.5 to 17.5 weeks after planting. In addition, soil samples were taken after harvest of the primary and of the rotational crops from each plot. The fate of glyphosate and its metabolites in soil in primary and in rotational crops was investigated.

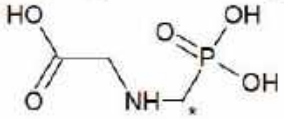
The radioactive residues were quantified by LSC following combustion. In primary crops the residues were higher for plants grown on Ray soil (silt loam; up to 1.07 mg/kg) than for plants grown on Norfolk soil (sandy loam; up to 0.22 mg/kg). The radioactive residues detected in rotational crops grown on the two different soils were comparable (all between about 0.040 and 0.280 mg/kg. The extractabilities of plant material with water were between 55.7 – 92.2 % TRR, except for some matrices of corn, carrots and bean leaves, from which lower amounts (23.8 – 51.6 % TRR) were extracted.

For crops grown on the sandy loam soil, glyphosate was the major component detected in the plant extracts of primary and rotational crops. Glyphosate was detected at to up to 0.137 mg/kg (primary crops) and up to 0.128 mg/kg (rotational crops). AMPA was less abundant in these extracts and was detected between 0.002 and 0.044 mg/kg. Components that were characterised upon their elution behaviour and are designated as “neutrals”, “others” or “indeterminates”, were detected at up to 0.037 mg/kg in the extracts of primary and rotational crops from Norfolk soil.
In plant extracts from primary crops grown on the silt loam soil, the amounts of AMPA (found at up to 0.041 mg/kg), were generally about twice as high as the concentrations of glyphosate. Glyphosate was not present in the extracts of rotational crops and AMPA was found at only low amounts (up to 0.004 mg/kg). The major part of radioactive residues were neutrals and / or indeterminates, representing up to 0.140 mg/kg.

Analyses of the soil samples revealed that glyphosate was relatively stable in the sandy loam soil. About 82 % of the applied radioactivity was still left after 4 weeks and 27 % after 31 weeks. In contrast, in the silt loam soil about 70 % of the applied radioactivity dissipated within 4 - 7 weeks and after about 25 weeks ≤10 % of the applied radioactivity was detected. The main component in all extracts of the sandy loam soil was unchanged glyphosate (67 - 93 %), AMPA was detected between 4 - 23 %. For the silt loam soil, taken after 4 - 7 weeks about 20 % glyphosate and about 70 % AMPA were identified in the soil extracts, while after 24 weeks no glyphosate and about 40 % AMPA were detected.

I. Materials and Methods

A. Materials

1. Test material	N-(phosphono-^{14}C-methyl)glycine
Chemical structure:	
	* Position of radiolabel
Radiochemical purity:	>99.9 % after purification (by AG-50W-X8 chromatography)
Specific activityof the test substance applied:	Batch I: 1.98 MBq/mg (9.07 mCi/mmol) Batch II: 1.76 MBq/mg (8.03 mCi/mmol) Batch III: 0.41 MBq/mg (1.87 mCi/mmol)
CAS No:	1071-83-6
Log $P_{o/w}$ for glyphosate:	- 3.2
2. Test material	
Soil:	Ray silt loam (pH: 8.1; cation exchange capacity: 10.4 %; sand: 4.6 %; silt: 84.2 %; clay: 10.0 %, organic matter: 1.2 %) Norfolk sandy loam (pH: 5.7; sand: 86.0 %; silt: 11 %; clay: 2.3 %; organic matter: 1.0 %)
Crop:	Carrot (Nantes) Cabbage (Wisconsin Golden Acres cabbage) Peas (Alaska peas) String beans (bush type; Burpees' Stringless and Tendergreen varieties) Sweet corn (variety DeKalb XL-45)
Botanical name:	*Daucus carota* subsp. *sativus* *Brassica oleracea* var. *Capitata* *Pisum sativum* *Phaseolus vulgaris* *Zea mays*
Crop part(s):	String bean and pea (pods and leaves), carrot (roots and leaves), cabbage (head and leaves), corn (leaf, kernels, cob)

B. Study design

1. In-life phase

Three batches of ^{14}C-glyphosate were used in the study, having specific activities of 1.98 MBq/mg (9.07 mCi/mmol; batch I), 1.76 MBq/mg (8.03 mCi/mmol; batch II) and 0.41 MBq/mg (1.87 mCi/mmol; batch III).

For the experiments with peas, string beans and cabbage in Norfolk sandy loam soil, the treatment solution was a mixture of 80 % of batch III and 20 % of batch I. For experiments with carrots in sandy loam soil, a mixture of 55.7 % batch III, 24.7 % batch II and 19.6 % of non-radioactive glyphosate was used. For the treatment of Ray silt loam soil (growing of carrots), the mixture was comprised of 10.7 % batch I and 89.3 % batch III. For experiments with string beans, silt loam soil was treated entirely with batch III.

The soils of the test plots were treated on the surface with the ^{14}C-glyphosate in 0.1 M NH_4HCO_3 solution at target rates of 4.48 kg a.s./ha (4lbs/A) on bare soil which was equivalent to applying 7.8 mg ^{14}C-glyphosate per pot and 22.7 mg per bucket. Detailed information about application method and formulation of test item is missing within the study report.
For controls, similar crops were planted in corresponding containers in order to check for $^{14}CO_2$ fixation from the soil metabolism of the applied radiolabelled glyphosate and grown side by side with the treated crops. All plants were nourished with the modified Hoagland's solution about every 5 - 7 days.

The plants were grown in 15 cm (6 inch) diameter pots and/or 30.5 cm (12 inch) diameter buckets using sandy loam and silt loam soils. The soil of the test plots of the main crops (primary crops) were treated 16 - 96 days after planting, while the maximum growth of the plants was taking place. Treatments were carried out 18 days after planting the peas, 16 days for string beans, 81 days for carrots, and 53 - 60 days for cabbage for sandy loam soil and 15 days after planting the string beans and 81 -96 days after planting carrots for silt loam soil. The primary crops were harvested 4 - 11 weeks after treatment. Rotational crops were planted l - 23 days after harvesting the main crops. Planting of the primary and rotational crops was according to the following design:

Table 6.6.1-45: Overview of the different scenarios of crop rotation

Plot No.	Primary crop	Rotational crops
Norfolk soil (sandy loam)		
A	Pea	Carrot
		Cabbage
B	String bean	Corn, sweet
C	Carrot	Cabbage
		String bean
D	Cabbage	Carrot
		Pea
Ray soil (silt loam)		
E	String bean	Carrot
		Cabbage
F	Carrot	-

2. Sampling

Sampling of plants: The plants were cut off about 2.5 cm above the soil level and any visible soil and dirt were wiped off. String bean and pea pods were sampled separately from their respective leaves. Carrots were pulled out from the soil and washed in a series of three distilled water baths. In addition, the residual soils were removed with the aid of a toothbrush and water rinse.
Wet weights of each sample were taken. Cabbage and carrots were sliced. Samples were then frozen and lyophilised (freeze-dried) after freezing. After drying, the dry weights were determined and the samples were ground to 40 mesh size in a Wiley mill. Aliquots were combusted to determine the total ^{14}C-content.

Sampling of soils: Triplicate samples were taken from each pot and 5 - 7 samples from each bucket. Samples were taken with the aid of a cork borers, dug 8 - 9 cm deep into the soil. Each sample was placed in a tared vial, frozen and lyophilised.

In the following table the time intervals/ sampling times of treatment and harvest of primary crops, rotational crops and soil are given.

Table 6.6.1-46: Overview of the different scenarios of crop rotation

Rotation	Plot No.	Crop	Age of crop at treatment (days)	PBI (days)		Crop sampling (DALT)	Soil sampling (weeks [1])
				min	max		
Norfolk soil (sandy loam)							
Primary crop	A	Pea	18	-	-	28	4
	B	String bean	16	-	-	46	6.5
	C	Carrot	81	-	-	50	7
	D	Cabbage	53-60	-	-	78	11
Rotational crop	A	Carrot [2]	-	*29*	*51*	98	18
		Cabbage [2]	-	*29*	*51*	99	18
	B	Corn, sweet	-	*47*	*69*	70	16.5
						110	22
	C	Cabbage	-	*51*	*73*	45	13.5
		String bean	-	*51*	*73*	122	27
	D	Carrot	-	*79*	*101*	45	17.5
		Pea	-	*79*	*101*	122	31
Ray soil (silt loam)							
Primary crop	E	String bean	15	-	-	30	4
	F	Carrot	81-96	-	-	51	7
Rotational crop	E	Carrot	-	*52*	*74*	122	24.5
		Cabbage	-	*52*	*74*	122	24.5
	F	-	-	-	-	-	-

PBI Plant back interval in days (time interval between treatment and planting): PBI was calculated upon the statement in the report, that rotational crops were planted 1 - 23 days after harvesting the primary crops: DALT of primary crop + 1 day (min) or + 23 days (max). A more detailed calculation is not possible due to missing details within the report.
DALT Days after last application (called "duration of treatment" within the report)
1 Soil was sampled in different intervals after treatment, given in weeks.
2 Crops were transplanted to the treated soil at an age of 47 days of growing.
Italic figures were not part of the report, but correspond to values calculated upon figures given in the report.

3. Analytical procedures

Total radioactive residues (TRR) in the plant and soil samples were determined by combustion and LSC. To determine the radioactivities in soils and plant samples, the Petersen Automatic Combustion Apparatus (PACA) was used. This apparatus quantitatively oxidises carbon ^{14}C-containing materials to $^{14}CO_2$. For combustions, two homogeneous aliquots from the dried samples were weighed after determining the soil dry weights. The total ^{14}C-content of each pot/bucket was then calculated from the combustion results.

For extracting radioactive residues from vegetables, the ground plant samples were extracted for 2 hours with water. The remaining solids were removed by centrifugation. The extracts were assayed by liquid

scintillation counting (LSC). Water extracts from vegetable samples were analyzed by AG-50W-X8 and AG-1-X8 column chromatography.
In the analysis of crops from the silt loam soil, AG-50 column chromatography was not applicable. A large amount of so called "neutral materials" eluting in front of glyphosate overshadowed the glyphosate present such that partitioning between these two entities was not possible. Therefore analysis was carried out by HVE (high voltage electrophoresis) and AG-1-X8 (HCO_3^-) chromatography in these cases.

Soil samples were extracted for 2 hours with 0.5 M NH_4OH. Aliquots of the supernatant were concentrated under vacuum and analyzed by LSC and by AG-50W-X8 column chromatography as well as by HVE separately. Radioactive spots on TLC plates or paper electrophoretograms were located and quantified using the Beta Camera.

The assignments of glyphosate and AMPA was verified by analysis of reference items.

To verify the identity of glyphosate and its metabolite AMPA, the radioactive components were isolated from selected plant samples (peas, carrots and cabbage from the sandy loam soil and string beans from the silt loam soil). Different ion exchange and size exclusion chromatographic resins (AG-50W-X4, AG-1-X8, AG-50W-X8, Bio-Gel P-2) were used for purification of ^{14}C-glyphosate and ^{14}C-AMPA.
Depending on the purity of the compound being isolated, the sample was either derivatised or purified further by HVE, another AG-1-X8 column and finally AG-50W-X8 chromatography.
The purified samples were derivatised to give either trimethyl N-trifluoroacetyl glyphosate or dimethyl N-trifluoroacetyl AMPA followed by determination using GC with phosphorous specific detection (PFD). In addition, detection was by flame ionisation detection coupled with radioactive detection (FID/RAD) as well as GC-MS for metabolite identification. The identification was verified by comparison of the analyses performed with (derivatised) reference compounds.

II. Results and Discussion

A. Total radioactive residues (TRRs)

The results of the soil uptake experiment are summarised in **Error! Reference source not found.**. The values are given in % of the applied radioactivity (% AR) and in mg/kg of the total radioactive residues (TRR).

Regarding the primary crops, uptake from sandy loam soil ranged from 0.051 to 0.27 % of the applied radioactivity for treated plants. Control plants showed an uptake of 0.006 to 0.02 %. The uptake by rotational crops from sandy loam soil ranged from 0.023 to 0.26 % of the applied radioactivity. Comparatively high values were found in some of the control plants, reaching from 0.002 to 0.11 %.
Uptake from treated silt loam soil was found to be higher than in the sandy loam soil. The uptake ranged from 0.05 % for bean pods to 1.05 % for the bean leaves. Uptake of the control plants was 0.006 to 0.07 %. Rotational crops in the silt loam soil showed low residues (0.047 to 0.074 %); the control plants were all <0.01 % of the applied radioactivity.

After harvest, total radioactive residues (TRR) in the primary crop samples ranged from 0.080 to 1.070 mg/kg. The highest residue levels were detected in string bean leaves (1.07 mg/kg), carrot leaves (0.494 mg/kg) and carrot root (0.31 mg/kg) grown on silt loam soil. For crops grown on sandy loam soil, highest values were found in pea tops (0.22 mg/kg). Generally, residue levels were lower in the analysed plants from the sandy loam soil (see **Error! Reference source not found.**).

For rotational crops, the highest residue levels were detected in pea pods (0.28 mg/kg) and in pea leaves (0.19 mg/kg) grown on sandy loam soil. All other TRR values were between 0.039 mg/kg and 0.094 mg/kg for plants grown on either soil.

Table 6.6.1-47: Radioactivity found in primary crops and rotational crops after application of ^{14}C-glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha

Rotation	PBI (days)	Plot No.	Crop	Sampled commodity	Sampling (DALT)	Treated plants		Control plants (% AR)
						% AR	TRR (mg/kg)	
Norfolk soil (sandy loam)								
Primary crop	-	A	Pea	Tops	28	0.051	0.22	0.006
		B	String bean	Tops	46	0.13	0.13 [2]	0.02
				Pods		0.07		n.a.
		C	Carrot	Leaves	50	0.21	0.17	0.01
				Root		0.15	0.11	0.018
		D	Cabbage	Head	78	0.04	0.08 [1]	0.02 [1]
				Leaves		0.27		
Rotational crop	29-101	A	Carrot	Leaves	98	0.14	0.086	n.a.
				Root		0.16	0.094	n.a.
			Cabbage	Cabbage [1]	99	0.26	0.056	0.11
		B	Sweet corn	Leaves	70	0.14	0.04	0.12
				Leaves	110	0.11	0.09	0.11
				Kernel	110	0.016	0.06	0.022
				Cob	110	0.023	0.05	0.043
		C	Cabbage	Cabbage [1]	45	0.20	0.045	0.03
			String bean	Leaves	122	0.044	0.08	0.012
				Pods		0.016	0.04	0.003
		D	Carrot	Leaves	45	0.06	0.08	0.02
				Root		0.09	0.05	0.04
			Pea	Leaves	122	0.07	0.19	0.008
				Pods		0.046	0.28	0.004
Ray soil (silt loam)								
Primary crop	-	E	String bean	Leaves	30	1.05	1.07	0.07
				Pods		0.05	0.19	0.006
		F	Carrot	Leaves	51	0.516	0.494	0.039
				Root		0.57	0.31	0.027
Rotational crop	52-74	E	Carrot	Leaves	122	0.038	0.061	0.005
				Root		0.047	0.039	0.007
			Cabbage	Cabbage [1]	122	0.074	0.051	0.006
		F	-	-	-	-	-	-

PBI Plant back interval in days (time interval between treatment and planting): PBI was calculated upon the statement in the report, that rotational crops were planted 1 - 23 days after harvesting the primary crops: DALT of primary crop + 1 day (min) or + 23 days (max). A more detailed calculation is not possible due to missing details within the report.
DALT Days after last application (called "duration of treatment" within the report)
AR Applied radioactivity
TRR Total radioactive residue
n.a. Not analysed
1 Sampled commodity was designated as "cabbage"; information about separation of head and leaves is missing.
2 Sampled commodity was designated as "string bean"; information about separation of tops and pods is missing.

In Norfolk soil 81.86 % of the applied radioactivity was still left after 4 weeks and 27.07 % after 31 weeks. Ray soil showed a faster biodegradability and about 70 % of the applied radioactivity dissipated in

4 - 7 weeks. At the end of the experiment ≤10 % of the applied were detected in Ray soil. For further details see the following table.

Table 6.6.1-48: Radioactivity found in soil after application of ^{14}C-glyphosate to bare soil at a dose rate of 4.48 kg ^{14}C-glyphosate/ha

Rotation	Plot No.	Crop	Soil sampling (weeks [1])	% AR [2]
Norfolk soil (sandy loam)				
Primary crop	A	Pea	4	81.86
	B	String bean	6.5	71.66
	C	Carrot	7	67.8
	D	Cabbage	11	52.59
Rotational crop	A	Carrot	18	58.11
		Cabbage	18	62.82
	B	Sweet corn	16.5	40.36
			22	53.04
	C	Cabbage	13.5	28.65
		String bean	27	61.80
	D	Carrot	31	27.07
		Peas	17.5	57.43
Ray soil (silt loam)				
Primary crop	E	String bean	4	30.8
	F	Carrot	7	29.4
Rotational crop	E	Carrot	24.5	6.43
		Cabbage	24.5	10.00
	F	-	-	-

AR Applied radioactivity
1 Soil was sampled in different intervals after treatment, given in weeks.
2 % AR values were calculated within the report as mean values of several samples per pot/bucket; each taken sample was measured twice.

B. Extraction and characterisation of residues

The mean extractabilities of plant samples and the composition of radioactive components in these extracts are shown in Table 6.6.1-49 to Table 6.6.1-54.

Sandy loam soil: The extractabilities with water of plants grown on the Norfolk sandy loam soil were good (all between 57.5 and 92.2 % TRR). Only the extractabilities of some corn and carrot matrices were somewhat lower (39.9 to 59.4 % TRR). The values are depicted in Table 6.6.1-49 to Table 6.6.1-52.

Upon chromatography, two components, namely glyphosate and AMPA, were identified.
Glyphosate was the predominant compound in extracts from all matrices of the **primary crops** (pea tops, string beans, carrots and cabbage) from Norfolk soil, ranging from 0.026 to 0.137 mg/kg (30.2 to 62.1 % TRR). AMPA was detected in the range from 0.005 to 0.013mg/kg representing 3.2 to 7.0 % TRR. Further components that were designated as "neutrals", "others" and "indeterminates" were characterised by their chromatographic properties and amounted to up to 0.021 mg/kg (neutrals), 0.010 mg/kg (others) and up to 0.029 mg/kg (indeterminates). The RRR in primary crops was between 0.017 mg/kg (7.8 % TRR, pea tops) and 0.073 mg/kg (43.0 % TRR, carrot leaves).
Glyphosate was also the most abundant compound in **rotational crops** grown on Norfolk soil, accounting for 0.016 to 0.128 mg/kg (19.6 to 46.4 % TRR), with the exception of corn, carrot (after cabbage) and cabbage (after carrots). In these matrices, comparable amounts of glyphosate and AMPA were detected (0.002 to 0.008 mg/kg or 2.9 to 12.0 % TRR). In the latter matrices, components designated as "neutrals" represented the majority of radioactive residues. (0.005 to 0.022 mg/kg, 12.8 to 43.2 % TRR). Altogether, components in the extract designated as "neutrals", "others" and "indeterminates", that were not identified but characterised by their chromatographic properties were detected at up to 0.026 mg/kg

(neutrals) up to 0.013 mg/kg (others) and 0.037 mg/kg (indeterminates). The RRR in rotational crops was between 0.004 mg/kg or 9.7 % TRR (bean pods) and 0.055 mg/kg or 58.6 % TRR (carrots). No attempts were done to further solubilise this bound radioactivity.

Silt loam soil: The extractabilities with water of plants grown on the Ray silt loam soil were moderate (51.3 – 74.6 % TRR), with the exception of string bean leaves (primary crop) and carrot leaves (rotational crop), that had poor extractabilities with water (23.8 % and 35.8 %). The individual values are shown in Table 6.6.1-53 to Table 6.6.1-54.

In the analysis of extracts from Ray soil, AG-50 column chromatography was not applicable. The large amount of neutral materials, eluting in front of glyphosate overshadowed the amount of glyphosate present. Analysis was therefore carried out by HVE and AG-1 chromatography in these cases. Since uptake by rotation crops grown in Ray soil was very low, analytical problems were formidable, and only the AG-1 column chromatography was applicable.

In the extracts of **primary crops** grown on Ray soil (string beans and carrots), the amounts of glyphosate ranged from 0.008 to 0.047 mg/kg showing significantly lower percentages (1.0 to 9.0 % TRR) than plant extracts from the Norfolk soil (compare section above). AMPA was more prominent than glyphosate, amounting to 0.017 to 0.041 mg/kg (1.9 to 22.5 % TRR). Altogether, characterised components in the extract designated as “neutrals”, “others” and “indeterminates” were detected at amounts up to 0.140 mg/kg (neutrals), up to 0.037 mg/kg (others) and up to 0.130 mg/kg (indeterminates). The highest amount of neutrals (45.5 % TRR) was detected in the extract of carrots. The radioactive residues that were not extracted with water (RRR) were comparatively high and were detected between 0.080 mg/kg and 0.805 mg/kg (representing between 25.4 and 76.2 % TRR). No attempts were done to further identify this not extracted radioactivity.
In the extracts from **rotational crops** from Ray soil (cabbage and carrot) no glyphosate was detected, while AMPA ranged from 0.001 to 0.004 mg/kg (2.4 to 9.0 % TRR).
Components in the extracts designated as “neutrals”, “others” and “indeterminates”, that were not identified but characterised were detected at up to 0.019 mg/kg (neutrals) and up to 0.007 mg/kg (others and indeterminates). The residues not extracted with water (RRR) ranged between 0.013 and 0.430 mg/kg or 34.1 to 64.2 % TRR. No attempts were done to further identify this not extracted radioactivity.

Table 6.6.1-49: Distribution of radioactive residues of glyphosate and its metabolites in primary crop (pea) and rotational crops (cabbage, carrot) after application of glyphosate to Norfolk sandy loam soil

Scenario type A	Primary crop		Rotational crops					
	Pea tops		Cabbage		Carrot, root		Carrot, leaves	
DALT	28		99		98		98	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.22	100.0	0.056	100.0	0.094	100.0	0.086
Extraction with water								
Aqueous extract	92.2	0.203	91.8	0.051	41.4	0.039	45.7	0.039
Glyphosate	*62.1*	0.137	*46.4*	0.026	*19.6*	0.018	*21.1*	0.018
AMPA	*5.9*	0.013	*4.5*	0.002	*5.6*	0.005	*2.3*	0.002
Total identified	***68.0***	***0.150***	***50.9***	***0.028***	***25.2***	***0.024***	***23.4***	***0.020***
Neutrals	*6.2*	*0.014*	*15.9*	*0.009*	*12.5*	*0.012*	*14.0*	*0.012*
Others	*4.7*	*0.010*	*8.4*	*0.005*	*3.8*	*0.004*	*4.6*	*0.004*
Indeterminate	*13.4*	*0.029*	*16.7*	*0.009*	*n.d.*	*n.d.*	*3.3*	*0.003*
Total characterised	***24.2***	***0.053***	***40.9***	***0.023***	***16.2***	***0.015***	***21.8***	***0.020***

Table 6.6.1-49: Distribution of radioactive residues of glyphosate and its metabolites in primary crop (pea) and rotational crops (cabbage, carrot) after application of glyphosate to Norfolk sandy loam soil

Scenario type A	Primary crop		Rotational crops					
	Pea tops		Cabbage		Carrot, root		Carrot, leaves	
DALT	28		99		98		98	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.22	100.0	0.056	100.0	0.094	100.0	0.086
ERR	92.2	0.203	91.8	0.051	41.4	0.039	45.7	0.039
RRR	*7.8*	*0.017*	*8.2*	*0.005*	*58.6*	*0.055*	*54.3*	*0.047*

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
n.d. Not detected
Remark: Values in % TRR were recalculated during dossier compilation, since the given values were based on a 100 % value of the extract. Input values in % of radioactivity in the extract were taken from table 7 of the report and used for the recalculation of % TRR. Additionally mg/kg values of "neutrals", "others" and "indeterminate" were calculated. Minor deviations to values in % TRR given in table 10 of the report may occur due to rounding.
Remark: Data presented for pea tops originate from a small scale experiment. Results of the large scale experiment (using AG-50 column) were comparable (see report, table 9).
Total identified was calculated as sum of glyphosate and AMPA. Total characterised was calculated as aqueous extract – identified.
Other fractions were only characterised by their chromatographic behaviour. They were designated as "neutrals", others" and "indeterminate".
Values calculated upon dossier compilation are presented in *italics*.

Table 6.6.1-50: Distribution of radioactive residues of glyphosate and its metabolites in primary crops (string bean) and rotational crop (corn) after application of glyphosate to Norfolk sandy loam soil

Scenario type B	Primary crop		Rotational crops							
	String beans		Corn first harvest		Corn second harvest		Corn kernel		Corn cob	
DALT	46		70		110		110		110	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.13	100.0	0.04	100.00	0.09	100.0	0.06	100.0	0.05
Extraction with water										
Aqueous extract	**87.5**	**0.110**	**48.0**	**0.020**	**59.4**	**0.053**	**39.9**	**0.024**	**51.5**	**0.026**
Glyphosate	*54.2*	0.068	*12.0*	0.005	*5.6*	0.005	--	--	*3.2*	0.002
AMPA	*6.0*	0.007	*11.1*	0.003	*9.0*	0.008	--	--	*9.0*	0.005
Total identified	***60.1***	***0.075***	***23.2***	***0.008***	***14.5***	***0.013***	--	--	***12.3***	***0.007***
Neutrals	*12.4*	*0.016*	*12.8*	*0.005*	*23.3*	*0.021*	*17.6*	*0.011*	*26.6*	*0.013*
Others	*7.2*	*0.009*	*12.0*	*0.005*	*7.6*	*0.007*	--	--	*10.8*	*0.005*
Indeterminate	*7.8*	*0.010*	*0.0*	*0.000*	*14.0*	*0.012*	--	--	*1.9*	*0.001*
Total characterised	***27.4***	***0.034***	***24.8***	***0.010***	***44.9***	***0.040***	***17.6***	***0.011***	***39.2***	***0.020***
ERR	**87.5**	**0.110**	**48.0**	**0.020**	**59.4**	**0.053**	**39.9**	**0.024**	**51.5**	**0.026**
RRR	***12.5***	***0.020***	***52.0***	***0.020***	***40.6***	***0.037***	***60.1***	***0.036***	***48.5***	***0.024***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

Table 6.6.1-50: Distribution of radioactive residues of glyphosate and its metabolites in primary crops (string bean) and rotational crop (corn) after application of glyphosate to Norfolk sandy loam soil

Scenario type B	Primary crop		Rotational crops							
	String beans		Corn first harvest		Corn second harvest		Corn kernel		Corn cob	
DALT	46		70		110		110		110	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.13	100.0	0.04	100.00	0.09	100.0	0.06	100.0	0.05

Remark: Values in % TRR were recalculated during dossier compilation, since the given values were based on a 100 % value of the extract. Input values in % of radioactivity in the extract were taken from table 7 of the report and used for the recalculation of % TRR. Additionally mg/kg values of "neutrals", "others" and "indeterminate" were calculated. Minor deviations to values in % TRR given in table 10 of the report may occur due to rounding.

Remark: Data presented for string beans originate from a small scale experiment. Results of the large scale experiment (using AG-50 column) were comparable (see report, table 9).

Total identified was calculated as sum of glyphosate and AMPA. Total characterised was calculated as aqueous extract – identified.

Other fractions were only characterised by their chromatographic behaviour. They were designated as "neutrals", others" and "indeterminate".

Values calculated upon dossier compilation are presented in italics.

Table 6.6.1-51: Distribution of radioactive residues of glyphosate and its metabolites in primary crops (carrot) and rotational crops (string bean and cabbage) after application of glyphosate to Norfolk sandy loam soil

Scenario type C	Primary crop				Rotational crops					
	Carrot		Carrot leaves		String bean leaves		String bean pod		Cabbage	
DALT	50				122				45	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.11	100.0	0.17	100.0	0.08	100.0	0.04	100.0	0.045
Extraction with water										
Aqueous extract	**77.2**	**0.085**	**57.0**	**0.097**	**66.2**	**0.053**	**90.3**	**0.036**	**57.5**	**0.026**
Glyphosate	*34.5*	0.038	*30.2*	0.051	*23.8*	0.019	*40.1*	0.016	*8.3*	0.004
AMPA	*5.0*	0.006	*3.2*	0.005	*3.5*	0.003	*7.9*	0.003	*6.7*	0.003
Total identified	***39.5***	***0.044***	***33.3***	***0.056***	***27.3***	***0.022***	***48.0***	***0.019***	***15.0***	***0.007***
Neutrals	*17.8*	*0.020*	*12.5*	*0.021*	*14.4*	*0.012*	*20.0*	*0.008*	*24.0*	*0.011*
Others	*5.0*	*0.006*	*3.6*	*0.006*	*5.0*	*0.004*	*8.9*	*0.004*	*9.3*	*0.004*
Indeterminate	*14.8*	*0.016*	*7.6*	*0.013*	*19.6*	*0.016*	*13.4*	*0.005*	*9.3*	*0.004*
Total characterised	***37.7***	***0.041***	***23.7***	***0.040***	***38.9***	***0.031***	***42.3***	***0.017***	***42.5***	***0.019***
ERR	**77.2**	**0.085**	**57.0**	**0.097**	**66.2**	**0.053**	**90.3**	**0.036**	**57.5**	**0.026**
RRR	***22.8***	***0.025***	***43.0***	***0.073***	***33.8***	***0.027***	***9.7***	***0.004***	***42.5***	***0.019***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)

Remark: Values in % TRR were recalculated during dossier compilation, since the given values were based on a 100 % value of the extract. Input values in % of radioactivity in the extract were taken from table 7 of the report and used for the recalculation of % TRR. Additionally mg/kg values of "neutrals", "others" and "indeterminate" were calculated. Minor deviations to values in % TRR given in table 10 of the report may occur due to rounding.

Remark: Data presented for carrot originate from a small scale experiment. Results of the large scale experiment (using AG-50 column) were comparable (see report, table 9).

Table 6.6.1-51: Distribution of radioactive residues of glyphosate and its metabolites in primary crops (carrot) and rotational crops (string bean and cabbage) after application of glyphosate to Norfolk sandy loam soil

Scenario type C	Primary crop				Rotational crops					
	Carrot		Carrot leaves		String bean leaves		String bean pod		Cabbage	
DALT	50				122				45	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.11	100.0	0.17	100.0	0.08	100.0	0.04	100.0	0.045

Total identified was calculated as sum of glyphosate and AMPA. Total characterised was calculated as aqueous extract – identified.
Other fractions were only characterised by their chromatographic behaviour. They were designated as "neutrals", others" and "indeterminate".
Values calculated upon dossier compilation are presented in *italics*.

Table 6.6.1-52: Distribution of radioactive residues of glyphosate and its metabolites in primary crop (cabbage) and rotational crops (pea and carrot) after application of glyphosate to Norfolk sandy loam soil

Scenario type D	Primary crop				Rotational crops							
	Cabbage		Cabbage head		Pea leaves		Pea pods		Carrot		Carrot leaves	
DALT	78				122				45			
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.08	100	n.d.	100.0	0.19	100.0	0.28	100.0	0.05	100.0	0.08
Extraction with water												
Aqueous extract	**69.1**	**0.055**	**65.9**	n.d.	**88.7**	**0.168**	**88.5**	**0.248**	**73.4**	**0.037**	**51.6**	**0.042**
Glyphosate	*32.1*	0.026	*39.2*	**n.d.**	*40.4*	0.076	*45.8*	0.128	*6.9*	0.003	*7.7*	0.006
AMPA	*7.0*	0.006	*2.9*	n.d.	*11.1*	0.021	*15.6*	0.044	*7.6*	0.004	*2.9*	0.002
Total identified	***39.0***	***0.032***	***42.1***	*n.d.*	***51.4***	***0.097***	***61.4***	***0.172***	***14.5***	***0.007***	***10.6***	***0.008***
Neutrals	*17.0*	*0.014*	*18.5*	*n.d.*	*13.6*	*0.026*	*9.0*	*0.025*	*43.2*	*0.022*	*17.9*	*0.015*
Others	*4.2*	*0.003*	*2.0*	*n.d.*	*5.5*	*0.010*	*4.8*	*0.013*	*8.5*	*0.004*	*8.6*	*0.007*
Indeterminate	*8.8*	*0.007*	*2.9*	*n.d.*	*18.2*	*0.034*	*13.3*	*0.037*	*7.2*	*0.004*	*14.5*	*0.012*
Total characterised	***30.1***	***0.024***	***23.4***	*n.d.*	***37.3***	***0.071***	***27.1***	***0.076***	***58.9***	***0.030***	***41.0***	***0.033***
ERR	**69.1**	**0.055**	**65.9**	n.d.	**88.7**	**0.168**	**88.5**	**0.248**	**73.4**	**0.037**	**51.6**	**0.042**
RRR	***30.9***	***0.025***	***34.1***	*n.d.*	***11.3***	***0.022***	***11.5***	***0.032***	***26.6***	***0.013***	***48.4***	***0.038***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
n.d. Not determined
Remark: Values in % TRR were recalculated during dossier compilation, since the given values were based on a 100 % value of the extract. Input values in % of radioactivity in the extract were taken from table 7 of the report and used for the recalculation of % TRR. Additionally mg/kg values of "neutrals", "others" and "indeterminate" were calculated. Minor deviations to values in % TRR given in table 10 of the report may occur due to rounding.
Remark: Data presented for cabbage originate from a small scale experiment. Results of the large scale experiment (using AG-50 column) were comparable (see report, table 9).
Total identified was calculated as sum of glyphosate and AMPA. Total characterised was calculated as aqueous extract – identified.
Other fractions were only characterised by their chromatographic behaviour. They were designated as "neutrals", others" and "indeterminate".

Values calculated upon dossier compilation are presented in *italics*.

Table 6.6.1-53: Distribution of radioactive residues of glyphosate and its metabolites in primary crops (string bean) and rotational crops (cabbage and carrot) after application of glyphosate to Ray silt loam soil

Scenario type E	Primary crop				Rotational crops					
	String bean leaves		String bean pod		Cabbage		Carrot		Carrot leaves	
DALT	30				122		122			
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
	100.0	1.07	100.0	0.19	100.0	0.051	100.0	0.039	100.0	0.061
Extraction with water										
Aqueous extract	23.8	0.262	57.1	0.104	55.7	0.028	65.9	0.026 [1]	35.8	0.018
Glyphosate	*1.0*	0.012	*7.9*	0.014	*n.d.*	n.d.	*n.d.*	n.d.	*n.d.*	n.d.
AMPA	*1.9*	0.020	*22.5*	0.041	*8.4*	0.004	*9.0*	0.003	*2.4*	0.001
Total identified	***2.9***	***0.032***	***30.4***	***0.055***	***8.4***	***0.004***	***9.0***	***0.003***	***2.4***	***0.001***
Neutrals	*6.1*	*0.068*	*20.2*	*0.037*	*20.3*	*0.010*	*48.2*	*0.019*	*22.5*	*0.011*
Others	*3.4*	*0.037*	*5.5*	*0.010*	*13.4*	*0.007*	*8.7*	*0.003*	*10.9*	*0.005*
Indeterminate	*11.3*	*0.125*	*1.0*	*0.002*	*13.6*	*0.007*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Total characterised	***20.9***	***0.230***	***26.7***	***0.049***	***47.3***	***0.024***	***56.9***	***0.022***	***33.4***	***0.017***
ERR	**23.8**	**0.262**	**57.1**	**0.104**	**55.7**	**0.028**	**65.9**	**0.026**	**35.8**	**0.018**
RRR	***76.2***	***0.808***	***42.9***	***0.086***	***44.3***	***0.023***	***34.1***	***0.013***	***64.2***	***0.043***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
[1] This value was recalculated because the value 0.020 mg/kg as given in the report does not fit to 65.9 % TRR.
n.d. Not detected
Remark: Values in % TRR were recalculated during dossier compilation, since the given values were based on a 100 % value of the extract. Input values in % of radioactivity in the extract were taken from table 7 of the report and used for the recalculation of % TRR. Additionally mg/kg values of "neutrals", "others" and "indeterminate" were calculated. Minor deviations to values in % TRR given in table 10 of the report may occur due to rounding.

Total identified was calculated as sum of glyphosate and AMPA. Total characterised was calculated as aqueous extract – identified.

Other fractions were only characterised by their chromatographic behaviour. They were designated as "neutrals", others" and "indeterminate".

Values calculated upon dossier compilation are presented in *italics*.

Table 6.6.1-54: Distribution of radioactive residues of glyphosate and its metabolites in primary crops (carrots) after application of glyphosate to Ray silt loam soil

Scenario type F	Primary crop			
	Carrot		Carrot leaves	
DALT	51			
	% TRR	mg/kg	% TRR	mg/kg
	100.0	0.31	100.0	0.494
Extraction with water				
Aqueous extract	74.6	0.230	51.3	0.269
Glyphosate	*2.5*	0.008	*9.0*	*0.047* [1]
AMPA	*5.6*	0.017	*3.3*	0.017
Total identified	***8.1***	***0.025***	***12.4***	***0.065***
Neutrals	*45.5*	*0.140*	*8.1*	*0.043*
Others	*2.8*	*0.009*	*6.0*	*0.031*
Indeterminate	*18.2*	*0.056*	*24.8*	*0.130*
Total characterised	***66.5***	***0.205***	***38.9***	***0.204***
ERR	**74.6**	**0.230**	**51.3**	**0.269**
RRR	***25.4***	***0.080***	***48.7***	***0.225***

DALT Days after last treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue (calculated by subtraction: TRR – ERR)
[1] Value out of the report (0.005 mg/kg) did not fit to the value given in % TRR, therefore this value was recalculated.
Remark: Values in % TRR were recalculated during dossier compilation, since the given values were based on a 100 % value of the extract. Input values in % of radioactivity in the extract were taken from table 7 of the report and used for the recalculation of % TRR. Additionally mg/kg values of "neutrals", "others" and "indeterminate" were calculated. Minor deviations to values in % TRR given in table 10 of the report may occur due to rounding.
Total identified was calculated as sum of glyphosate and AMPA. Total characterised was calculated as aqueous extract – identified.
Other fractions were only characterised by their chromatographic behaviour. They were designated as "neutrals", others" and "indeterminate".
Values calculated upon dossier compilation are presented in *italics*.

Soil samples were extracted with NH_4OH-solution. The extractabilities and the composition of radioactive components in the extracts are shown in Table 6.6.1-55 and Table 6.6.1-56.

Generally, the extractability of soil samples was high (in all cases ≥73.6 %), except for soil samples taken after harvest of rotational crops from Ray soil. From these samples, only 25.4 and 28.8 % were extracted. Analyses showed that the predominant compound in Norfolk soil extracts was still the parent. It represented above 84.0 % of the of the radioactive residues in the extracts of soil taken within 11 weeks and more than 66.5 % in samples taken within 31 weeks. AMPA was detected between 4.4 and 22.5 %.
In Ray soil the predominant compound in the extracts was AMPA (68.0 and 74.5 %) after 4-7 weeks, while the parent compound glyphosate represented about 20 %. After 24.5 weeks nearly no glyphosate, was detected (up to 2.0 %) in the extracts of two soil samples, AMPA was found at 36.6 and 37.1 %. In the soil extract scenario E (cabbage) besides glyphosate and AMPA, the metabolite N-methylaminomethylphosphonic acid was detected at 6.6 %.

Table 6.6.1-55: Extraction of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil (after harvest of primary crops)

	Norfolk sandy loam soil				Ray silt loam soil	
Scenario Type	A	B	C	D	E	F
Primary crop	Pea	String bean	Carrot	Cabbage	String bean	Carrot
DALT (weeks)	4	6.5	7	11	4	7
	% AR	% AR	% AR	% AR	% AR	% AR
Radioactivity left in soil [1]	81.86	71.66	67.8	52.59	30.8	29.4
Extraction with 0.5 M NH_4OH						
Aqueous extract [2]	n.a.	91.8	96.5	98.9	74.1	73.6
Glyphosate [3]	n.a.	92.5	92.9	84.5	18.9	19.2
AMPA [3]	n.a.	4.4	5.0	13.0	74.5	68.0
Identified [4]	n.a.	*96.9*	*97.9*	*97.5*	*93.4*	*87.2*

DALT Days after last treatment given in weeks
AR Applied radioactivity
n.a. not analysed
1 Given values refer to % applied radioactivity.
2 These values correspond to mean values calculated within the report for experiments with different analytical methods used (AG-50, AG-1, HVE, TLC, and FID/RAD).
3 Given values refer to % of the compound in the extract.
4 Identified was calculated as sum of glyphosate and AMPA.
Values calculated upon dossier compilation are presented in *italics*.

Table 6.6.1-56: Extraction of the radioactive residues of glyphosate in soil after application of glyphosate to bare soil (after harvest of rotational crops)

	Norfolk sandy loam soil							Ray silt loam soil	
Scenario Type	A	B		C		D		E	
Rotational crop	Carrot/ cabbage	Corn	Corn	Cabbage	String bean	Carrot	Pea	Carrot	Cabbage
DALT (weeks)	18	16.5	22	27	13.5	31	17.5	24.5	24.5
	% AR	% AR	% AR	% AR	% AR	% AR	% AR	% AR	% AR
Radioactivity left in soil [1]	59.95/ 62.82[4]	40.36	53.04	28.65	61.80	27.07	57.43	6.43	10.00
Extraction with 0.5 M NH_4OH									
Aqueous extract	97.2	124.0	90.8	81.1	90.7	94.0	100.5	25.4	28.8
Glyphosate [2]	90.8	78.9	66.5	67.0	79.5	68.0	67.8	0.0	2.0
AMPA [2]	6.7	11.2	15.1	8.5	8.0	8.1	22.5	37.1	36.6
CP 70948 [5]	-	-	-	-	-	-	-	-	6.6
Identified [3]	*97.5*	*90.1*	*81.6*	*75.5*	*87.5*	*76.1*	*90.3*	*37.1*	*45.2*

DALT Days after last treatment given in weeks
AR Applied radioactivity
1 Given values refer to % of the applied radioactivity.
2 Given values refer to % of the compound in the extract.
3 Identified was calculated as sum of glyphosate and AMPA; for ray soil the metabolite CP 70948 was summed up, too.
4 For the scenario type A only results for one soil sample were presented, no information which pots were sampled.
5 CP 70948 = N-methylaminomethylphosphonic acid
Values calculated upon dossier compilation are presented in *italics*.

C. Storage stability

Storage intervals for frozen samples and extracts are not reported. No information on storage stability is reported.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants supporting uses in cereals at the end of this chapter.

III. Conclusion

Plants were grown on two different soils, a sandy loam (Norfolk soil) and a silt loam (Ray soil). For Norfolk soil the primary crops were string beans, peas, carrots and cabbage and for Ray soil string beans and carrots. At the maximum growth of the primary crops, ^{14}C-glyphosate was applied to the bare soil at 4.48 kg a.s./ha. The plants were sampled 4 - 11 weeks after treatment. After harvesting of the primary crops, rotational crops (same as primary plants, and sweet corn) were planted within a 1 - 23 day interval (PBI 29 - 79 / 101 days) and harvested 45 to 122 days after treatment (DALT). In addition, soil samples were taken after harvest of the primary crops as well as after harvest of the rotational crops from each plot. The recovered radioactivity and its composition in soil, in primary crops and in rotational crops was determined.

The uptake of glyphosate by the plants reflects the amount of glyphosate and/or metabolites present in the soils. Glyphosate appears to be relatively stable in the sandy loam soil (Norfolk soil) with $t_{1/2}$ of 17 - 19 weeks in contrast to $t_{1/2}$ of 3 - 4 weeks in the silt loam soil (Ray soil).

The residue levels in the investigated commodities of primary crops from Norfolk soil were lower (0.08 - 0.22 mg/kg) than those from primary crops grown on Ray soil (0.19 - 1.07 mg/kg). The amounts of radioactive residues in rotational crops were comparable for plants from both soils and ranged between (0.04 - 0.094 mg/kg), except for pea leaves and pots, where radioactive residues up to 0.28 mg/kg were detected.
Generally, 55.7 – 92.2 % of the TRR were extractable with water from the plant material. Somewhat lower amounts (23.8 – 51.6 % TRR) were extracted from leaves of string beans and carrots of primary crops from Ray soil and from carrot leaves and corn (kernel and cob) of the rotational crops from both soils. The remaining residues after solvent extraction (TRR) amounted to 0.004 - 0.808 mg/kg (7.8 – 76.2 % TRR) and were not investigated.

Glyphosate was the major component detected in the plant extracts of primary crops (0.026 - 0.137 mg/kg and rotational crops (0.003 - 0.128 mg/kg from Norfolk soil). AMPA was less abundant in these extracts (0.002 - 0.044 mg/kg). Components that were characterised according to their elution behaviour (designated as “neutrals”, “others” or “indeterminates”) amounted to 0.007 - 0.037 mg/kg.
In plant extracts of primary crops from Ray soil, AMPA was found at higher amounts than glyphosate (0.017 - 0.041 mg/kg), except for carrot leaves. Glyphosate amounted to 0.008 - 0.046 mg/kg. Glyphosate was not detected in the extracts of rotational crops from Ray soil, while AMPA was found at low amounts (up to 0.004 mg/kg). In the extracts of primary and rotational crops of Ray soil, high amounts of neutrals and / or indeterminates were found, representing up to 0.140 mg/kg.

The higher uptake from silt loam (Ray soil) was seen as a result of rapid degradation of ^{14}C-glyphosate to $^{14}CO_2$ which was fixed by the plants, resulting in the high amount of neutral materials and non-extractable components which were proposed to represent incorporation of ^{14}C into natural products.

3. Assessment and conclusion

Assessment and conclusion by applicant:

This study assessing the metabolic behavior of glyphosate in two different soils, in primary crops (beans, peas, carrots and cabbage) and rotational crops (same as primary crops plus corn) has been previously evaluated at EU level. It was not performed under GLP (as in 1976 GLP was not yet established at the test facility) but is considered to be scientifically valid.
The study is deemed to largely comply with current requirements as laid down in Reg. (EU) No 283/2013 and OECD Guideline for the Metabolism in Rotational Crops, 502with some deficits.
The study is not a typical rotational crop study as it included analysis of primary crops and only one rotation was conducted; nevertheless it contains supportive data on the uptake of glyphosate/metabolites by primary crops and rotational crops from two different soils.
Information about the application method and formulation of test item is missing. Developmental stages of the crops at application and harvesting are not reported, but could be roughly estimated based on planting and sampling dates.
No information on storage duration of plant samples and aqueous plant extracts is given in the study report.
Relevant amounts of non-extractable residues were not characterised / not investigated (residual radioactive residues were 11.3 - 76.2 % TRR (0.013 - 0.808 mg/kg)).
Relevant amounts of residues in the extracts (11.3 - 45.5 % TRR (0.012 - 0.140 mg/kg)) were not investigated.

Despite the shortcomings, the present study is considered scientifically valid and supportive of the whole package of studies on the metabolism of glyphosate in primary and rotational crops.

Assessment and conclusion by RMS:

Overall assessment and conclusion on confined rotational crop studies

Overall summaries are given in the following. Data are summarised also in Appendix G, and those studies where the nature of residues was investigated are considered for the definition of residues (see CA 6.7.1).

In the following for the classification of a metabolite into major and minor the following definitions were used:

- Major metabolite in food ≥10 % TRR and ≥0.01 mg/kg or if TRR <10 % amount >0.05 mg /kg
- Major metabolite in processing study: TRR ≥10 %
- Major metabolite in feed and non food/non feed related commodities ≥10 % TRR and ≥0.01 mg/kg

In total six metabolism studies are available (four using *N*-(phosphono-^{14}C-methyl)glycine and two using *N*-(phosphono-^{14}C-methyl)glycine as trimesium salt) investigating the fate and nature of glyphosate-derived residues in **confined rotational crops**. An overview on the studies is given in the following table:

Table 6.6.1-57: Overview on available confined rotational crop studies

Plant	Application	Application rate	Reference
Rotational crops (lettuce, wheat and radish)	Soil application (soil was aged 30, 120 and 365 days until planting of rotational crops)	*N*-(phosphono-^{14}C-methyl)glycine, 6.5 kg a.s./ha	CA 6.6.1/001 [redacted] 1998, LX1146-02 (Glyphosate technical) Confined Rotational Crop Study on lettuce, radish, and wheat in California, 1651-91-146-01-09B-17
Rotational crops (lettuce, wheat and radish)	Soil application (soil was aged 35, 63 and 308 days until planting of rotational crops)	*N*-(phosphono-methyl)glycine as trimesium salt, 5.617 kg a.s./ha (3.87 kg a.s./ha expressed as glyphosate equivalents) or 9.51 kg a.s./ha (three monthly applications, 6.56 kg a.s./ha expressed as glyphosate equivalents)	CA 6.6.1/002: [redacted] 1993, [^{14}C-Anion] Glyphosate-Trimesium: Confined Accumulation Studies on Rotational Crops, Report No. RR92-096B
Rotational crops (lettuce, barley and carrot)	Soil application (soil was aged 30, 119 and 364 days until planting of rotational crops)	*N*-(phosphono-^{14}C-methyl)glycine, 4.16 kg a.s./ha	CA 6.6.1/003: [redacted] 1990, Confined Rotational Crop Study of Glyphosate. Part I: In-Field Portion, Report No. MSL-9810, McMullan, P. C. *et al.*, 1990, Confined Rotational Crop Study of Glyphosate. Part II: Quantitation, Characterisation, and Identification of Glyphosate and Its Metabolites in Rotational Crops, Report No. MSL-9811
Rotational crops (wheat and turnip)	Soil application (soil was aged 35, 95 and 370 days until planting of rotational crops)	*N*-(phosphono-methyl)glycine as trimesium salt, 6 kg a.s./ha (4.12 kg a.s./ha expressed as glyphosate equivalents)	CA 6.6.1/004: [redacted] 1989, [^{14}C-Anion] ICIA0224 - Confined Accumulation Studies on Rotational Crops, Report No. WRC-89-25
Primary crops (soybean, cabbage, wheat and beet) Rotational crops (beet, cabbage and wheat)	Soil application (soil was aged 30, 120 and 365 days until planting of rotational crops)	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application was performed 3 days before planting of primary crops)	CA 6.6.1/005: [redacted] 1978, Uptake and metabolism of glyphosate in root, leaf and cereal type rotational crops, Report No. MSL-0882
Primary crops (pea, string bean, carrot, cabbage) Rotational crops (pea, string bean, carrot, cabbage, sweet corn)	Soil application (rotational crops were planted at PBI of 29 – 79/101 days)	*N*-(phosphono-^{14}C-methyl)glycine, 4.48 kg a.s./ha (application at the maximum plant growth of primary crops)	CA 6.6.1/006: [redacted] 1976, Metabolism of CP 67573 in representative vegetables and rotation crops, Report No.: 406

In the first confined rotational crop study ([redacted] **1998**) the metabolism of *N*-(phosphono-methyl)glycine was examined in rotational crops lettuce, wheat and radish. The treated soil was planted at a plant-back interval of 30 days (PBI 30), simulating a crop failure, at 120 days simulating a second crop planting in the same year (PBI 120), and at 365 days simulating a yearly rotational planting (PBI 365).

The rotational crops from the PBI 30 contained 0.24 – 2.0 mg/kg in edible matrices (lettuce leaves, radish roots as well as wheat grain) and 1.3 – 4.8 mg/kg in inedible matrices (radish leaves, wheat forage and wheat chaff). Crops from the PBI 120 contained 0.15 – 0.7 mg/kg and 0.17 – 1.4 mg/kg for edible and inedible matrices, respectively. Crops from the PBI 360 contained 0.02 – 0.16 mg/kg and 0.01 – 0.19 mg/kg for edible and inedible matrices, respectively.
Only glyphosate and AMPA are reported to be identified in very low amounts in all crop samples but the vast majority of remaining radioactivity was not further analysed. In mature, edible samples of all three rotations, **glyphosate** was present only to amounts of < 0.05 mg/kg. In mature samples of wheat forage and chaff, **glyphosate** accounted for < 0.05 mg/kg, 0.3 – 0.4 mg/kg and < 0.05 – 0.06 mg/kg for the first, second and third rotation, respectively. **AMPA** was found as major metabolite only in mature 30 and 120 day wheat forage, chaff, and seed, accounting for up to 0.4 mg/kg.

In the second confined rotational crop study ([redacted], **1993**) the uptake and metabolism of glyphosate was examined in rotational crops. *N*-(phosphono-methyl)glycine, labelled in the methylene position (^{14}C-PMG-label) was applied to two plots at different application rates as its trimesium salt.
A primary crop of soybeans was planted prior to treatment in all plots containing sandy loam soil. After removal of the primary crop the rotational crops lettuce, radish, and wheat were planted into the subplots at 35, 63, and 308 days after herbicide treatment (35, 63, and 308 plant-back intervals, PBI).
The soybean cover crop was not analysed. The TRR levels in matrices obtained from rotational crops were relatively low, not exceeding 0.1 mg/kg, except for lettuce (0.127 mg/kg). The rotational crops from the 35 and 63 PBI plots contained TRR levels of 0.020 – 0.076 mg/kg and 0.021 – 0.127 mg/kg, respectively. Crops from the 308 PBI contained TRR levels of 0.010 – 0.038 mg/kg.
Following analysis of rotational crop samples, **AMPA** was found as major metabolite at levels of 8.7 - 34 % of the TRR. **Glyphosate** was also detected in most samples, however its levels were <2.3 % of the TRR. Residues after extraction with water and chloroform were further investigated; they were identified as being carbohydrates as **glucose**, **fructose** and **malic acid** and characterised as being **starch, lignins, amino acids** and **cellulose**.

In the third rotational crop study *N*-(phosphono-^{14}C-methyl)glycine formulated as Roundup® was applied to plots of bare sandy loam soil ([redacted] **1990**). As primary crop soybeans were planted 7 days after application. The primary crop was harvested and the plots rototilled before planting rotational crops of lettuce, carrots, and barley into the subplots at 30, 119, and 364 days after herbicide treatment (PBI).
The primary and rotational crops were sampled for analysis. The primary crop soybean contained residues of 0.0822 – 0.4329 mg/kg; no further investigations were conducted for the primary crop. The rotational crops from the 30 PBI plots contained 0.037 – 0.188 mg/kg of glyphosate equivalent residues. Crops from the 119 PBI planting contained residues of 0.017 – 0.078 mg/kg. Carrots, barley, and lettuce from the 364 PBI contained residues of 0.0096 to 0.061 mg/kg.
Analysis of rotational crop samples revealed two residue components, **AMPA** and a polar metabolite (called Metabolite 1 within the report) characterised as being a mixture of sugars, primarily glucose and fructose. **Glyphosate** was present only in lettuce, barley straw and grain of the first rotation at 1.0 – 9.8 % of the TRR and in lettuce DALT 167 of the second rotation at 1.6 % of the TRR. **AMPA** ranged from 3.7 – 17.9, 1.1 – 14.2 and 7.7 – 20.0 % of the TRR in the matrices of the crops of the first, second and third rotation, respectively. Metabolite 1 (characterised as mixture of sugars) amounted to 7.7 – 40.8, 6.3 – 24.9, 6.6 – 31.9 % of the TRR in the matrices of the crops of the first, second and third rotation, respectively.
Residues after extraction with water and chloroform were further investigated and were characterised as being **starch, lignin and cellulose**, as well as **biopolymers of glucose**.

In the fourth rotational crop uptake study the uptake of glyphosate from soil by rotational crops, namely wheat and turnip, and to determine was investigated ([redacted] **1989**).
For the investigations, a loamy sand soil was treated with *N*-(phosphono-^{14}C-methyl)glycine as its trimesium salt (^{14}C-glyphosate-trimesium). No primary crop was planted.

Rotational crops wheat and turnip were planted at plant-back intervals of 35, 95 and 370 days after treatment. The plants were harvested at maturity and the radioactive residues were determined in commodities of wheat (seed, chaff and stalks/leaves) and of turnips (leaves and bulbs).
Total radioactive residues in wheat chaff, seeds and stalks/leaves were 0.29, 0.25 and 0.46 mg/kg (35 PBI), 0.25, 0.28 and 0.51 mg/kg (95 PBI) and 0.1, 0.06 and 0.11 mg/kg (270 PBI). In turnip leaves and bulbs the radioactive residues amounted to 0.02 mg/kg for both commodities of turnips for the 35 PBI, to 0.09 and 0.03 mg/kg (95 PBI) and were detected at 0.03 and 0.02 mg/kg (270 PBI).
The radioactive residues in the plant matrices were not extracted and investigated for their identity.

In the fifths study ([redacted] **1978**) *N*-(phosphono-^{14}C-methyl)glycine was applied to sandy loam soil in pots. Primary crops of soybean, cabbage, wheat and beet, were planted 3 days after application. After harvesting the primary crop, different succeeding crops were planted at plant-back intervals of 30, 120 or 365 days. To simulate crop failure, some of the pots containing the primary crop received a second treatment of 4.48 kg a.s./ha and were replanted with the same crops as before (except for soybean where beet was replanted).
Radioactive residues were extracted with water from respective plant materials. Concerning radioactive residues there was a reduction in the amount of uptake of ^{14}C-activity with time. There was no increase in the uptake of ^{14}C-activity in any of the rotational crops with the exception of emergency crops beet and cabbage, where a slight increase in TRRs was determined. The rotational crops from the 30 PBI scenario contained 0.002 – 0.018 mg/kg of **glyphosate** and 0.003 – 0.041 mg/kg **AMPA**. Only for wheat residues were higher (0.046 mg/kg for **glyphosate** and 0.128 mg/kg for **AMPA**). Residues from respective plant materials for the 120 PBI decreased to <0.001 – 0.014 mg/kg for glyphosate and 0.001 – 0.010 mg/kg for **AMPA**. Residues from respective plant materials for the 365 PBI further decreased to <0.001 – 0.004 mg/kg for **glyphosate** and <0.001 – 0.004 mg/kg for **AMPA**. The extracts were analysed by ion-exchange column chromatography. Many ^{14}C-products with elution patterns similar to glyphosate and AMPA were observed.

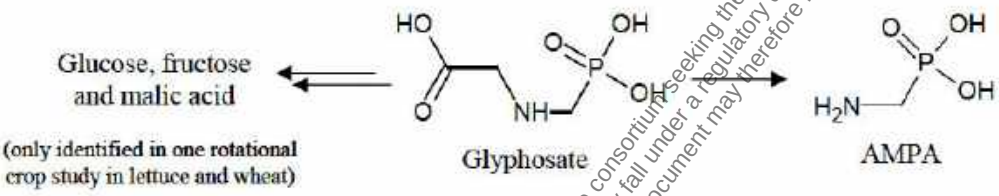
In the sixth study the uptake of *N*-(phosphono-^{14}C-methyl)glycine ([redacted] **1976**) from two different soils [sandy loam (Norfolk soil) and a silt loam (Ray soil)] into representative crops, like carrots (root type), cabbage (leafy type), string beans and peas (both legumes) and sweet corn was investigated. At the maximum plant growth of the primary crops, *N*-(phosphono-^{14}C-methyl)glycine was applied to bare soil. The primary crops (carrot, cabbage, string beans, peas) were sampled 4 - 11 weeks after treatment. Rotational crops (same as primary plants plus corn) were planted within a 1 - 23 day interval after harvest of the primary crops (PBI 29 - 79 - 101 days) and harvested 6.5 to 17.5 weeks after planting.
In primary crops the residues were higher for plants grown on Ray soils (up to 1.07 mg/kg) than for plants grown on Norfolk soil (up to 0.22 mg/kg). The radioactive residues detected in rotational crops grown on the two different soils were comparable (all between about 0.040 and 0.280 mg/kg).
For crops grown on Norfolk soil, **glyphosate** was the major component detected in the plant extracts of primary and rotational crops. Glyphosate was detected at up to 0.137 mg/kg in primary crops and up to 0.128 mg/kg in rotational crops. **AMPA** was less abundant in these extracts and was detected between 0.002 and 0.044 mg/kg. Components that were characterised upon their elution behaviour and are designated as “neutrals”, “others” or “indeterminates”, were detected at up to 0.037 mg/kg in the extracts of primary and rotational crops from Norfolk soil. No further investigations were conducted to identify those other ^{14}C-products.
In plant extracts from primary crops grown on Ray soil, the amounts of **AMPA** (found at up to 0.041 mg/kg), were generally about twice as high as the concentrations of **glyphosate**. Glyphosate was not present in the extracts of rotational crops and AMPA was found at only low amounts (up to 0.004 mg/kg). The major part of radioactive residues were neutrals and / or indeterminates, representing up to 0.140 mg/kg. No further investigations were conducted to identify those other ^{14}C-products.

Overall conclusion on metabolism in rotational crops

Within all studies investigating the metabolism of ^{14}C-glyphosate in rotational crops a similar picture on metabolism was found. **Glyphosate** parent compound and **AMPA** were the only radioactive compounds identified during the course of the different rotational crop studies (with one exception, see below). In some cases glyphosate was the dominant radioactive residue; in some cases **AMPA** was identified or at least indicated as main or major metabolite. **Glucose, fructose** and **malic acid** were identified in one study ([redacted] 1993). In addition natural products were characterised as starch, lignin, cellulose or biopolymer of glucose.

The confined rotational crop studies also included investigations of the nature of residues in primary crops (soybean, cabbage, wheat, beet, carrot, cabbage, string bean and pea) which were planted before rotational crops. These findings support the studies on the nature of residues in primary crops for crop categories root crops (carrot and beet), leafy crops (cabbage), cereals (wheat) and pulses and oilseeds (soybean, string bean and pea).

Pathway for rotational crops



AMPA:	Major metabolite in wheat grain, lettuce leaves, barley grain, wheat whole plant, beet root, string bean pods, pea leaves and pea pods
Glucose:	Indicated in lettuce leaves, wheat grain, and wheat straw
Fructose:	Indicated in lettuce leaves and wheat straw
Malic acid:	Indicated in lettuce leaves and wheat straw

CA 6.6.2 Magnitude of residues in rotational crops

The confined rotational crop studies above show that the uptake of glyphosate and AMPA from treated soil into the plants is limited.

Overall, during these studies the highest residue level of parent glyphosate in edible commodities was 0.128 mg/kg and this level was found in pea pods from peas planted about 79-101 days after application of glyphosate at a rate of 4.48 kg ae/ha to the primary crop (CA 6.6.1/006; [redacted] L.A., 1976). In all other studies the residues of glyphosate in the edible commodities from rotational crops were below 0.05 mg/kg.

The PEC_{soil} values of parent glyphosate for the various representative uses in annual crops are shown in the table below (for details please refer to CP 9.1.3).

Table 6.6.2-1: Summary of PEC_{soil} of glyphosate

Application rate (g a.s./ha)	Frequency	$PEC_{soil,ini}$ (mg/kg)	Soil depth for $PEC_{soil,plateau}$ (cm)	$PEC_{soil,plateau}$ (mg/kg)	$PEC_{soil,accu}$ (mg/kg)
720	Every year	0.960	5	0.452	1.412
			20	0.113	1.073
	Every 3rd year		5	0.151	1.111
			20	0.038	0.998
1440	Every year	1.920	5	0.904	2.824
			20	0.226	2.146
540	Every year	0.720	5	0.339	1.059
			20	0.085	0.805
	Every 3rd year		5	0.113	0.833
			20	0.028	0.748
2160	Every year	2.880	5	1.356	4.236
			20	0.339	3.219
2880	Every year	3.840	5	1.808	5.648
3600	Every year	4.800	5	2.260	7.060
1800	Every year	2.400	5	1.130	3.530
			20	0.283	2.683

Comparison of the PEC $_{soil\ plateau}$ for the 0-20 cm soil layer with the PEC $_{soil\ ini}$ (for the same layer of 0-20 cm) shows that the PEC $_{soil\ plateau}$ represents less than 25 % of the PEC $_{soil\ ini}$. Therefore, glyphosate does not accumulate in soil and the PEC $_{soil\ accu}$ remains within +/- 25 % of the PEC $_{soil\ ini}$. It results that the residues of parent glyphosate in rotational crops after repeated application at the maximum seasonal rate for many years may be estimated based on field residue trials with soil application at the maximum seasonal rate.

Suitable residue trials are available to support pre-emergence or pre-planting application of glyphosate at a rate of 2.16 kg ae/ha in a variety of crops from different crop groups (root & tubers, bulbs, fruiting vegetables, brassica, leafy vegetables). All the trials showed residues of parent glyphosate below the LOQ of 0.05 mg/kg (and usually below the LOD). Therefore, the residues of parent glyphosate in rotational crops are unlikely to exceed the LOQ of 0.05 mg/kg. Pre-emergence residue trials in cereals, oilseeds and pulses will be conducted in the near future to further support this argument.

During the confined rotational crop studies the highest residue level of AMPA in edible commodities was 0.3 mg/kg and this level was found in grain from wheat sown 30 days after application of glyphosate at a rate of 6.5 kg ae/ha to bare soil ([redacted] 1998). In all other studies the residues of AMPA in the edible commodities from rotational crops were below 0.05 mg/kg.

In two of the confined rotational crop studies (CA 6.6.1/002 ([redacted] 1993) and CA 6.6.1/003 (McMullan, P. C. *et al.*, 1990)) the residues were determined in soil. In the former study the highest residue level of AMPA was 0.844 mg/kg and this level was found in the soil layer of 0-10 cm 34 days after the application of 3.87 kg ae/ha of glyphosate. Assuming that the residues of AMPA in the layer of 10-20 cm are negligible, this corresponds to a residue level of AMPA of 0.422 mg/kg in the soil layer of 0-20 cm. All the rotational crops planted at the corresponding plant back interval (actually 35 days after soil treatment) showed residues of AMPA below 0.05 mg/kg (maximum of 0.026 mg/kg in wheat grain).

The PEC_{soil} values of AMPA for the various representative uses in annual crops are shown in the table below (for details please refer to CP 9.1.3).

Table 6.6.2-2: Summary of PEC_{soil} of AMPA

Application rate (g a.s./ha)	Frequency	$PEC_{soil,ini}$ (mg/kg)	Soil depth for $PEC_{soil,plateau}$ (cm)	$PEC_{soil,plateau}$ (mg/kg)	$PEC_{soil,accu}$ (mg/kg)
720	Every year	0.397	5	0.810	1.207
			20	0.203	0.600
	Every 3rd year		5	0.270	0.667
			20	0.068	0.465
1440	Every year	0.794	5	1.620	2.414
			20	0.405	1.199
540	Every year	0.298	5	0.607	0.905
			20	0.152	0.450
	Every 3rd year		5	0.203	0.500
			20	0.051	0.349
2160	Every year	1.191	5	2.429	3.621
			20	0.607	1.799
2880	Every year	1.589	5	3.239	4.828
3600	Every year	1.986	5	4.049	6.035
1800	Every year	0.993	5	2.025	3.017
			20	0.506	1.499

Based on the level of 0.844 mg/kg in the 0-10 cm layer (*i.e.* 0.422 mg/kg in the 0-20 cm layer) The use with a maximum annual application rate of 0.540 kg a.s./ha and an application every third year results in a $PEC_{soil,\ accu}$ of 0.349 mg/kg, which is below the concentration of AMPA in the 0-20 cm soil layer in the study of [redacted] 1993. Therefore, this use is not expected to result in residues > 0.05 mg/kg in rotational crops.

The above table also shows that with an interval of three years between subsequent applications of glyphosate, the plateau level of residues of AMPA in soil does not exceed 25 % of the initial level. Using this application interval, the residues of AMPA in rotational crops are not expected to significantly exceed the residues of AMPA in the same crops after pre-emergence application.

Suitable residue trials are available to support pre-emergence or pre-planting application of glyphosate at a rate of 2.16 kg ae/ha in a variety of crops from different crop groups (root & tubers, bulbs, fruiting vegetables, brassica, leafy vegetables). All the trials showed residues of AMPA below the LOQ of 0.05 mg/kg (and usually below the LOD). Therefore, if glyphosate is only applied every third year the residues of AMPA in rotational crops are unlikely to exceed the LOQ of 0.05 mg/kg. Pre-emergence residue trials in cereals, oilseeds and pulses will be conducted in the near future to further support this argument.

A limited field rotational crop study will also be conducted in the near future to cover the maximum yearly application rate of 2.16 kg a.s./ha and repeated uses every year. The planned study design involves treatment of soil with a mixture of parent glyphosate and AMPA.

CA 6.7 Proposed Residue Definitions and Maximum Residue Levels

CA 6.7.1 Proposed residue definitions

Proposed residue definition for enforcement

The conclusion on the residue definition based on separate assessments of the results for conventional crops, CP4 EPSPS and GOX modified crops and GAT modified crops is presented below, followed by the overall proposal for plant and animal matrices.

Conventional crops and rotational crops (relevant to the representative uses):

The residue definition for risk assessment for conventional crops and rotational crops includes glyphosate and AMPA (see further below for details).

The proposed residue definition was used in residue trials relevant to this submission. Based on the findings within the residue trials performed according to GAP no residues of AMPA (LOQ 0.05 mg/kg) were determined. Therefore, it is considered sufficient to include glyphosate only in the residue definition for enforcement of conventional and rotational crops.

The following residue definition for enforcement is proposed:

Glyphosate

CP4 EPSPS as well as CP4 EPSPS and GOX modified crops (not supported in the EU and only relevant to imported commodities):

In CP4 EPSPS as well as CP4 EPSPS and GOX modified crops, glyphosate and AMPA generally represent the majority of the identified compounds in food and feed commodities and are considered relevant for both risk assessment (see further below for details) and enforcement.

Therefore, the following residue definition for enforcement is proposed:

Sum of glyphosate and AMPA, expressed as glyphosate

GAT modified crops (not supported in the EU and only relevant to imported commodities):

In GAT modified crops, *N*-acetyl glyphosate represents the majority of the identified compounds in food commodities and is considered relevant for both risk assessment (see further below for details) and enforcement. Glyphosate as parent compound should be included by default and is also major in most food and feed commodities.

Therefore, the following residue definition for enforcement is proposed:

Sum of glyphosate and N-acetyl glyphosate, expressed as glyphosate

Processed commodities:

The same residue definitions as for unprocessed commodities are proposed.

Conclusion for plant matrices

Combining the proposals for a suitable residue definition for enforcement in both non-tolerant/conventional and tolerant/genetically modified crops to derive a single residue definition that covers all possible uses, the following proposal is made:

For plants with glyphosate tolerant genetically modified varieties currently available on the market (sweet corn, cotton seeds, sugar beets, rapeseeds, maize and soybeans)

Sum of glyphosate, AMPA and N-acetyl glyphosate, expressed as glyphosate

For all other plant commodities

Glyphosate

This is in line with the latest EFSA Reasoned Opinion (EFSA, 2019. Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862, 211 pp.).

Conclusion for animal commodities

After gavage of glyphosate or glyphosate + AMPA, the only major compounds in animal matrices were glyphosate and AMPA, while after *N*-acetyl glyphosate feeding (only relevant in case of feeding of GAT modified crops), *N*-acetyl glyphosate, glyphosate or AMPA serve best as marker compounds, depending on the matrix. Under monitoring conditions, it is unclear what the respective animals have fed on; consequently the residue definition should cover all possible modifications of crops that can serve as feedstuffs.

Therefore, the following residue definition for enforcement is proposed:

Sum of glyphosate, AMPA and N-acetyl glyphosate expressed as glyphosate

This is in line with the latest EFSA Reasoned Opinion (EFSA, 2019. Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862, 211 pp.).

Proposed residue definition for risk assessment

Because the derivation of the residue definition for risk assessment is rather extensive, first a summary of the proposed residue definitions is presented below for better overview, followed by the actual derivation of this conclusion.

Summary of proposed residue definitions for risk assessment

The metabolism of glyphosate in primary crops and rotational crops was assessed in conventional crops. For primary crops the metabolism was also investigated in genetically modified crops containing CP4 EPSPS as well as CP4 EPSPS and GOX modifications belonging to different crop groups as well as in genetically modified crops (soybean, maize and rape) containing the GAT modification.

The following residue definitions for risk assessment are proposed for conventional crops and rotational crops, CP4 EPSPS, CP4 EPSPS and GOX modified crops as well as for GAT modified crops:

- Conclusion for conventional crops and rotational crops (relevant to the representative uses):

Two major compounds were identified in food and feed commodities of conventional crops and rotational crops, i.e. glyphosate and AMPA.

Therefore, the following residue definition for risk assessment is proposed:

Sum of glyphosate and AMPA, expressed as glyphosate

- Conclusion for CP4 EPSPS as well as CP4 EPSPS and GOX modified crops (not supported in the EU and only relevant to imported commodities):

In CP4 EPSPS as well as CP4 EPSPS and GOX modified crops, glyphosate and AMPA generally represent the majority of the identified compounds in food and feed commodities and are considered relevant for risk assessment.

Therefore, the following residue definition for risk assessment is proposed:

Sum of glyphosate and AMPA, expressed as glyphosate

- Conclusion for GAT modified crops (not supported in the EU and only relevant to imported commodities):

In GAT modified crops, glyphosate, *N*-acetyl glyphosate and/or *N*-acetyl AMPA represent the majority of the identified compounds in all food and feed commodities. AMPA was also found in significant amounts.

Therefore, the following residue definition for risk assessment is proposed:

Sum of glyphosate, AMPA, N-acetyl glyphosate and N-acetyl AMPA, expressed as glyphosate

In the latest EFSA Reasoned Opinion (EFSA, 2019. Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862, 211 pp.), a common residue definition for risk assessment for all crops (conventional and genetically modified) was proposed:

Sum of glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA, expressed as glyphosate.

This definition includes the two metabolites *N*-acetyl glyphosate and *N*-acetyl AMPA which are not relevant to all crops. Metabolism studies on conventional crops as well as metabolism studies on CP4 EPSPS or CP4 EPSPS and GOX modified crops prove the absence of these compounds. The two *N*-acetylic metabolites were found only relevant to GAT modified crops.

Also in the EFSA Reasoned Opinion it was concluded that the two acetyl metabolites do not need to be considered in the residue trials for conventional or EPSPS crops.

Therefore, the proposal of two different residue definitions for risk assessment (as given above) is only a formal difference to the latest EFSA Reasoned Opinion, but is the favoured since it is a clearer approach.

Conclusion for processed commodities:

Three high temperature hydrolysis studies were performed, one with glyphosate, one with *N*-acetyl glyphosate and one with AMPA and *N*-acetyl AMPA. Each compound was stable and no degradation products were identified.

Therefore, the **same residue definitions as for unprocessed commodities** are proposed.

Conclusion for animal commodities:

Several livestock metabolism studies on goat and hen using glyphosate or a mixture of glyphosate and AMPA were assessed. In addition, in order to address the animal metabolism of residues derived from GAT modified crops, metabolism studies on goat and hen using *N*-acetyl glyphosate were performed.

After gavage of glyphosate or glyphosate + AMPA, two major compounds were identified, i.e. glyphosate and AMPA.

Therefore, the following residue definition for risk assessment is proposed:

Sum of glyphosate and AMPA, expressed as glyphosate

For *N*-acetyl glyphosate gavage (**only relevant in case of feeding of GAT modified crops**), to cover all relevant identified compounds for livestock, the following residue definition for risk assessment is proposed:

Sum of glyphosate, AMPA, N-acetyl glyphosate and N-acetyl AMPA, expressed as glyphosate

In the latest EFSA Reasoned Opinion (EFSA, 2019. Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862, 211 pp.), a common residue definition for risk assessment for animal commodities is was proposed:

Sum of glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA, expressed as glyphosate

This definition includes the two metabolites *N*-acetyl glyphosate and *N*-acetyl AMPA which are only relevant when genetically GAT modified plants are feed to livestock. Metabolism studies on glyphosate and AMPA prove the absence of these compounds in animal commodities.

Also in the EFSA Reasoned Opinion it was noted that the two acetyl metabolites are specific for GAT-modified crops only and that GAT-modified crops are currently not on the EU market.

Therefore, the proposal of two different residue definitions for risk assessment (as given above) is only a formal difference to the latest EFSA Reasoned Opinion, but is the favoured since it is a clearer approach.

Derivation of residue definition for risk assessment

For derivation of the residue definition for risk assessment the metabolites identified in the primary and rotational crop metabolism studies, high temperature hydrolysis studies and livestock metabolism studies are summarised in the tables below. The information can also be found in CA 6.2 and 6.6 but is repeated here for ease of review.

Generally, metabolism experiments where the application method adequately reflects the normal uses of glyphosate are considered relevant. Those with more "artificial" setups, i.e. the hydroponic experiments, which were conducted to favour the uptake of the active substance by the plants and its metabolism, are also considered qualitatively, but quantitatively they are only treated as supportive information because they do not mirror realistic conditions.

Only those studies are summarised in which identification of glyphosate-related residues was performed and where values were available as %TRR (mg/kg) as only these terms allow a classification into minor and major metabolites according to OECD guideline 501.

Those studies where identification was done, but values were only available in % applied dose or only as % in extract were not considered here.

Commodities relevant as food items are highlighted in grey, those which are relevant as feed items (according to Animal model 2017) are coloured blue. As there is no specific GAP for glyphosate on forage, forage items from primary crops are not relevant for animal feeding. However, cereal forage metabolism data may be used to support (pre-emergence) uses in other forage crops such as e.g. grass. Likewise, soybean forage metabolism data may be used to support uses in other forage crops such as e.g. alfalfa. In case of rotational crops, data on lettuce, radish and beet may be extrapolated to e.g. kale, turnip and sugar beet.

Those commodities which are neither food nor feed item are not highlighted. These are only considered for the genotoxicity assessment.

Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).

Non-tolerant crops – primary crops

Crop category fruit crops

Table 6.7.1-1: Identified components of the radioactive residues of glyphosate in fruit crops – tree nut (primary crop)

Reference	CA 6.2.1/003, [redacted] 1976					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	100 µg per leaf surface of two trees		100 µg per leaf surface of two trees		100 µg per leaf surface of two trees	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 14		DALT 14		DALT 14	
Commodity	**Walnut, treated leaves**		**Walnut, other tops**		**Walnut, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	*94.76*	n.a.	*81.60*	n.a.	*85.36*
AMPA	n.a.	*3.09*	n.a.	*1.70*	n.a.	*1.76*
Reference	CA 6.2.1/003, [redacted] 1976					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	100 µg per leaf surface of two trees		100 µg per leaf surface of two trees		100 µg per leaf surface of two trees	
Number of applications	1[1]		1[1]		1[1]	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 35		DALT 35		DALT 35	
Commodity	**Walnut, treated leaves**		**Walnut, other tops**		**Walnut, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	*63.22*	n.a.	*18.28*	n.a.	*41.76*
AMPA	n.a.	*6.56*	n.a.	*1.20*	n.a.	*1.92*
Reference	CA 6.2.1/003, [redacted] 1976					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	100 µg per leaf surface of two trees		100 µg per leaf surface of two trees		100 µg per leaf surface of two trees	
Number of applications	1[1]		1[1]		1[1]	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 35		DALT 35		DALT 35	
Commodity	**Almond, treated leaves**		**Almond, other tops**		**Almond, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	*41.58*	n.a.	*13.70*	n.a.	*62.65*
AMPA	n.a.	*4.32*	n.a.	*2.47*	n.a.	*4.66*

Table 6.7.1-1: Identified components of the radioactive residues of glyphosate in fruit crops – tree nut (primary crop)

Reference	CA 6.2.1/003, [redacted] 1976					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	100 µg per leaf surface of two trees		100 µg per leaf surface of two trees		100 µg per leaf surface of two trees	
Number of applications	1[1]		1[1]		1[1]	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 35		DALT 35		DALT 35	
Commodity	**Pecan, treated leaves**		**Pecan, other tops**		**Pecan, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	*62.06*	n.a.	*61.74*	n.a.	*59.85*
AMPA	n.a.	*4.32*	n.a.	*1.4*	-	-

n.a.: not available. Recalculation in mg/kg expressed in glyphosate equivalents is not possible based on the info given in the report.
TRR: Total radioactive residue
DALT: days after last treatment
[1] In a separate experiment, soil application was performed in the same growth chamber as this experiment, leading to high residues in control samples
mg/kg: mg/kg expressed as glyphosate parent equivalents
values given in *italics* were recalculated upon dossier compilation

Table 6.7.1-2: Identified components of the radioactive residues of glyphosate in fruit crops – apple (primary crop)

Reference	CA 6.2.1/004, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10 µg per leaf		10 µg per leaf		10.7 µg per leaf	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 21		DALT 28	
Commodity	**Apple, treated leaves**		**Apple, treated leaves**		**Apple, treated leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*128.1*	*88.73*	*119.6*	*91.16*	*94.23*	*96.09*
AMPA/*N*-methyl AMPA	*0.99*	*0.69*	*0.94*	*0.72*	*3.97*	*4.05*

Table 6.7.1-2: Identified components of the radioactive residues of glyphosate in fruit crops – apple (primary crop)

Reference	CA 6.2.1/004, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10 µg per leaf		10 µg per leaf		10.7 µg per leaf	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 28		DALT 49		DALT 70	
Commodity	**Apple, treated leaves**		**Apple, treated leaves**		**Apple, treated leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*126.1*	*92.69*	*108.1*	*90.60*	*103.3*	*83.85*
AMPA/*N*-methyl AMPA	*3.74*	*2.75*	*6.68*	*5.59*	*7.949*	*6.45*
Reference	CA 6.2.1/004, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10 µg per leaf		10 µg per leaf		10.7 µg per leaf	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 21		DALT 28	
Commodity	**Apple, new growth above treatment (leaves and stem)**		**Apple, new growth above treatment (leaves and stem)**		**Apple, new growth above treatment (leaves and stem)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*1.563*	*85.91*	*1.485*	*101.3*	1.021	*94.96*
AMPA/*N*-methyl AMPA	*0.009*	*0.47*	*0.010*	*0.67*	*0.002*	*0.21*
Reference	CA 6.2.1/004, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10 µg per leaf		10 µg per leaf		10.7 µg per leaf	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 28		DALT 49		DALT 70	
Commodity	**Apple, new growth above treatment (leaves and stem)**		**Apple, new growth above treatment (leaves and stem)**		**Apple, new growth above treatment (leaves and stem)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*1.842*	*89.79*	*1.072*	*87.42*	*1.153*	*87.14*
AMPA/*N*-methyl AMPA	*0.045*	*2.21*	*0.030*	*2.46*	*0.067*	*5.04*

Table 6.7.1-2: Identified components of the radioactive residues of glyphosate in fruit crops – apple (primary crop)

Reference	CA 6.2.1/004, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10 µg per leaf		10.7 µg per leaf		10 µg per leaf	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 21		DALT 28		DALT 28	
Commodity	**Apple, other new growth (leaves and stem)**		**Apple, other new growth (leaves and stem)**		**Apple, other new growth (leaves and stem)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*0.980*	*87.30*	*0.346*	*66.82*	*0.783*	*84.32*
AMPA/*N*-methyl AMPA	*0.008*	*0.69*	*0.005*	*1.03*	*0.008*	*0.83*

Reference	CA 6.2.1/004, [redacted] 1974			
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10.7 µg per leaf		10 µg per leaf	
Number of applications	1		1	
Application route	Foliar		Foliar	
Growth stage at sampling	DALT 49		DALT 70	
Commodity	**Apple, other new growth (leaves and stem)**		**Apple, other new growth (leaves and stem)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*0.363*	*92.20*	*0.368*	*95.08*
AMPA/*N*-methyl AMPA	*0.011*	*2.89*	*0.011*	*2.72*

Reference	CA 6.2.1/004, [redacted] 1974			
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	10.7 µg per leaf		10 µg per leaf	
Number of applications	1		1	
Application route	Foliar		Foliar	
Growth stage at sampling	DALT 28		DALT 28	
Commodity	**Apple, trunk and branches**		**Apple, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*0.014*	*64.43*	*0.027*	*66.39*

TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
DALT: days after last treatment
values given in *italics* were recalculated upon dossier compilation

Table 6.7.1-3: Identified components of the radioactive residues of glyphosate in fruit crops – grape (primary crop)

Reference	CA 6.2.1/005, [redacted] 1991	
Label	*N*-(phosphono-^{14}C-methyl)glycine (as trimesium salt)	
Application rate	9.9 mg glyphosate per 10 bunches per vine	
Number of applications	1	
Application route	Spray	
Growth stage at sampling	DALT 14 (maturity)	
Commodity	**Grape fruit**	
	mg/kg	**% TRR**
Glyphosate	**0.964**	77.1
AMPA	0.031	2.5

TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
DALT: days after last treatment
Grey: Food
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).

Table 6.7.1-4: Identified components of the radioactive residues of glyphosate in fruit crops – grape (primary crop)

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Concord Treated leaves**		**Grape Concord Treated leaves**		**Grape Concord Treated leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	**n.a.**	**74.8**	n.a.	**70.5**	**n.a.**	**78.8**
AMPA	**n.a.**	3.9	n.a.	1.5	**n.a.**	4.4

Table 6.7.1-4: Identified components of the radioactive residues of glyphosate in fruit crops – grape (primary crop)

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Concord New growth**		**Grape Concord New growth**		**Grape Concord New growth**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	74.3	n.a.	78.1	n.a.	100.5
AMPA	n.a.	-	n.a.	-	n.a.	-

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		180 µg per 6 leaves (12 surfaces)		180 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 28		DALT 28		DALT 28	
Commodity	**Grape Concord Roots and old stock**		**Grape Concord New growth**		**Grape Concord Grape fruit**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	87.6	n.a.	74.6	*0.01*	**64.6**
AMPA	-	-	-	-	-	-

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Sauvignon Blanc Treated leaves**		**Grape Sauvignon Blanc Treated leaves**		**Grape Sauvignon Blanc Treated leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	87.7	n.a.	88.2	n.a.	84.3
AMPA	n.a.	2.8	n.a.	2.9	n.a.	9.2

Table 6.7.1-4: Identified components of the radioactive residues of glyphosate in fruit crops – grape (primary crop)

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Sauvignon Blanc New growth**		**Grape Sauvignon Blanc New growth**		**Grape Sauvignon Blanc New growth**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	103.1	n.a.	80.1	n.a.	84.3
AMPA	-	-	n.a.	1	n.a.	2

Reference	CA 6.2.1/007, [redacted] 1974			
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1	
Application route	Foliar		Foliar	
Growth stage at sampling	DALT 28		DALT 28	
Commodity	**Grape Sauvignon Blanc Grape fruit**		**Grape Sauvignon Blanc Roots and old stock**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	79.5	n.a.	90.2
AMPA	n.a.	< 1.0	-	-

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Thompson seedless Treated leaves**		**Grape Thompson seedless Treated leaves**		**Grape Thompson seedless Treated leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	97.1	n.a.	89.6	n.a.	70.6
AMPA	-	-	-	-	n.a.	1.8

Table 6.7.1-4: Identified components of the radioactive residues of glyphosate in fruit crops – grape (primary crop)

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Thompson seedless New growth**		**Grape Thompson seedless New growth**		**Grape Thompson seedless New growth**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	88.5	n.a.	89.3	n.a.	74.4
AMPA	n.a.	1.2	n.a.	≤2.0	n.a.	≤1.0
Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces) for 7 days		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 28		DALT 7		DALT 14	
Commodity	**Grape Thompson seedless Roots and old stock**		**Grape Concord Treated leaves**		**Grape Concord Treated leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	87.7	n.a.	85.6	n.a.	93.8
AMPA	-	-	n.a.	3.4	n.a.	2.5

Table 6.7.1-4: Identified components of the radioactive residues of glyphosate in fruit crops – grape (primary crop)

Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces) for 7 days		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 7		DALT 14		DALT 28	
Commodity	**Grape Concord New growth**		**Grape Concord New growth**		**Grape Concord New growth**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	98.5	n.a.	82.9	n.a.	77.1
AMPA	n.a.	1.0	n.a.	1.0	n.a.	1.0
Reference	CA 6.2.1/007, [redacted] 1974					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	120 µg per 6 leaves (12 surfaces)		120 µg per 6 leaves (12 surfaces) for 7 days		120 µg per 6 leaves (12 surfaces)	
Number of applications	1		1		1	
Application route	Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 42		DALT 56		DALT 70	
Commodity	**Grape Concord New growth**		**Grape Concord New growth**		**Grape Concord New growth**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	82.8	n.a.	70.4	n.a.	58.5
AMPA	n.a.	1.0	n.a.	1.0	n.a.	1.0

TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
DALT: days after last treatment
Grey: Food
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).

Crop category root and tuber crops

Table 6.7.1-5: Identified components of the radioactive residues of glyphosate in root and tuber crops – potato (primary crop)

Reference	CA 6.2.1/008, [redacted] 1975					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		Amino-^{14}C-methyl-phosphonic acid	
Application rate	23.8 mg/pot		23.3 mg/pot		23.9 mg/pot	
Number of applications	1		1		1	
Application route	Soil		Soil & foliar to weeds, followed by incorporation of weeds after 3 weeks		Soil	
Growth stage at sampling	DALT 67		DALT 67		DALT 67	
Commodity	Potato tuber		Potato tuber		Potato tuber	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate[1]	-	-		-	-	-
AMPA	n.a.	***35.3***	n.a.	***31.0***	n.a.	***44.8***

Reference	CA 6.2.1/008, [redacted] 1975	
Label	*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	23.8 mg ^{14}C-glyphosate per pot	
Number of applications	1	
Application route	Soil, pre-emergence	
Growth stage at sampling	DALT 67/101	
Commodity	Potato tuber	
	mg/kg	**% TRR**
Glyphosate	-	-
AMPA	n.a.	*6.6*

TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
n.a.: not available
DALT: days after last treatment
1 only characterised
Grey: Food
Blue: Feed
Values given in *italics* were recalculated upon dossier compilation.
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).

Crop category cereal/grass crops

Table 6.7.1-6: Identified components of the radioactive residues of glyphosate in cereals / grass crops – wheat (primary crop)

Reference	CA 6.2.1/010, [redacted] 1989					
Label	*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt		*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt		*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt	
Application rate	5.640 kg ha glyphosate-trimesium corresponding to 3.89 kg a.s./ha expressed as glyphosate equivalents		5.640 kg ha glyphosate-trimesium corresponding to 3.89 kg a.s./ha expressed as glyphosate equivalents		5.640 kg ha glyphosate-trimesium corresponding to 3.89 kg a.s./ha expressed as glyphosate equivalents	
Number of applications	1		1		1	
Application route	Foliar spray		Foliar spray		Foliar spray	
Growth stage at sampling	DALT 7		DALT 7		DALT 7	
Commodity	Wheat grain		Wheat chaff		Wheat straw	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	2.43	90.8	37.4	85.0	102.60	82.6
AMPA	**0.08**	2.8	1.2	3.9	4.10	3.3

TRR Total radioactive residue
DALT Days after last treatment
mg/kg mg/kg expressed as glyphosate parent equivalents
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Metabolism experiments with more "artificial" setups, i.e. the hydroponic experiments

Table 6.7.1-7: Identified components of the radioactive residues of glyphosate in cereals / grass crops – barley, oats, rice, sorghum (primary crop)

Reference	CA 6.2.1/011, [redacted] 1974							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	0.183 mg/mL		0.183 mg/mL		0.183 mg/mL		0.183 mg/mL	
Number of applications	1		1		1		1	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 28		DALT 28		DALT 28	
Commodity	Barley aerial parts		Oats aerial parts		Rice aerial parts		Sorghum aerial parts	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	***0.418***	73.25	***0.541***	76.63	*2.076*	73.75	***0.165***	76.23
AMPA	***0.080***	13.97	*0.046*	6.51	*0.243*	8.62	***0.027***	12.67
N-methyl AMPA	*0.020*	3.50	*0.012*	1.69	*0.040*	1.41	*0.012*	5.43

Table 6.7.1-7: Identified components of the radioactive residues of glyphosate in cereals / grass crops – barley, oats, rice, sorghum (primary crop)

Reference	CA 6.2.1/011, [redacted] 1974							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	0.183 mg/mL		0.183 mg/mL		0.183 mg/mL		0.183 mg/mL	
Number of applications	1		1		1		1	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 28		DALT 28		DALT 28	
Commodity	**Barley roots**		**Oats roots**		**Rice roots**		**Sorghum roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*3.221*	52.60	*2.312*	35.70	*0.703*	19.10	*0.766*	44.80
AMPA	*0.231*	3.77	*0.165*	2.54	*0.273*	7.42	*0.037*	2.18
N-methyl AMPA	*0.027*	0.43	*0.071*	1.09	*0.058*	1.56	*0.008*	0.50

DALT Days after last treatment
TRR Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Blue: Feed

Table 6.7.1-8: Identified components of the radioactive residues of glyphosate in cereals / grass crops – maize (primary crop)

Reference	CA 6.2.1/012, [redacted] 1973							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1 (28 days duration)		1 (28 days duration)		1 (28 days duration)		1 (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 12		DALT 20		DALT 28	
Commodity	**Maize forage**		**Maize forage**		**Maize forage**		**Maize forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**70.6**	n.a.	**19.7**	n.a.	**23.7**	n.a.	**28.1**
AMPA	-	-	n.a.	**16.2**	n.a.	**22.2**	n.a.	**27.0**
N-methyl AMPA	-	-	n.a.	4.2	n.a.	1.9	n.a.	2.0

Table 6.7.1-8: Identified components of the radioactive residues of glyphosate in cereals / grass crops – maize (primary crop)

Reference	CA 6.2.1/012, [redacted] 1973							
Label	*N*-(phosphono-^{14}C-methyl)glycine							
Application rate	24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine							
Number of applications	1 (28 days duration)							
Application route	Hydroponic							
Growth stage at sampling	DALT 28							
Commodity	**Maize forage**							
	mg/kg				**% TRR**			
Glyphosate	n.a.				**21.1**			
AMPA	n.a.				***27.9***			
Reference	CA 6.2.1/012, [redacted] 1973							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1 (28 days duration)		1 (28 days duration)		1 (28 days duration)		1 (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 12		DALT 20		DALT 28	
Commodity	**Maize roots**		**Maize roots**		**Maize roots**		**Maize roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	61.1	n.a.	56.6	n.a.	45.5	n.a.	40.8
AMPA	n.a.	7.4	n.a.	10.1	n.a.	8.4	n.a.	8.0
N-methyl AMPA	-	-	-	-	n.a.	0.6	-	-
Reference	CA 6.2.1/012, [redacted] 1973							
Label	*N*-(phosphono-^{14}C-methyl)glycine							
Application rate	24 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine							
Number of applications	1 (28 days duration)							
Application route	Hydroponic							
Growth stage at sampling	DALT 28							
Commodity	**Maize roots**							
	mg/kg				**% TRR**			
Glyphosate	n.a.				38.1			
AMPA	n.a.				4.4			
N-methyl AMPA	-				-			

Table 6.7.1-8: Identified components of the radioactive residues of glyphosate in cereals / grass crops – maize (primary crop)

DALT Days after last treatment
TRR Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Blue: Feed

Table 6.7.1-9: Identified components of the radioactive residues of glyphosate in cereals / grass crops – wheat (primary crop)

Reference	CA 6.2.1/012, [redacted] 1973					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	72 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		72 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		72 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1 (10 days duration)		1 (10 days duration)		1 (10 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 10		DALT 10	
Commodity	**Wheat forage**		**Wheat forage**		**Wheat forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**69.3**	n.a.	**70.7**	n.a.	**55.3**
AMPA	n.a.	8.0	n.a.	6.6	n.a.	*6.0*
Reference	CA 6.2.1/012, [redacted] 1973					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	72 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		72 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine		72 plants in 5 L hydroponic solution, 3 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1 (10 days duration)		1 (10 days duration)		1 (10 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 10		DALT 10	
Commodity	**Wheat root**		**Wheat root**		**Wheat root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**33.5**	n.a.	**38.5**	n.a.	***65.1***
AMPA	n.a.	8.8	n.a.	5.2	n.a.	*6.0*
N-methyl AMPA	-	-	n.a.	2.0	-	-

DALT Days after last treatment
TRR Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Blue: Feed

Crop category pulses and oilseeds

Table 6.7.1-10: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/014, [redacted], 1992					
Label	*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt		*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt		*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt	
Application rate	8.4 kg a.s./ha		8.4 kg a.s./ha		8.4 kg a.s./ha	
Number of applications	1		1		1	
Application route	Soil drench		Soil drench		Soil drench	
Growth stage at sampling	DALT 31		DALT 97 (maturity)		DALT 97 (maturity)	
Commodity	Soybean forage		Soybean straw		Soybean hull	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.058	3.30	0.005	0.57	0.020	4.10
AMPA	0.100	5.70	0.023	2.70	0.007	1.50
Reference	CA 6.2.1/014, [redacted], 1992					
Label	*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt		*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt		*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt	
Application rate	8.4 kg a.s./ha		8.4 kg a.s./ha		8.4 kg a.s./ha	
Number of applications	1		1		1	
Application route	Soil drench		Soil drench		Soil drench	
Growth stage at sampling	DALT 97		DALT 97 (maturity)		DALT 97 (maturity)	
Commodity	Soybean hay		Soybean seed green		Soybean seed yellow	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.02	2.08	0.020	2.60	0.034	2.60
AMPA	0.02	1.97	0.012	1.60	0.021	1.60

TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Grey: Food
Blue: Feed

Metabolism experiments with more "artificial" setups, i.e. the hydroponic experiments

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1 (28 days duration)		1 (28 days duration)		1 (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 12		DALT 20		DALT 28	
Commodity	**Soybean forage**		**Soybean forage**		**Soybean forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**65.4**	n.a.	**78.5**	n.a.	**70.3**
AMPA	n.a.	5.4	n.a.	3.8	n.a.	5.1

Reference	CA 6.2.1/015, [redacted] 1973									
Label	*N*-(phosphono-^{14}C-methyl)glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine		N-(phosphono-methyl)-^{14}C-carboxy-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine	
Number of applications	1 (28 days duration)		1 (28 days duration)		1 (28 days duration)		1 (28 days duration)		1 (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 6		DALT 12		DALT 20		DALT 28	
Commodity	**Soybean forage**		**Soybean forage**		**Soybean forage**		**Soybean forage**		**Soybean forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**63.1**	n.a.	**53.1**	n.a.	**65.8**	n.a.	**69.6**	n.a.	**82.6**
AMPA	n.a.	9.2	-	-	-	-	-	-	-	-

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973							
Label	N-(phosphono-methyl)-^{14}C-methyl-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine		N-(phosphono-methyl)-^{14}C-methyl-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine	
Number of applications	1 (25 days duration)		1 (25 days duration)		1 (25 days duration)		1 (25 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 12		DALT 20		DALT 25	
Commodity	**Soybean forage**		**Soybean forage**		**Soybean forage**		**Soybean forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**66.8**	n.a.	**78.6**	n.a.	**71.6**	n.a.	**85.5**
Reference	CA 6.2.1/015, Rueppel, 1973							
Label	N-(phosphono-^{14}C-methyl)glycine & N-(phosphono-^{13}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine & N-(phosphono-^{13}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine & N-(phosphono-^{13}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine & N-(phosphono-^{13}C-methyl)glycine	
Application rate	198 plants in 20 L hydroponic solution, 2.96 mg *N*-(phosphono-^{14}C-methyl)glycine & 50.12 mg *N*-(phosphono-^{13}C-methyl)glycine		198 plants in 20 L hydroponic solution, 2.96 mg *N*-(phosphono-^{14}C-methyl)glycine & 50.12 mg *N*-(phosphono-^{13}C-methyl)glycine		198 plants in 20 L hydroponic solution, 2.96 mg *N*-(phosphono-^{14}C-methyl)glycine & 50.12 mg *N*-(phosphono-^{13}C-methyl)glycine		198 plants in 20 L hydroponic solution, 2.96 mg *N*-(phosphono-^{14}C-methyl)glycine & 50.12 mg *N*-(phosphono-^{13}C-methyl)glycine	
Number of applications	1 (26 days duration)		1 (26 days duration)		1 (26 days duration)		1 (26 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 12		DALT 20		DALT 26	
Commodity	**Soybean forage**		**Soybean forage**		**Soybean forage**		**Soybean forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**60.6**	n.a.	**54.4**	n.a.	**56.2**	n.a.	**55.1**
AMPA/*N*-methyl AMPA	n.a.	**16.6**	n.a.	7.3	n.a.	5.2	n.a.	7.8

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973							
Label	N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine	
Application rate	24 plants in 5 L hydroponic solution, 11.85 mg *N*-(phosphono-^{14}C-methyl)glycine pulse treatment for first 6 days only		24 plants in 5 L hydroponic solution, 11.85 mg *N*-(phosphono-^{14}C-methyl)glycine pulse treatment for first 6 days only		24 plants in 5 L hydroponic solution, 11.85 mg *N*-(phosphono-^{14}C-methyl)glycine pulse treatment for first 6 days only		24 plants in 5 L hydroponic solution, 11.85 mg *N*-(phosphono-^{14}C-methyl)glycine pulse treatment for first 6 days only	
Number of applications	1, pulse treatment for first 6 days only (56 days duration)		1, pulse treatment for first 6 days only (56 days duration)		1, pulse treatment for first 6 days only (56 days duration)		1, pulse treatment for first 6 days only (56 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 12		DALT 20		DALT 28	
Commodity	**Soybean forage**		**Soybean forage**		**Soybean forage**		**Soybean forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**55.3**	n.a.	**46.5**	n.a.	**39.3**	n.a.	**29.4**
AMPA/*N*-methyl AMPA	n.a.	8.8	n.a.	4.7	n.a.	5.5	n.a.	5.8

Reference	CA 6.2.1/015, [redacted] 1973					
Label	N-(phosphono-^{14}C-methyl)glycine		*N*-(phosphonomethyl)-^{14}C-carboxy-glycine		*N*-(phosphonomethyl)-^{14}C-methyl-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg N (phosphonomethyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg N (phosphonomethyl)-^{14}C-methyl-glycine	
Number of applications	1 (28 days duration)		1 (28 days duration)		1 (25 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 28		DALT 25	
Commodity	**Soybean forage**		**Soybean forage**		**Soybean forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	**69.2**	n.a.	**57.1**	n.a.	**48.8**
AMPA	n.a.	9.0	-	-	-	-
N-methyl AMPA	n.a.	1.1	-	-	-	-

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973							
Label	N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine		N-(phosphono-^{14}C-methyl)glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1, (28 days duration)		1, (28 days duration)		1, (28 days duration)		1, (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 12		DALT 20		DALT 28	
Commodity	**Soybean root**		**Soybean root**		**Soybean root**		**Soybean root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	29.3	n.a.	54.1	n.a.	30.2	n.a.	44.3
AMPA	n.a.	2.3	n.a.	5.6	n.a.	3.5	n.a.	1.4
N-methyl AMPA	n.a.	1.0	-	-	-	-	-	-

Reference	CA 6.2.1/015, [redacted] 1973							
Label	N-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-methyl)-^{14}C-carboxy-glycine		*N*-(phosphono-methyl)-^{14}C-carboxy-glycine		*N*-(phosphono-methyl)-^{14}C-carboxy-glycine	
Application rate	99 plants in 20 L hydroponic solution, 50 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine	
Number of applications	1, (28 days duration)		1, (28 days duration)		1, (28 days duration)		1, (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 6		DALT 12		DALT 20	
Commodity	**Soybean root**		**Soybean root**		**Soybean root**		**Soybean root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	35.4	n.a.	35.0	n.a.	73.0	n.a.	59.3
AMPA	n.a.	3.0	-	-	-	-	-	-
N-methyl AMPA	n.a.	0.3	-	-	-	-	-	-
Methyl-phosphonic acid	n.a.	0.3	-	-	-	-	-	-

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973							
Label	*N*-(phosphono-methyl)-^{14}C-carboxy-glycine		*N*-(phosphono-methyl)-^{14}C-methyl-glycine		*N*-(phosphono-methyl)-^{14}C-methyl-glycine		*N*-(phosphono-methyl)-^{14}C-methyl-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine	
Number of applications	1, (28 days duration)		1, (25 days duration)		1, (25 days duration)		1, (25 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 6		DALT 12		DALT 20	
Commodity	**Soybean root**		**Soybean root**		**Soybean root**		**Soybean root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	65.0	n.a.	30.3	n.a.	53.7	n.a.	28.3
N-methyl AMPA	-	-	-	2.3	-	-	-	-

Reference	CA 6.2.1/015, [redacted] 1973							
Label	*N*-(phosphonomethyl)-^{14}C-methyl-glycine		N-(phosphono-^{14}C-methyl)glycine		*N*-(phosphonomethyl)-^{14}C-carboxy-glycine		*N*-(phosphonomethyl)-^{14}C-methyl-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-methyl-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphonomethyl)-^{14}C-carboxy-glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-methyl)-^{14}C-methyl-glycine	
Number of applications	1, (25 days duration)		1 (28 days duration)		1 (28 days duration)		1 (25 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 25		DALT 28		DALT 28		DALT 25	
Commodity	**Soybean root**		**Soybean root**		**Soybean root**		**Soybean root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	21.5	n.a.	37.3	n.a.	46.9	n.a.	28.3
AMPA	-	-	n.a.	3.1	-	-	-	-
N-methyl AMPA	-	-	n.a.	0.3	-	-	n.a.	0.4

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphonomethyl)-^{14}C-methyl-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1, (28 days duration)		1, (28 days duration)		1, (28 days duration)		1, (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 6		DALT 13		DALT 20		DALT 28	
Commodity	**Cotton forage**		**Cotton forage**		**Cotton forage**		**Cotton forage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	73.8	n.a.	80.0	n.a.	70.8	n.a.	70.5
AMPA	-	-	n.a.	4.6	n.a.	5.3	n.a.	8.0

Reference	CA 6.2.1/015, [redacted] 1973					
Label	*N*-(phosphonomethyl)-^{14}C-methyl-glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1, (28 days duration)		1, (28 days duration)		1, (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 28		DALT 6		DALT 13	
Commodity	**Cotton forage**		**Cotton root**		**Cotton root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	61.5	n.a.	38.8	n.a.	21.3
AMPA	n.a.	*6.8*	n.a.	8.9	n.a.	4.2
N-methyl AMPA	n.a.	*2.0*	-	-	n.a.	0.8

Table 6.7.1-11: Identified components of the radioactive residues of glyphosate in pulses and oilseeds – soybean (primary crop)

Reference	CA 6.2.1/015, [redacted] 1973					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphonomethyl)-^{14}C-methyl-glycine		*N*-(phosphonomethyl)-^{14}C-methyl-glycine	
Application rate	24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine		24 plants in 5 L hydroponic solution, 12 mg *N*-(phosphono-^{14}C-methyl)glycine	
Number of applications	1, (28 days duration)		1, (28 days duration)		1, (28 days duration)	
Application route	Hydroponic		Hydroponic		Hydroponic	
Growth stage at sampling	DALT 20		DALT 28		DALT 28	
Commodity	**Cotton root**		**Cotton root**		**Cotton root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.a.	24 5	n.a.	11.1	n.a.	7.6
AMPA	n.a.	4.4	n.a.	3.0	n.a.	2.8
N-methyl AMPA	n.a.	0.2	n.a.	0.4	n.a.	0.4
Methyl-phosphonic acid	n.a.	2.0	n.a.	0.4	-	-
N-methyl glyphosate	-	-	n.a.	0.3	-	-

DALT Days after last treatment
TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Blue: Feed

Crop category miscellaneous

Table 6.7.1-12: Identified components of the radioactive residues of glyphosate in miscellaneous crops – coffee (primary crop)

Reference	CA 6.2.1/016, [redacted] 1975							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	0.608 mg/plant		0.608 mg/plant		0.608 mg/plant		0.608 mg/plant	
Number of applications	1		1		1		1	
Application route	Lower leaf surface of lower branches treatment		Lower leaf surface of lower branches treatment		Lower leaf surface of lower branches treatment		Lower leaf surface of lower branches treatment	
Growth stage at sampling	DALT 5 weeks		DALT 5 weeks		DALT 5 weeks		DALT 5 weeks	
Commodity	**Coffee, treated leaves**		**Coffee stems**		**Coffee untreated leaves**		**Coffee, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*>30.35*	*>91.1*	*>3.69*	*>90.0*	*>0.45*	*>71.7*	*>4.09*	*>95.0*
AMPA/*N*-methyl AMPA	*<0.30*	*<0.9*	*<0.04*	*<0.9*	*<0.004*	*<0.7*	*<0.04*	*<1*

Table 6.7.1-12: Identified components of the radioactive residues of glyphosate in miscellaneous crops – coffee (primary crop)

Reference	CA 6.2.1/016, [redacted] 1975							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	1.9 mg/plant		1.9 mg/plant		1.9 mg/plant		1.9 mg/plant	
Number of applications	1		1		1		1	
Application route	Lower leaf surface of lower branches treatment		Lower leaf surface of lower branches treatment		Lower leaf surface of lower branches treatment		Lower leaf surface of lower branches treatment	
Growth stage at sampling	DALT 4 weeks		DALT 8 weeks		DALT 23 weeks		DALT 23 weeks	
Commodity	Coffee beans		Coffee beans		Coffee ripe beans		Coffee ripe pods	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	*0.091*	*98.0*	*0.054*	*97.0*	*0.134*	*91.2*	*0.199*	*94.0*
AMPA/*N*-methyl AMPA	*0.001*	*0.99*	*0.001*	*0.98*	*0.002*	*4.8*	*0.011*	*5.0*

Metabolism experiments with more "artificial" setups, i.e. the hydroponic experiments

Reference	CA 6.2.1/016, [redacted] 1975							
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	1.1 mg/L for 3 weeks		1.1 mg/L for 3 weeks		3.6 mg/L for 3 weeks		3.6 mg/L for 3 weeks	
Number of applications	1		1		1		1	
Application route	Hydroponic treatment		Hydroponic treatment		Hydroponic treatment		Hydroponic treatment	
Growth stage at sampling	DALT 3 weeks		DALT 3 weeks		DALT 3 weeks		DALT 3 weeks	
Commodity	Coffee roots		Coffee aerial		Coffee roots		Coffee aerial	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	*5.7*	*81.9*	*0.033*	*74.0*	*9.65*	*79.2*	*0.093*	*59.9*
AMPA/*N*-methyl AMPA	*0.1*	*8.1*	*0.005*	*12.0*	*0.95*	*7.8*	*0.022*	*14.1*

Reference	CA 6.2.1/016, [redacted] 1975			
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	11.1 mg/L for 3 weeks		11.1 mg/L for 3 weeks	
Number of applications	1		1	
Application route	Hydroponic treatment		Hydroponic treatment	
Growth stage at sampling	DALT 3 weeks		DALT 3 weeks	
Commodity	Coffee roots		Coffee aerial	
	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	*9.46*	*31.9*	*0.323*	*38.4*
AMPA/*N*-methyl AMPA	*1.81*	*6.1*	*0.081*	*9.6*

Table 6.7.1-12: Identified components of the radioactive residues of glyphosate in miscellaneous crops – coffee (primary crop)

DALT Days after last treatment
TRR Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Grey: Food
Values given in *italics* were recalculated upon dossier compilation.
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Genetically modified plants

CP4 EPSPS modified and CP4 EPSPS and GOX modified crops

Crop category root and tuber crops

Table 6.7.1-13: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops – sugar beet (primary crop)

Reference	CA 6.2.1/018, [redacted] 2000			
Label	*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	2x 1.08 kg a.s./ha		2x 1.08 kg a.s./ha	
Number of applications			2	
Application route	Foliar application (with soil protection)		Foliar application (with soil protection)	
Growth stage at sampling	DALT 91		DALT 91	
Commodity	Sugar beet tops		Sugar beet roots	
	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	2.74	79.65	1.33	95.31
AMPA	0.06	1.84	0.05	3.79

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Crop category cereals/grass crops

Table 6.7.1-14: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops – wheat (primary crop)

Reference	CA 6.2.1/019, [redacted] 2000							
Label	*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	2x 0.84 kg a.s./ha		2x 0.84 kg a.s./ha		2x 0.84 kg a.s./ha		2x 0.84 kg a.s./ha	
Number of applications	2		2		2		2	
Application route	Foliar		Foliar		Foliar		Foliar	
Growth stage at sampling	DALT 5		DALT 24		DALT 84		DALT 84	
Commodity	Wheat forage		Wheat hay		Wheat straw		Wheat grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	18.09	89.44	23.34	83.86	24.09	69.19	8.78	72.40
AMPA	0.15	0.76	0.96	3.45	1.77	5.08	**1.31**	**10.77**
N-glyceryl AMPA	-	-	-	-	-	-	0.04	0.34

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Table 6.7.1-15: Identified components of the radioactive residues of glyphosate in CP4 EPSPS and GOX modified crops – maize (corn) (primary crop)

Reference	CA 6.2.1/020, [redacted] 1995							
Label	*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha	
Number of applications	2		2		2		2	
Application route	Foliar (with soil protection)		Foliar (with soil protection)		Foliar (without soil protection)		Foliar (without soil protection)	
Growth stage at sampling	DALT 37		DALT 53		DALT 37		DALT 49	
Commodity	Maize forage		Maize silage		Maize forage		Maize silage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	10.8	80.9	7.09	77.9	7.77	71.9	6.43	67.1
AMPA	1.25	9.4	0.82	9.0	**1.72**	**15.9**	**1.26**	**13.1**
N-glyceryl AMPA	0.05	0.4	0.11	1.2	0.06	0.5	0.14	1.5

Table 6.7.1-15: Identified components of the radioactive residues of glyphosate in CP4 EPSPS and GOX modified crops – maize (corn) (primary crop)

Reference	KCA 6.2.1/020, [redacted] 1995							
Label	*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha	
Number of applications	2		2		2		2	
Application route	Foliar (with soil protection)		Foliar (with soil protection)		Foliar (without soil protection)		Foliar (without soil protection)	
Growth stage at sampling	DALT 83		DALT 83		DALT 83		DALT 83	
Commodity	Maize fodder		Maize grain		Maize fodder		Maize grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	**12.4**	83.3	0.05	7.4	**14.27**	**74.8**	0.03	2.6
AMPA	0.73	4.9	**0.37**	**54.1**	**2.13**	**11.2**	**0.63**	**60.3**
N-glyceryl AMPA	0.17	1.2	0.05	6.9	0.31	1.6	0.07	6.9

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10% of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10% of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Crop category pulses and oilseeds

Table 6.7.1-16: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops – oilseed rape (canola) (primary crop)

Reference	CA 6.2.1/021, [redacted] *et al.*, 1994			
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	0.455 kg a.s./ha		0.908 kg a.s./ha 0.905 kg a.s./ha	
Number of applications	1		1	
Application route	Foliar		Foliar	
Growth stage at sampling	DALT 87		DALT 79	
Commodity	Rape seed		Rape seed	
	mg/kg	% TRR	mg/kg	% TRR
AMPA	0.037	7.7	**0.58**	7.1
N-acetyl AMPA	0.004	0.9	**0.06**	0.7
N-glyceryl AMPA	0.017	3.4	**0.31**	3.9

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10% of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR), feed ≥ 10% of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Table 6.7.1-17: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops –soybean (primary crop)

Reference	CA 6.2.1/022, [redacted] *et al.*, 1994					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	0.84 kg a.s./ha		0.84 kg a.s./ha		0.84 kg a.s./ha	
Number of applications	1		1		1	
Application route	Early post-emergent		Early post-emergent		Early post-emergent	
Growth stage at sampling	DALT 35		DALT 63		DALT 83	
Commodity	Soybean forage		Soybean hay		Soybean seed	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	**0.764**	**88.5**	**0.354**	**64.7**	**0.041**	**10.1**
AMPA	**0.020**	2.3	0.029	5.3	**0.093**	**22.9**
N-methyl AMPA	-	-	0.003	0.6	-	-
N-acetyl AMPA	-	-	-	-	0.004	1.0
N-glyceryl AMPA	-	-	-	-	0.005	1.2
N-malonyl AMPA	-	-	-	-	0.003	0.9

Table 6.7.1-17: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops –soybean (primary crop)

Reference	CA 6.2.1/022, [redacted] *et al.*, 1994					
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	0.84 kg a.s./ha 1.68 kg a.s./ha		0.84 kg a.s./ha 1.68 kg a.s./ha		0.84 kg a.s./ha 1.68 kg a.s./ha	
Number of applications	2		2		2	
Application route	Sequential post-emergent		Sequential post-emergent		Sequential post-emergent	
Growth stage at sampling	DALT 13		DALT 41		DALT 61	
Commodity	Soybean forage		Soybean hay		Soybean seed	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	21.078	89.1	5.582	53.6	4.402	25.2
AMPA	1.619	6.8	1.328	12.8	8.579	49.1
N-methyl AMPA	0.140	0.6	0.130	1.3	0.131	0.8
N-acetyl AMPA	-	-	-	-	0.235	1.4
N-glyceryl AMPA			0.084	0.8	0.278	1.6
N-malonyl AMPA	-	-	-	-	0.309	1.8

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Table 6.7.1-18: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops – cotton (primary crop)

Reference	CA 6.2.1/023, [redacted] 1997			
Label	*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	0.93 kg a.s./ha 1.27 kg a.s./ha		0.93 kg a.s./ha 1.27 kg a.s./ha	
Number of applications	2		2	
Application route	Foliar (without soil protection)		Foliar (with soil protection)	
Growth stage at sampling	DALT 27		DALT 27	
Commodity	Cotton forage		Cotton forage	
	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	13.9	91.5	29.1	95.7
AMPA	0.243	1.60	0.201	0.66

Table 6.7.1-18: Identified components of the radioactive residues of glyphosate in CP4 EPSPS modified crops – cotton (primary crop)

Reference	CA 6.2.1/023, [redacted] 1997			
Label	*N*-(phosphono-^{13} and ^{14}C-methyl)glycine		*N*-(phosphono-^{13} and ^{14}C-methyl)glycine	
Application rate	0.93 kg a.s./ha 1.27 kg a.s./ha		0.93 kg a.s./ha 1.27 kg a.s./ha	
Number of applications	2		2	
Application route	Foliar (without soil protection)		Foliar (with soil protection)	
Growth stage at sampling	DALT 158		DALT 158	
Commodity	Cotton seed		Cotton seed	
	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	**0.022**	**12.0**	**0.025**	**23.7**
AMPA	<0.002	<1	0.001	1.4

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

GAT modified crops

Crops category cereals/grass crops

Table 6.7.1-19: Identified components of the radioactive residues of glyphosate in GAT modified crops – maize (corn) (primary crop)

Reference	CA 6.2.1/024, [redacted] 2007							
Label	*N*-(phosphono-^{14}C-methyl)glycine							
Application rate	4.37 kg a.s./ha soil application prior to emergence + 1.12, 1.11 and 1.10 kg a.s./ha foliar application							
Number of applications	3		4		4		4	
Application route	Soil and 2x foliar		Soil and 3x foliar		Soil and 3x foliar		Soil and 3x foliar	
Growth stage at sampling	V19, R5; 59 days after the second foliar application		Maturity R6; 7 days after the third foliar application		Maturity R6; 7 days after the third foliar application		Maturity R6; 7 days after the third foliar application	
Commodity	Maize forage		Maize stover		Maize cobs		Maize grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	**2.016**	**58.0**	**9.166**	**74.9**	-	-	<0.001	0.1
AMPA	0.140	4.0	0.422	3.4	-	-	0.016	6.1
N-acetyl glyphosate	**0.937**	**27.0**	**2.188**	**17.8**	0.435	63.8	**0.141**	**51.2**
N-acetyl AMPA	0.060	1.7	0.152	1.3	0.034	5.0	0.026	9.4

Table 6.7.1-19: Identified components of the radioactive residues of glyphosate in GAT modified crops – maize (corn) (primary crop)

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Crop category pulses and oilseeds

Table 6.7.1-20: Identified components of the radioactive residues of glyphosate in GAT modified crops – oilseed rape (canola) (primary crop)

Reference	CA 6.2.1/025, [redacted] 2010							
Label	*N*-(phosphono-^{14}C-methyl)glycine							
Application rate	4.502 kg a.s./ha to bare soil, 0.984 and 1.025 kg a.s./ha foliar						4.502 kg a.s./ha to bare soil, 0.984, 1.025 and 0.935 kg a.s./ha foliar	
Number of applications	3		3		3		4	
Application route	Soil and 2x foliar		Soil and 2x foliar		Soil and 2x foliar		Soil and 3x foliar	
Growth stage at sampling	BBCH 69 38 days after the second foliar application		BBCH 87; 90 days after the second foliar application, immediately prior to the last foliar application		BBCH 87; 90 days after the second foliar application, immediately prior to the last foliar application		Maturity, BBCH 89 7 days after the third foliar application	
Commodity	Rape immature foliage		Rape pods (with seeds)		Rape foliage		Rape seed	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.179	3.0	-	-	-	-	**0.448**	**20.8**
AMPA	0.084	1.4	-	-	-	-	0.041	1.9
N-acetyl glyphosate	**5.351**	**89.5**	1.013	79.6	**1.442**	93.0	**1.101**	**51.1**
N-acetyl AMPA	0.203	3.4					**0.316**	14.7

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Table 6.7.1-21: Identified components of the radioactive residues of glyphosate in GAT modified crops – soybean (primary crop)

Reference	CA 6.2.1/026, [redacted] 2007													
Label	*N*-(phosphono-^{14}C-methyl)glycine													
Application rate	3.290 kg a.s./ha		3.290 kg a.s./ha and 1.410 kg a.s./ha		3.290 kg a.s./ha (soil) and 1.410 + 2.284 kg a.s./ha (foliar)				3.290 kg a.s./ha (soil) and 1.410 + 2.284 + 0.880 kg a.s./ha (foliar)							
Number of applications	1		2		3				4					
Application route	Soil immediately prior to emergence		Soil immediately prior to emergence and 1 foliar application		Soil immediately prior to emergence and 2 foliar applications				Soil immediately prior to emergence and 3 foliar applications					
Growth stage at sampling	V6, unifoliolate and six trifoliolate leaves are fully developed		R1-R2 (open flower at any node on the main stem or open flower at one of the two uppermost nodes on the main stem with a fully developed leaf		R7 (one normal pod on the main stem that has reached its mature pod colour				R8 (95 % of the pods have reached their mature pod colour					
Commodity	Soybean forage		Soybean hay		Soybean seeds		Soybean foliage		Soybean seeds		Soybean pod		Soybean foliage	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.039	9.1	9.740	72.5	**0.434**	**22.7**	**4.894**	**43.6**	**0.102**	3.2	10.101	56.9	**11.791**	**53.4**
AMPA	0.166	39.3	0.704	5.3	**0.103**	5.3	0.819	7.4	**0.351**	**11.2**	1.794	10.2	**2.250**	**10.3**
N-acetyl glyphosate	0.009	1.9	2.581	19.2	**1.156**	**60.6**	**4.699**	**42.0**	**1.788**	**56.9**	4.906	27.7	**7.039**	**31.9**
N-acetyl AMPA	-	-	0.096	0.7	n.d.	n.d.	0.255	2.2	**0.738**	**23.5**	0.574	3.3	0.308	1.4

DALT Days after last treatment
mg/kg: mg/kg expressed as glyphosate parent equivalents
n.d.: not detected
TRR Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Processing (high temperature hydrolysis studies)

Three HTH studies were performed, one with on glyphosate (CA 6.5.1/01, [redacted] 2010), one with *N*-acetyl glyphosate (CA 6.5.1/02, [redacted] 2006) and one with AMPA and *N*-acetyl AMPA ([redacted] 2020).

Each compound was stable and no degradation products were identified.

Confined rotational crop studies

Table 6.7.1-22: Identified components of the radioactive residues of glyphosate in rotational crops (lettuce, wheat and radish) at plant back intervals of 30, 120 and 365 days

Reference	CA 6.6.1/001, [redacted] 1998									
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha	
Number of applications	1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	30		30		30		30		30	
Growth stage at sampling	DALT 75 (maturity)		DALT 120 (maturity)		DALT 120 (maturity)		DALT 120 (maturity)		DALT 75 (maturity)	
Commodity	Lettuce, leaves		Wheat, forage		Wheat, chaff		Wheat grain		Radish, roots	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	<0.05	n.c.	<0.05	n.c.	<0.05	n.c.	<0.05	n.c.	<0.05	n.c.
AMPA	<0.05	n.c.	0.2	n.c.	0.4	n.c.	0.3	n.c.	<0.05	n.c.

Reference	CA 6.6.1/001, [redacted] 1998									
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha	
Number of applications	1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	120		120		120		120		120	
Growth stage at sampling	DALT 165 (maturity)		DALT 210 (maturity)		DALT 210 (maturity)		DALT 210 (maturity)		DALT 165 (maturity)	
Commodity	Lettuce, leaves		Wheat, forage		Wheat, chaff		Wheat grain		Radish, roots	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	<0.05	n.c.	0.4	n.c.	0.3	n.c.	<0.05	n.c.	<0.05	n.c.
AMPA	<0.05	n.c.	0.1	n.c.	0.2	n.c.	0.2	n.c.	<0.05	n.c.

Table 6.7.1-22: Identified components of the radioactive residues of glyphosate in rotational crops (lettuce, wheat and radish) at plant back intervals of 30, 120 and 365 days

Reference	CA 6.6.1/001, [redacted] 1998									
Label	*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine		*N*-(phosphono-^{14}C-methyl)glycine	
Application rate	6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha		6.5 kg a.s./ha	
Number of applications	1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	365		365		365		365		365	
Growth stage at sampling	DALT 410 (maturity)		DALT 455 (maturity)		DALT 455 (maturity)		DALT 455 (maturity)		DALT 410 (maturity)	
Commodity	Lettuce, leaves		Wheat, forage		Wheat, chaff		Wheat grain		Radish, roots	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	<0.05	n.c.	<0.05	n.c.	0.06	n.c.	<0.05	n.c.	<0.05	n.c.
AMPA	<0.05	n.c.	<0.05	n.c.	<0.05	n.c.	<0.05	n.c.	<0.05	n.c.

DALT Days after last treatment
TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
n.c.: Calculation not possible due to missing details within the report
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)
Values given in *italics* were recalculated upon dossier compilation

Table 6.7.1-23: Identified components of the radioactive residues of glyphosate in rotational crops (lettuce, wheat and radish) at plant back intervals of 35, 63 and 308 days

Reference	CA 6.6.1/002, [redacted] 1993											
Label	*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt											
Application rate	5.617 kg a.s./ha glyphosate-trimesium corresponding to 3.87 kg a.s./ha expressed as glyphosate equivalents											
Number of applications	1		1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	35		35		35		35		35		35	
Growth stage at sampling	DALT 147 (maturity)		DALT 187 (maturity)		DALT 187 (maturity)		DALT 147 (milk stage)		DALT 132 (maturity)		DALT 132 (maturity)	
Commodity	Lettuce, leaves		Wheat, grain		Wheat, straw		Wheat forage		Radish, roots		Radish, tops	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.001	0.7	<0.001	n.d.	0.0002	0.4	0.0001	0.5	0.0004	1.8	0.0002	0.9
AMPA	0.015	20.4	0.026	34.0	0.006	11.7	0.005	20.5	0.002	8.7	0.002	12.3
Glyphosate/ AMPA	0.003	4.4	0.003	3.7	-	-	-	-	-	-	-	-
Glucose	0.007	10.0	0.025	32.3	-	-	-	-	-	-	-	-
Fructose	0.005	7.5	<0.001	n.d.	-	-	-	-	-	-	-	-
Malic acid	0.005	7.2	-	-	-	-	-	-	-	-	-	-
Reference	CA 6.6.1/002, [redacted] 1993											
Label	*N*-(phosphono-^{14}C-methyl)glycine as trimesium salt											
Application rate	Total rate of 9.51 kg a.s./ha glyphosate-trimesium corresponding to 6.56 kg a.s./ha expressed as glyphosate equivalents											
Number of applications	3		3		3		3		3		3	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	63		63		63		63		63		63	
Growth stage at sampling	DALT 175 (maturity)		DALT 215 (maturity)		DALT 215 (maturity)		DALT 175 (milk stage)		DALT 160 (maturity)		DALT 160 (maturity)	
Commodity	Lettuce, leaves		Wheat, grain		Wheat, straw		Wheat forage		Radish, roots		Radish, tops	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.001	0.9	0.002	2.3	0.0002	0.3	<0.001	n.d.	0.0004	1.7	0.0002	1.1
AMPA	0.024	18.5	0.024	25.8	0.008	12.7	0.007	20.5	0.002	11.0	0.002	9.5
Glyphosate/ AMPA	0.006	4.9	0.003	2.3	-	-	-	-	-	-	-	-
Glucose	0.033	26.0	0.037	39.8	0.002	3.5	-	-	-	-	-	-
Fructose	0.018	13.8	<0.001	n.d.	0.002	3.7	-	-	-	-	-	-
Malic acid	0.008	6.5	-	-	0.003	4.6	-	-	-	-	-	-

Table 6.7.1-23: Identified components of the radioactive residues of glyphosate in rotational crops (lettuce, wheat and radish) at plant back intervals of 35, 63 and 308 days

DALT Days after last treatment
TRR Total radioactive residue
mg/kg mg/kg expressed as glyphosate parent equivalents
n.d. not determined
n.a. not analysed as the found total TRR was low (<0.01 mg/kg).
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).
Values given in *italics* were recalculated upon dossier compilation.

For the third rotation (plant back interval of 308 days) only the TRR was determined by combustion. As residues found were at or below 0.01 mg/kg no further extraction / analysis were conducted.

Table 6.7.1-24: Identified components of the radioactive residues of glyphosate in rotational crops (lettuce, barley and carrot) at plant back intervals of 30, 119 and 364 days

Reference	CA 6.6.1/003, [redacted] 1990													
Label	*N*-(phosphono-^{14}C-methyl)glycine													
Application rate	4 x 1.64 kg a.s./ha													
Number of applications	1		1		1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	30		30		30		30		30		30		30	
Growth stage at sampling	DALT 70 (maturity)		DALT 90 (maturity)		DALT 105 (maturity)		DALT 125 (maturity)		DALT 125 (maturity)		DALT 154 (maturity)		DALT 154 (maturity)	
Commodity	Lettuce, leaves		Lettuce, leaves		Lettuce, leaves		Barley, straw		Barley, grain		Carrots, tops		Carrots, roots	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.0041	3.8	n.d.	n.d.	0.0028	2.9	0.0018	1.0	0.0184	9.8	n.d.	n.d.	n.d.	n.d.
AMPA	**0.0158**	**14.6**	0.0039	8.1	**0.0137**	**14.1**	0.0065	3.7	**0.0336**	**17.9**	0.0007	1.4	0.0041	11.1

Table 6.7.1-24: Identified components of the radioactive residues of glyphosate in rotational crops (lettuce, barley and carrot) at plant back intervals of 30, 119 and 364 days

Reference	CA 6.6.1/003, [redacted] 1990													
Label	*N*-(phosphono-^{14}C-methyl)glycine													
Application rate	4.16 kg a.s./ha													
Number of applications	1		1		1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	119		119		119		119		119		119		119	
Growth stage at sampling	DALT 147 (maturity)		DALT 167 (maturity)		DALT 181 (maturity)		DALT 314 (maturity)		DALT 314 (maturity)		DALT 210 (maturity)		DALT 210 (maturity)	
Commodity	**Lettuce, leaves**		**Lettuce, leaves**		**Lettuce, leaves**		**Barley, straw**		**Barley, grain**		**Carrots, tops**		**Carrots, roots**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.d.	n.d.	0.0009	1.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMPA	0.0027	4.6	0.0050	9.1	0.0046	12.4	0.0054	9.6	**0.0111**	**14.2**	0.0003	1.1	0.0014	8.2

Reference	CA 6.6.1/003, [redacted] 1990													
Label	*N*-(phosphono-^{14}C-methyl)glycine													
Application rate	4.16 kg a.s./ha													
Number of applications	1		1		1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	364		364		364		364		364		364		364	
Growth stage at sampling	DALT 399 (maturity)		DALT 425 (maturity)		DALT 455 (maturity)		DALT 412 (intermediate)		DALT 482 (maturity)		DALT 482 (maturity)		DALT 482 (maturity)	
Commodity	**Lettuce, leaves**		**Lettuce, leaves**		**Lettuce, leaves**		**Barley, forage**		**Barley, straw**		**Barley, grain**		**Carrots, roots and tops**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.a.	
AMPA	0.0076	13.3	0.0045	10.5	0.0056	20.0	0.0093	16.6	0.0047	7.7	0.0074	15.7		

DALT Days after last treatment
TRR: Total radioactive residue
mg/kg: **mg/kg expressed as glyphosate parent equivalents**
n.d.: **not determined**
n.a.: not analysed as the found TRR in extraction phase was low (<0.01 mg/kg).
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).
Values given in *italics* were recalculated upon dossier compilation.

Table 6.7.1-25: Identified components of the radioactive residues of glyphosate in primary crops (soybean, cabbage, wheat and beet) planted in a confined rotational crop study

Reference	CA 6.6.1/005, [redacted] 1978											
Label	*N*-(phosphono-^{14}C-methyl)glycine											
Application rate	4.48 kg a.s./ha (application was performed 3 days before planting of the primary crops)											
Number of applications	1		1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Scenario type [1]	A				B		C		D			
Growth stage at sampling	DALT 115 (maturity)				DALT 93 (maturity)		DALT 93 (maturity)		DALT 93 (maturity)			
Commodity	**Soybean, foliage**		**Soybean, pods**		**Cabbage**		**Wheat [3]**		**Beet, foliage**		**Beet, root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	Only limited information available [2]		Only limited information available [2]		0.008	6.2	0.362	9.9	0.008	1.7	0.022	7.1
AMPA					0.005	3.8	0.116	3.2	0.021	4.6	0.030	9.7

1 Each different scenario type stands for one planting set up (different planting order of crops).
2 The chromatograms of the extracts of soybean showed ^{14}C-activity eluting in the glyphosate and AMPA regions. Detailed information on chromatographic results on these sample materials are missing within the report.
DALT Days after last treatment
TRR Total radioactive residue
mg/kg mg/kg expressed as glyphosate parent equivalents
3 For wheat details on sampling were missing, it is assumed that whole plants were sampled without any separating.
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).
Values given in *italics* were recalculated upon dossier compilation.

Table 6.7.1-26: Identified components of the radioactive residues of glyphosate in rotational crops (cabbage, wheat and beet) at plant back intervals of 30, 120 and 365 days

Reference	CA 6.6.1/005, [redacted] 1978											
Label	*N*-(phosphono-^{14}C-methyl)glycine											
Application rate	Each application 4.48 kg a.s./ha (total rate of 8.96 kg a.s./ha) [2]											
Number of applications	2		2		2		2		2		2	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	30		30		30		30		30		30	
Scenario type [1]	A				B		C		D			
Growth stage at sampling	DALT 123 (maturity)				DALT 123 (maturity)		DALT 123 (maturity)		DALT 123 (maturity)			
Commodity	**Beet, foliage**		**Beet, root**		**Cabbage**		**Wheat[3]**		**Beet, foliage**		**Beet, root**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	0.005	*1.6*	0.018	*3.7*	0.007	*3.9*	0.046	*3.5*	0.002	*1.0*	0.012	*3.2*
AMPA	0.006	*1.9*	0.041	*8.4*	0.003	*1.7*	0.128	*9.8*	0.004	*2.0*	0.036	*9.7*

Reference	CA 6.6.1/005, [redacted] 1978											
Label	*N*-(phosphono-^{14}C-methyl)glycine											
Application rate	4.48 kg a.s./ha											
Number of applications	1		1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	120		120		120		120		120		120	
Scenario type [1]	A				B		C				D	
Growth stage at sampling	DALT 240 (maturity)				DALT 240 (maturity)		DALT 240 (maturity)				DALT 240 (maturity)	
Commodity	**Beet, foliage**		**Beet, root**		**Wheat[3]**		**Beet, foliage**		**Beet, root**		**Cabbage**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	<0.001	*2.0*	0.002	*2.5*	0.014	*1.3*	<0.001	*1.3*	0.002	*2.0*	0.008	*10.0*
AMPA	0.001	*2.0*	0.010	*12.5*	0.003	*0.3*	0.001	*1.3*	0.008	*8.0*	0.005	*6.3*

Table 6.7.1-26: Identified components of the radioactive residues of glyphosate in rotational crops (cabbage, wheat and beet) at plant back intervals of 30, 120 and 365 days

Reference	CA 6.6.1/005, [redacted] 1978									
Label	*N*-(phosphono-^{14}C-methyl)glycine									
Application rate	4.48 kg a.s./ha									
Number of applications	1		1		1		1		1	
Application route	Bare soil		Bare soil		Bare soil		Bare soil		Bare soil	
Plant back interval	365		365		365		365		365	
Scenario type [1]	A		B				C		D	
Growth stage at sampling	DALT 462 (maturity)		DALT 485 (maturity)				DALT 462 (maturity)		DALT 485 (maturity)	
Commodity	Cabbage		Beet, foliage		Beet, root		Cabbage		Wheat[3]	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.002	*6.7*	<0.001	*2.0*	0.002	*4.0*	0.004	*6.7*	<0.001	*0.5*
AMPA	0.002	*6.7*	0.002	*4.0*	0.004	*8.0*	0.002	*3.3*	<0.001	*0.5*

1 Each different scenario type stands for one planting set up (different planting order of crops).
2 For emergency crops (planted 30 days after first application) a second treatment was conducted before planting. Further information on timing of second application and formulation of test item for application is missing. Given plant back intervals refers to the time interval between first application and planting of emergency crops.
DALT Days after last treatment
TRR Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
3 For wheat details on sampling were missing, it is assumed that whole plants were sampled without any separating.
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).
Values given in *italics* were recalculated upon dossier compilation.

Table 6.7.1-27: Identified components of the radioactive residues of glyphosate in primary crops (pea, string bean, carrot and cabbage) planted in a confined rotational crop study

Reference	CA 6.6.1/006, [redacted] 1976											
Label	*N*-(phosphono-^{14}C-methyl)glycine											
Application rate	4.48 kg a.s./ha (application at the maximum plant growth of the primary crops)											
Number of applications	1											
Application route	Bare soil											
Soil type	Norfolk soil (sandy loam)											
Plot No.	A		B		C				D			
Growth stage at sampling	DALT 28 (maturity)		DALT 46 (maturity)		DALT 50 (maturity)				DALT 78 (maturity)			
Commodity	**Pea, tops**		**String bean**		**Carrot**		**Carrot, leaves**		**Cabbage**		**Cabbage, head**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	0.137	*62.1*	0.068	*54.2*	**0.038**	***34.5***	**0.051**	***30.2***	0.026	*32.1*	n.d.	*39.2*
AMPA	0.013	*5.9*	0.007	*6.0*	**0.006**	*5.6*	0.005	*3.2*	0.006	*7.0*	n.d.	*2.9*

Reference	CA 6.6.1/006, [redacted] 1976							
Label	*N*-(phosphono-^{14}C-methyl)glycine							
Application rate	4.48 kg a.s./ha (application at the maximum plant growth of the primary crops)							
Number of applications	1							
Application route	Bare soil							
Soil type	Ray soil (silt loam)							
Plot No.	E				F			
Growth stage at sampling	DALT 30 (maturity)				DALT 51 (maturity)			
Commodity	**String bean, leaves**		**String bean, pods**		**Carrot**		**Carrot, leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	0.012	*1.0*	0.014	*7.9*	0.008	*2.5*	*0.047*	*9.0*
AMPA	0.020	*1.9*	**0.041**	***22.5***	0.017	*5.6*	0.017	*3.3*

DALT Days after last treatment
TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
n.d. not determined
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).
Values given in *italics* were recalculated upon dossier compilation.

Table 6.7.1-28: Identified components of the radioactive residues of glyphosate in rotational crops (carrot, cabbage, corn, string bean, pea) at plant back intervals of between 29 to 101 days

Reference	CA 6.6.1/006, [redacted] 1976													
Label	*N*-(phosphono-^{14}C-methyl)glycine													
Application rate	4.48 kg a.s./ha													
Number of applications	1													
Application route	Bare soil													
Soil type	Norfolk soil (sandy loam)													
Plant back interval [1]	29-51						57-69							
Plot No.	A						B							
Growth stage at sampling	DALT 99 (maturity)		DALT 98 (maturity)				DALT 70 (intermediate)		DALT 110 (maturity)					
Commodity	Cabbage		Carrot, root		Carrot, leaves		Sweet corn, first harvest		Sweet corn, second harvest		Sweet corn, kernel		Sweet corn, cob	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.026	*46.4*	0.018	*19.6*	0.018	*21.1*	0.008	*12.0*	0.005	*5.6*	-	-	0.002	*3.2*
AMPA	0.002	*4.5*	0.005	*5.6*	0.002	*2.3*	0.003	*11.1*	0.008	*9.0*	-	-	0.005	*9.0*

Reference	CA 6.6.1/006, [redacted] 1976													
Label	N-(phosphono-14C-methyl)glycine													
Application rate	4.48 kg a.s./ha													
Number of applications	1													
Application route	Bare soil													
Soil type	Norfolk soil (sandy loam)													
Plant back interval [1]	51-73						79-101							
Plot No.	C						D							
Growth stage at sampling	DALT 45 (maturity)		DALT 122 (maturity)				DALT 45 (intermediate)				DALT 122 (maturity)			
Commodity	Cabbage		String bean, leaves		String bean, pods		Carrot		Carrot, leaves		Pea, leaves		Pea, pod	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.004	*8.3*	0.019	*23.8*	0.016	*40.1*	0.003	*6.9*	0.006	*7.7*	0.076	*40.4*	0.128	*45.8*
AMPA	0.003	*6.7*	0.003	*3.5*	0.003	*7.9*	0.004	*7.6*	0.002	*2.9*	0.021	*11.1*	0.044	*15.6*

Table 6.7.1-28: Identified components of the radioactive residues of glyphosate in rotational crops (carrot, cabbage, corn, string bean, pea) at plant back intervals of between 29 to 101 days

Reference	CA 6.6.1/006, [redacted] 1976					
Label	*N*-(phosphono-^{14}C-methyl)glycine					
Application rate	4.48 kg a.s./ha					
Number of applications	1					
Application route	Bare soil					
Soil type	Ray soil (silt loam)					
Plant back interval [1]	52-74					
Plot No.	E					
Growth stage at sampling	DALT 122 (maturity)		DALT 122 (maturity)			
Commodity	**Cabbage**		**Carrot**		**Carrot, leaves**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
AMPA	0.004	*8.4*	0.003	*9.0*	0.001	*2.4*

1 Plant back interval in days (time interval between treatment and planting): PBI was calculated upon the statement in the report, that rotational crops were planted 1 - 23 days after harvesting the primary crops: DALT of primary crop + 1 day (min) or + 23 days (max). A more detailed calculation is not possible due to missing details within the report.

DALT Days after last treatment
TRR: Total radioactive residue
mg/kg: mg/kg expressed as glyphosate parent equivalents
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR); feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg).
Values given in *italics* were recalculated upon dossier compilation.

Livestock studies

Laying hens

Table 6.7.1-29: Identified components of the radioactive residues of glyphosate in poultry (dosing with glyphosate / AMPA)

Reference	CA 6.2.2/02, [redacted] 1988 + CA 6.2.2/03, [redacted]													
Label	*N*-(phosphono-$^{13}C/^{14}C$-methyl)glycine and amino-$^{13}C/^{14}C$-methylphosphonic acid													
Dose (mg/kg bw/day)	9.84 (8.86 glyphosate/0.98 AMPA)													
Dosing days	7 (once daily)		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily	
Commodity	**Kidney**		**Liver**		**Gizzard**		**Fat**		**Thigh muscle**		**Breast muscle**		**Egg yolk (day 1- sacrifice)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*1.663*	92.0	*0.384*	68.6	*0.210*	59.6	*0.014*	71.6	*0.018*	68.1	*0.011*	63.1	*0.071*	67.1
AMPA	*0.084*	4.6	*0.150*	26.7	*0.132*	37.4	*0.002*	10.6	*0.003*	12.7	*0.002*	13.8	*0.014*	13.1
Reference	CA 6.2.2/02, [redacted] 1988 + CA 6.2.2/03, [redacted]													
Label	*N*-(phosphono-$^{13}C/^{14}C$-methyl)glycine and amino-$^{13}C/^{14}C$-methylphosphonic acid													
Dose (mg/kg bw/day)	8.83 (7.95 glyphosate/0.88 AMPA)													
Dosing days	7 (once daily)		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily	
Commodity	**Kidney**		**Liver**		**Gizzard**		**Fat**		**Thigh muscle**		**Breast muscle**		**Egg yolk (day 1- sacrifice)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*1.596*	91.4	*0.340*	66.5	*0.199*	55.2	*0.011*	70.3	*0.018*	68.2	*0.012*	62.1	*0.054*	60.3
AMPA	*0.092*	5.3	*0.144*	28.1	*0.145*	40.1	*0.002*	11.7	*0.003*	13.2	*0.002*	11.6	*0.013*	14.3
Reference	CA 6.2.2/02, [redacted] 1988 + CA 6.2.2/03, [redacted]													
Label	*N*-(phosphono-$^{13}C/^{14}C$-methyl)glycine and amino-$^{13}C/^{14}C$-methylphosphonic acid													
Dose (mg/kg bw/day)	29.75 (26.78 glyphosate/2.98 AMPA)													
Dosing days	7 (once daily)		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily	
Commodity	**Kidney**		**Liver**		**Gizzard**		**Fat**		**Thigh muscle**		**Breast muscle**		**Egg yolk (day 1- sacrifice)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*6.528*	93.2	*1.225*	*64.0*	*0.645*	56.9	*0.048*	76.1	*0.067*	74.8	*0.039*	71.0	*0.218*	63.3
AMPA	*0.295*	4.2	*0.608*	*31.8*	*0.449*	39.6	*0.006*	10.1	*0.013*	14.8	*0.009*	17.3	*0.047*	13.7

Table 6.7.1-29: Identified components of the radioactive residues of glyphosate in poultry (dosing with glyphosate / AMPA)

Reference	CA 6.2.2/02, [redacted] 1988 + CA 6.2.2/03, [redacted]													
Label	*N*-(phosphono-$^{13}C/^{14}C$-methyl)glycine and amino-$^{13}C/^{14}C$-methylphosphonic acid													
Dose (mg/kg bw/day)	8.62 (7.76 glyphosate/0.86 AMPA)													
Dosing days	7 (once daily)		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily		7 (once daily	
Commodity	Kidney		Liver		Gizzard		Fat		Thigh muscle		Breast muscle		Egg yolk (day 1- sacrifice)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	***0.058***	83.9	***0.032***	**40.9**	*0.021*	66.7	*0.001*	28.1	*0.001*	37.9	*0.002*	41.1	***0.069***	**60.6**
AMPA	*0.007*	9.8	***0.042***	**53.1**	*0.005*	16.9	*0.001*	14.1	*0.001*	42.6	*0.003*	42.2	***0.013***	**11.1**

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)
Values in *italic* were calculated upon dossier compilation

Table 6.7.1-30: Identified components of the radioactive residues of glyphosate in poultry (dosing with glyphosate-trimesium)

Reference	CA 6.2.2/04, [redacted] 1994					
Label	*N*-(phosphono-^{14}C-methyl)glycine (as trimesium salt)					
Dose (mg/kg bw/day)	4.1/5.9 (as *N*-(phosphono-^{14}C-methyl)glycine or as trimesium salt)					
Dosing days	10 (once daily)		10 (once daily)		10 (once daily)	
Commodity	Liver		Thigh muscle		Breast muscle	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	**0.2684**	**60.97**	0.0245	61.00	**0.0114**	**39.05**
AMPA	**0.0992**	**22.53**	0.0016	4.06	0.0015	5.00
Reference	CA 6.2.2/04, [redacted] 1994					
Label	*N*-(phosphono-^{14}C-methyl)glycine (as trimesium salt)					
Dose (mg/kg bw/day)	4.1/5.9 (as *N*-(phosphono-^{14}C-methyl)glycine or as trimesium salt)					
Dosing days	10 (once daily)		10 (once daily)		10 (once daily)	
Commodity	Fat		Egg white (day 10)		Egg yolk (day 10)	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	0.0119	**40.66**	0.0043	21.48	**0.1429**	59.54
AMPA	0.0010	3.31	0.0002	0.82	0.0055	2.28

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)

Table 6.7.1-31: Identified components of the radioactive residues of glyphosate in poultry (dosing with glyphosate)

Reference	CA 6.2.2/01, [redacted] 1994											
Label	*N*-(phosphono-^{14}C-methyl)glycine											
Dose (mg/kg bw/day)	17.2											
Dosing days	5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)	
Commodity	**Liver**		**Skin**		**Fat**		**Muscle**		**Egg yolk (72 – 92 h)**		**Egg white (72 – 92 h)**	
	mg/kg	**% Total**	**mg/kg**	**% Total**	**mg/kg**	**% Total**	**mg/kg**	**% Total**	**mg/kg**	**% Total**	**mg/kg**	**% Total**
Glyphosate	***0.663*** ***0.966*** ***0.904***	**61.38** **89.41** **83.72**	***0.354*** ***0.342*** ***0.283***	98.65 95.34 78.78	***0.082*** ***0.052*** ***0.055***	**98.94** **62.21** **66.55**	***0.040*** ***0.040*** ***0.019***	**97.81** **97.91** **45.15**	***0.158*** ***0.119*** ***0.040***	**96.13** **72.60** **24.23**	- ***0.056*** ***0.056***	- **100** **100**
AMPA	- ***0.107*** ***0.154***	- **9.94** **14.26**	- ***0.003*** ***0.006***	- 0.79 1.76	-	-			-	-	-	-

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)
%values presented for this study are % total values, probably not %TRR; presented to show worst case; mg/kg values were calculated with % total values from the report, not recalculated; values in *italic* were calculated upon dossier compilation; the results of 3 different systems are shown (i.e. HPLC, TLC-system 1 and TLC-system 2)

Table 6.7.1-32: Identified components of the radioactive residues of glyphosate in poultry (dosing with *N*-acetyl glyphosate)

Reference	CA 6.2.2/05, [redacted] 2007									
Label	*N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine									
Dose (mg/kg bw/day)	3.513/4.393 (as *N*-(phosphono-^{14}C-methyl)glycine or as *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine)									
Dosing days	7 (twice daily)		7 (twice daily)		7 (twice daily)		7 (twice daily)		7 (twice daily)	
Commodity	**Egg white**		**Egg yolk**		**Liver**		**Muscle**		**Abdominal fat**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*0.001*	10.90	*0.010*	5.69	***0.067***	**16.34**	*0.002*	7.19	***0.018***	**39.43**
AMPA	-	-	*0.002*	**0.91**	*0.027*	6.74	*0.004*	16.69	*0.006*	11.29
N-acetyl glyphosate	*0.003*	41.48	***0.126***	**68.40**	***0.258***	**63.82**	*0.007*	25.22	***0.011***	**23.45**
N-acetyl AMPA	*<0.001*	4.34	*0.002*	1.10	*0.016*	4.04	*0.001*	1.89	*0.005*	10.18

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)
Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetyl glyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetyl glyphosate of 211.11 and molecular weight of glyphosate of 169.07.

Lactating goat

Table 6.7.1-33: Identified components of the radioactive residues of glyphosate in ruminants (dosing with glyphosate / AMPA)

Reference	CA 6.2.3/03, [redacted] 1988 + CA 6.2.3/04, [redacted]									
Label	*N*-(phosphono-$^{13}C/^{14}C$-methyl)glycine and amino-$^{13}C/^{14}C$-methylphosphonic acid									
Dose (mg/kg bw/day)	2.95 (2.65 glyphosate/0.29 AMPA)									
Dosing days	5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)	
Commodity	**Kidney**		**Liver**		**Muscle**		**Fat**		**Milk (day 1-sacrifice)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*6.429*	*89.6*	*0.384*	*77.8*	*0.020*	*74.5*	*0.008*	*83.7*	*0.009*	*47.8*
AMPA	*0.352*	*6.2*	*0.063*	*12.7*	*0.002*	*7.*	*0.001*	*9.6*	*0.001*	*4.9*
Reference	CA 6.2.3/03, [redacted] 1988 + CA 6.2.3/04, [redacted]									
Label	*N*-(phosphono-$^{13}C/^{14}C$-methyl)glycine and amino-$^{13}C/^{14}C$-methylphosphonic acid									
Dose (mg/kg bw/day)	2.83 (2.55 glyphosate/0.28 AMPA)									
Dosing days	5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)		5 (once daily)	
Commodity	**Kidney**		**Liver**		**Muscle**		**Fat**		**Milk (day 1-sacrifice)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*4.116*	81.5	*0.234*	**61.4**	*0.005*	51.8	*0.003*	75.8	*0.011*	**48.0**
AMPA	*0.67*	**13.4**	*0.117*	**30.7**	*0.001*	10.4	*0.000*	8.9	*0.002*	7.4

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)
Values in *italic* were calculated upon dossier compilation.

Table 6.7.1-34: Identified components of the radioactive residues of glyphosate in ruminants (dosing with glyphosate-trimesium)

Reference	CA 6.2.3/02, [redacted] 1994									
Label	*N*-(phosphono-^{14}C-methyl)glycine (as trimesium salt)									
Dose (mg/kg bw/day)	2.7/3.9 (as *N*-(phosphono-^{14}C-methyl)glycine or as trimesium salt)									
Dosing days	7 (twice daily)		7 (twice daily)		7 (twice daily)		7 (twice daily)		7 (twice daily)	
Commodity	**Liver**		**Kidney**		**Fat**		**Muscle**		**Milk (day 7)**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	0.139	59.4	**4.816**	**86.3**	0.029	91.3	**0.022**	**87.1**	0.005	22.3
AMPA	0.050	21.4	**0.418**	**7.5**	0.001	4.7	0.002	6.3	0.001	2.4
Lactose	-	-	-	-	-	-	-	-	*0.006*	25.2

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥0.01 mg/kg or ≥0.05 mg/kg (independent of %TRR)

Table 6.7.1-35: Identified components of the radioactive residues of glyphosate in ruminants (dosing with glyphosate)

Reference	CA 6.2.3/01, [redacted] 1994			
Label	*N*-(phosphono-^{14}C-methyl)glycine			
Dose (mg/kg bw/day)	6.4			
Dosing days	3 (twice daily)		3 (twice daily)	
Commodity	**Liver**		**Kidney**	
	mg/kg	**% Total**	**mg/kg**	**% Total**
Glyphosate	***0.215*** ***0.217*** ***0.216***	**95.52** **96.64** **95.89**	***11.777*** ***11.128*** ***11.431***	**96.93** **91.59** **94.08**
AMPA	-	-	- ***0.973*** ***0.595***	- 8.01 4.90

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)
%values presented for this study are % total values, probably not %TRR; presented to show worst case; mg/kg values were calculated with % total values from the report, not recalculated, values in *italic* were calculated upon dossier compilation, the results of 3 different systems are shown (i.e. HPLC, TLC-system 1 and TLC-system 2)

Table 6.7.1-36: Identified components of the radioactive residues of glyphosate in ruminants (dosing with *N*-acetyl glyphosate)

Reference	CA 6.2.3/05, [redacted] 2007							
Label	*N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine							
Dose (mg/kg bw/day)	6.74/8.42 (as *N*-(phosphono-^{14}C-methyl)glycine or as *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine)							
Dosing days	5 (twice daily)		5 (twice daily)		5 (twice daily)		5 (twice daily)	
Commodity	**Liver**		**Kidney**		**Milk**		**Muscle**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*0.095*	**14.71**	*0.194*	4.98	*≤0.001*	3.59	-	-
AMPA	*0.054*	8.45	-	-	*≤0.001*	[illegible]	-	-
N-acetyl glyphosate	***0.357***	**55.51**	***2.994***	**77.12**	*0.009*	**39.98**	***0.011***	**16.70**

Reference	CA 6.2.3/05, [redacted] 2007					
Label	*N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine					
Dose (mg/kg bw/day)	6.74/8.42 (as *N*-(phosphono-^{14}C-methyl)glycine or as *N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine)					
Dosing days	5 (twice daily)		5 (twice daily)		5 (twice daily)	
Commodity	**Omental fat**		**Renal fat**		**Subcutaneous fat**	
	mg/kg	**% TRR**	**mg/kg**	**% TRR**	**mg/kg**	**% TRR**
Glyphosate	*0.009*	6.03	*0.004*	5.02	*0.003*	2.65
AMPA	*≤0.001*	0.50	*≤0.001*	1.20	*0.006*	4.77
N-acetyl glyphosate	***0.032***	**21.43**	***0.062***	**73.19**	***0.072***	**64.73**
N-acetyl AMPA	*0.006*	4.31	*≤0.001*	0.59	***0.017***	**14.86**

TRR Total radioactive residue
Grey: Food
Emboldened values indicate major metabolites (≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR)
Values expressed as glyphosate equivalents. These values in italics were not included as part of the study report, but were calculated from the TRR expressed as *N*-acetyl glyphosate equivalents using a conversion factor of 0.8, based on molecular weight of *N*-acetyl glyphosate of 211.11 and molecular weight of glyphosate of 169.07.

Reference	CA 6.2.1/020 [redacted] 1995							
Label	*N* (phosphono-^{13}-and ^{14}C-methyl)glycine		*N* (phosphono-^{13}-and ^{14}C-methyl)glycine		*N* (phosphono-^{13}-and ^{14}C-methyl)glycine		*N* (phosphono-^{13}-and ^{14}C-methyl)glycine	
Application rate	0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha		0.93 kg a.s./ha 0.84 kg a.s./ha	
Number of applications	2		2		2		2	
Application route	Foliar (with soil protection)		Foliar (with soil protection)		Foliar (without soil protection)		Foliar (without soil protection)	
Growth stage at sampling	DALT 83		DALT 83		DALT 83		DALT 83	
Commodity	Maize fodder		Maize grain		Maize fodder		Maize grain	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Glyphosate	12.4	83.3	0.05	7.4	14.27	74.8	0.03	2.6
AMPA	0.72	4.9	0.37	54.1	2.13	11.2	0.63	60.3
N-glyceryl-AMPA	0.17	1.2	0.05	6.9	0.31	1.6	0.07	6.9

DALT = Days after last treatment
mg/kg = mg/kg expressed as glyphosate parent equivalents
TRR = Total radioactive residue
Grey: Food
Blue: Feed
Emboldened values indicate major metabolites (food ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg or ≥ 0.05 mg/kg (independent of %TRR), feed ≥ 10 % of the total radioactive residues (TRR) and ≥ 0.01 mg/kg)

Assessment

In the following the compounds **identified in plant metabolism and confined rotational crop studies** are summarised.

Non tolerant/conventional crops (relevant to the representative uses):

- Glyphosate
- Aminomethylphosphonic acid (AMPA)
- [(methylamino)methyl]phosphonic acid (*N*-methyl AMPA)
- Methyl-phosphonic acid
- 2-[methyl(phosphonomethyl)amino]acetic acid (*N*-methyl glyphosate)

Genetically modified/tolerant crops (not supported in the EU and only relevant to imported commodities):

- Glyphosate
- Aminomethylphosphonic acid (AMPA)
- [(methylamino)methyl]phosphonic acid (*N*-methyl AMPA) (CP4 EPSPS and GOX modified crops only)
- *N*-acetyl-*N*-(phosphonomethyl)glycine (*N*-acetyl glyphosate) (GAT modified crops only)
- [(acetylamino)methyl]phosphonic acid (*N*-acetyl AMPA)
- (2,3-dihydroxypropanoyl-amino)methylphosphonic acid (*N*-glyceryl AMPA) (CP4 EPSPS and GOX modified crops only)
- 3-oxo-3-(phosphonomethyl-amino)propanoic acid (*N*-malonyl AMPA) (CP4 EPSPS and GOX modified crops only)

The following compounds were **identified in animal metabolism studies**:

- Glyphosate
- Aminomethylphosphonic acid (AMPA)
- *N*-acetyl-*N*-(phosphonomethyl)glycine (*N*-acetyl glyphosate) (after gavage of *N*-acetyl glyphosate only)
- [(acetylamino)methyl]phosphonic acid (*N*-acetyl AMPA) (after gavage of *N*-acetyl glyphosate only)

Natural products identified in the different studies are not considered further as the compounds are of no toxicological concern can be excluded from further assessment.

Genotoxicity assessment

Genotoxicity classification

No harmonised classifications are available for the plant metabolites of glyphosate, however considering the available data with glyphosate and its major metabolite aminomethyl-phosphonic acid (AMPA), the compounds presented below of no toxicological concern:

Compound	Classification of genotoxicity[1)]
N-acetyl glyphosate	Not available
N-methyl glyphosate	Not available
AMPA	Not available
N-methyl AMPA	Not available
N-acetyl AMPA	Not available
N-glyceryl AMPA	Not available
N-malonyl AMPA	Not available
Methyl-phosphonic acid	Not available

1) According to Regulation (EC) 1272/2008

Assessment of the genotoxic potential based on available studies with the parent compound or with the metabolites

Compound	Bacterial reverse mutation	Mammalian forward mutation	Chromosome aberration	Micronucleous (in vivo)
Glyphosate	Yes	Yes	Yes	Yes
N-acetyl glyphosate	Yes	Yes	Yes	Yes
N-methyl glyphosate	Yes	No	No	No
AMPA	Yes	Yes	No	Yes
N-methyl AMPA	No	No	No	No
N-acetyl AMPA	Yes	Yes	Yes	Yes
N-glyceryl AMPA	No	No	No	No
N-malonyl AMPA	No	No	No	No
Methyl-phosphonic acid	No	No	No	No

Glyphosate

The bacterial gene mutation endpoint has been adequately addressed with several studies available that meet the full requirements of OECD 471. The available bacterial reverse mutation assays contain sufficient details regarding the batches of glyphosate used and lead to the conclusion that glyphosate is non-mutagenic in bacterial mutation studies.

The available evidences from *in vitro* mammalian cell gene mutation assays and chromosome aberration studies indicate that glyphosate does not cause chromosome aberrations *in vitro*, moreover does not lead to mammalian cell gene mutations in vitro.
The 11 studies examining *in vivo* chromosome damage and/or chromosome loss in bone marrow support negative conclusion supported by ADME data confirming systemic (and therefore bone marrow) exposure.
Currently two genotoxicity assays with glyphosate are being repeated following recently updated OECD test guidelines to confirm the above mentioned negative genotoxicity results, and are scheduled for completion in September 2020 (final reports).

Aminomethyl-phosphonic acid
AMPA was screened for genotoxic activity by the following data package of in vitro genotoxicity studies: Ames test, gene mutation test with mammalian cells and a chromosome aberration test/*in-vitro* micronucleus test.
With available data, the mutagenicity potential of AMPA has been tested in multiple assays. These included four Ames reverse mutation assays (MCA Section 5.8.1; Jensen 1993a, Callander 1988 and Mie 1996), a mammalian cell gene mutation test, MLA assay (MCA Section 5.8.1; Jensen, 1993) unscheduled DNA synthesis test (MCA Section 5.8.1; Bakke, 1991) and two mouse micronucleus assays (MCA Section 5.8.1; Jensen, 1993c, Kier & Stegman 1993). All the results were negative and support the conclusion that AMPA is non-genotoxic.
Currently all three required genotoxicity assays with AMPA are being repeated following recently updated OECD test guidelines to confirm the above mentioned negative genotoxicity. The scheduled completion is September 2020 (final reports).

N-acetyl glyphosate
A full data package (CA 5.8.1) is available to assess the genotoxicity potential of *N*-acetyl glyphosate. Although there are several deviations identified in the studies, the conclusions reported are considered valid. *N*-acetyl glyphosate is not mutagenic neither in bacteria or in mammalian cell systems, moreover does not lead to numerical or structural chromosome damage upon exposure.

N-methyl glyphosate (NMG)
Structurally, NMG is glyphosate with an *N*-methyl group added. NMG is considered as amino acid that has very similar physical properties to AMPA and glyphosate, and can be expected to behave similarly in environmental and biological systems. Although there is no direct evidence, *N*-dealkylation is a common metabolic transformation and it is probable that NMG may be converted to glyphosate in natural systems. Further details regarding the evaluation of the genotoxic potential is presented in Document J, PART A, Confidential Information.

N-Acetyl AMPA
A full data package (CA 5.8.1) is available to assess the genotoxicity potential is of *N*-acetyl AMPA. Although there are several deviations identified in the studies, the conclusions reported are considered valid. *N*-acetyl AMPA is not mutagenic neither in bacteria or in mammalian cell systems, moreover does not lead to numerical or structural chromosome damage upon exposure.

Genotoxicity studies on metabolites ***aminomethyl-phosphonic acid (AMPA), N-acetyl glyphosate and N-acetyl AMPA*** indicate no genotoxic concern in the conditions described in vitro testing or in in vivo testing conditions. The assessment therefore can be finalised on point mutation, moreover structural and numerical chromosome aberration.

In the following, an evaluation is presented for all metabolites for which the genotoxicity are not covered by experimental data. An in silico assessment is provided to extend the conclusion for N-methyl glyphosate, *N*-methyl AMPA, *N*-glyceryl AMPA, *N*-malonyl AMPA as well as methyl-phosphonic-acid by establishing read-across groups based on structural similarity with aminomethyl-phosphonic acid, *N*-acetyl glyphosate, *N*-acetyl AMPA and ultimately with glyphosate.

QSAR prediction of genotoxicity

In the following a reference is made to the QSAR Report supporting the Residue Definition related discussions ((Q)SAR and read-across genotoxicity evaluation of glyphosate and eight metabolites, Report No.: 110054/QSAR/1).

1. Information on the study

Data point	CA 6.7.1/001
Report author	knoell Germany GmbH
Report year	2020
Report title	(Q)SAR and read-across genotoxicity evaluation of Glyphosate and eight metabolites, using VEGA v1.1.5b22, DEREK Nexus v6.0.1, Toxtree v3.1.0 and OECD QSAR Toolbox v4.4
Report No	110054/QSAR/1
Guidelines followed in study	Not applicable
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

Data point	CA 6.7.1/002
Report author	knoell Germany GmbH
Report year	2020
Report title	Supplementary information for (Q)SAR and read-across genotoxicity evaluation of Glyphosate and seven metabolites, using VEGA v1.1.5b22, DEREK Nexus v6.0.1, Toxtree v3.1.0 and OECD QSAR Toolbox v4.4
Report No	110054/QSAR/1
Guidelines followed in study	Not applicable
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

To maximise the sensitivity and specificity of the prediction, two independent (Q)SAR models were chosen based on different training sets and/or algorithms (as knowledge-based and statistical-based models) to evaluate each genotoxicity endpoint. For gene mutation, the Mutagenicity (Ames test) model (CAESAR) v2.1.13 and the Mutagenicity (Ames test) model (SarPy/IRFMN) (version 1.0.7) (as implemented in VEGA v1.1.5b22) were combined. The combined results were then compared with the prediction of the expert system DEREK Nexus mutagenicity model for prediction of gene mutation v6.0.1 (as implemented in Lhasa Nexus v2.2). In the same way, two independent models were also used to evaluate chromosome aberration, i.e. DEREK Nexus chromosome damage model for prediction of

chromosomal aberrations v6.0.1 (as implemented in Lhasa Nexus v2.2) as well as Structure Alerts for the in vivo micronucleus assay in rodents (as implemented in Toxtree v3.1.0). The scientific validity of all models used in the assessment is fully documented and in line with the "OECD principles for the Validation, for Regulatory Purpose, of (Q)SAR Models".
Only one structural alert (hacceptor-path3-hacceptor) had been identified in the in vivo micronucleus model by Toxtree v3.1.0. in all metabolites except methylphosphonic acid (M08). This alert represents a molecular framework that could account for non-covalent interactions with proteins or DNA, however only a low positive predictive value of 34 % had been assigned by the developers of the model (Benigni *et al.*, 2010) to the this type of alert.
The overall conclusion is that the QSAR assessment supports the non-genotoxicity of all evaluated residues of glyphosate.

Read-across prediction of genotoxicity

In the second step, a read-across analysis was set up, considering the requirements and suggestions implemented within the EFSA guidance and the expert statements by Benigni *et al.* (2019). Glyphosate and its eight metabolites were organised into four groups, within which read-across strategies were implemented in order to fill data gaps for gene mutation and chromosome aberration. Grouping and genotoxicity evaluation strategies are summarised in Table 6.7.1-37.The proposed grouping intends to support the reliability of negative predictions obtained for the metabolites for which no data is available, since the (Q)SAR predictions were in agreement with the available experimental data for metabolites M02, M03, M04 and M09.
The available experimental results show that the glyphosate, AMPA (M02), *N*-acetyl glyphosate (M04), *N*-acetyl AMPA (M05) and *N*-methyl glyphosate (M09) do not exert gene mutation potential. These evidences are supported by the results obtained with the (Q)SAR models and from the genotoxicity profiling: all molecules are predicted as negative and no structural alerts have been identified.
No experimental data for gene mutation were available for *N*-methyl AMPA (M03), *N*-glyceryl AMPA (M06), *N*-malonyl AMPA (M07) and methyl phosphonic acid (M08). While the (Q)SAR results were negative for these metabolites, the reliability of the predictions obtained with statistical-based models were moderate or low. To substantiate the evaluation a two-step read-across analysis was designed to identify possible groupings, which could allow using available data for the parent and metabolites M02, M04 and M05, to cover the evaluation for genotoxicity endpoints of M03, M06, M07, M08 and M09. The read-across analysis allowed building four groups of substances:
- Group 1: M04, M05, M06 and M07
- Group 2: Parent, M02 and M03
- Group 3: Parent, M02 and M08
- Group 4: Parent, M04 and M09

Table 6.7.1-37: Read-across grouping

Substances	Gene mutation	Structural chromosome aberration	Numerical chromosome aberration	Final evaluation
Group 1				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-acetyl glyphosate (M04)**	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-acetyl AMPA (M05)**	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-glyceryl AMPA (M06)**	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M05) Negative	Non genotoxic
***N*-malonyl AMPA (M07)**	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M05) Negative	QSAR+RA(M04,M) Negative	Non genotoxic
Group 2				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
AMPA (M02)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
***N*-methyl AMPA (M03)**	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	Non genotoxic
Group 3				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
AMPA (M02)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
Methyl phosphonic acid (M08)	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	QSAR+RA(P01,M02)	Non genotoxic
Group 4				
Glyphosate (P01)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
N-acetyl glyphosate (M04)	EXP+QSAR Negative	EXP+QSAR Negative	EXP+QSAR Negative	Non genotoxic
N-methyl glyphosate (M09)	EXP+QSAR Negative	QSAR+RA(P01,M04)	QSAR+RA(P01,M04)	Non genotoxic

The grouping proposed above was developed for genotoxicity as a whole and therefore applies to chromosome (numerical and structural) aberration as well. With this strategy, chromosome aberration of metabolites M03, M06, M07, M08 and M09 can be covered with data available for the parent substance, M02, M04 and M05. Moreover, the grouping allows to disregard the structural alert identified by TOXTREE and supports the negative predictions (no alert) obtained by DEREK. To conclude, the

(Q)SAR results support non-genotoxicity of glyphosate and the metabolites for which experimental data are available (M02, AMPA; M04, *N*-acetyl glyphosate; M05, *N*-acetyl AMPA and *N*-methyl glyphosate, M09). *N*-methyl AMPA (M03), *N*-glyceryl-AMPA (M06), *N*-malonyl AMPA (M07), methyl phosphonic acid (M08) and *N*-methyl glyphosate (M09) are evaluated as non-genotoxic as well, based on the (Q)SAR and read-across evaluation ((Q)SAR and read-across genotoxicity evaluation of glyphosate and eight metabolites, Report No.: 110054/QSAR/1).

Major metabolites in food, processing and feed

As genotoxic potential was excluded for all compounds, only major components are further assessed.

Major metabolite in food ≥10 % TRR and ≥0.01 mg/kg or if TRR <10 % amount ≥0.05 mg/kg
Major metabolite in processing study: TRR ≥10 %
Major metabolite in feed ≥10 % TRR and ≥0.01 mg/kg

Conventional crops and animal commodities after gavage of glyphosate (relevant to the representative uses)

Compound	Plant compound in food or processing?	Plant compound in feed?[1]	Compound in animal commodities?
Glyphosate	Major	Major	Major
AMPA	Major	Major	Major
N-methyl AMPA	Minor[2]	Minor[3]	Not found
Methyl-phosphonic acid[4]	Not found	Not found	Not found
N-methyl glyphosate[4]	Not found	Not found	Not found

1 According to animal model 2017, including possible extrapolations
2 Only in coffee ripe beans (not chromatographically separated from AMPA)
3 Only after hydroponic treatment
4 Only found after hydroponic treatment and in commodities not relevant as food or feed

Tolerant crops and animal commodities after gavage of *N*-acetyl glyphosate (not supported in the EU and only relevant to imported commodities)

Compound	Plant compound in food or processing?	Plant compound in feed?[1]	Compound in animal commodities?
Glyphosate	Major	Major	Major
AMPA	Major	Major	Major
N-methyl AMPA	Major (but <10 % TRR)	Minor	Not found
N-acetyl glyphosate	Major	Major	Major
N-acetyl AMPA	Major	Major	Major
N-glyceryl AMPA	Major (but <10 % TRR)	Minor	Not found
N-malonyl AMPA	Major (but <10 % TRR)	Minor	Not found

1 According to animal model 2017, including possible extrapolations

Details on metabolites in primary and rotational crops, processed commodities and animal matrices are summarised in Table 6.7.1-1 to Table 6.7.1-36.

Three HTH studies were performed, one with on glyphosate, one with *N*-acetyl glyphosate and one with AMPA and *N*-acetyl AMPA. Each compound was stable and no degradation products were identified.

For conventional crops and animal commodities after gavage of glyphosate, the only major compounds in both plants and animals were glyphosate and AMPA.

For tolerant crops and animal commodities after gavage of *N*-acetyl glyphosate, the major compounds were also glyphosate and AMPA and in addition *N*-acetyl glyphosate and *N*-acetyl AMPA. *N*-methyl, *N*-glyceryl and *N*-malonyl AMPA were identified in food commodities of plant origin at <10 % TRR and only classified as major because of their mg/kg content (≥0.05 mg/kg).

Because the same metabolites were identified as major in feed and animal commodities, a feed burden calculation to assess the residue transfer from feed to livestock is not considered necessary in this case as it will not impact the proposed residue definition for animal matrices.

General toxicity assessment

Assessment of metabolites based on studies available with the parent compound or studies with metabolites

Compound	Occurrence in rat metabolism studies (% administered dose)	Toxicological properties covered by other than ADME
AMPA	Glyphosate is poorly metabolised with the only biotransformation product aminomethyl-phosphonic acid (AMPA) accounting for up to 1 % of the total excreted amount (probably resulting from bacterial metabolism in the gut).	Yes
N-acetyl glyphosate	*N*-acetyl glyphosate was metabolised to a very limited extent. One metabolite, glyphosate (< 1 % of the total radioactivity), was detected in faeces after a single oral dose exposure.	Yes
N-methyl glyphosate	No	No
N-methyl AMPA	No	No
N-acetyl AMPA	No	Yes
N-glyceryl AMPA	No	No
N-malonyl AMPA	No	No
Methyl-phosphonic acid	No	No

Rat metabolism studies conducted with *glyphosate* bring consistent result, namely that glyphosate is not metabolised in rats, lactating goats and laying hens and mainly excreted unchanged. Some traces of AMPA were found in these studies, possibly resulting from microbial degradation after oral absorption.
Metabolism studies with *N*-acetyl glyphosate, a major plant metabolite of glyphosate in glyphosate tolerant crops (GAT trait), were performed in rats, lactating goats and laying hens. This compound was not extensively metabolised. The two metabolic pathways proposed are leading to AMPA *via* formation of glyphosate or *N*-acetyl AMPA. Consequently, all four metabolites can be found with *N*-acetyl glyphosate being the major residue. These findings are confirmed in the farm animal feeding studies on ruminant and poultry with *N*-acetyl glyphosate where the only quantifiable residue was *N*-acetyl glyphosate in tissues and milk, except in kidney tissue of dairy cows in which case glyphosate, AMPA and *N*-acetyl AMPA were also detected; in eggs, glyphosate was found in addition to *N*-acetyl glyphosate.

Aminomethyl phosphonic acid (AMPA)
The metabolite AMPA was extensively investigated for acute and sub-chronic effects, moreover for skin sensitisation, mutagenicity and developmental toxicity. In acute oral rodent studies the median lethal dose had been identified with sign of no toxicity as greater than 2000 mg/kg bw/day in rats (Leuschner, 2002). Non-sensitizing potential had been demonstrated with guinea pigs in a Magnusson and Kligman Maximisation test (Leuschner, 2002). Sub-acute studies had been evaluated with rats as dogs as well. The lowest sub-acute NOAEL value of 100 mg/kg bw/day based on kidney weight increase in male rats and decreased bw gain in female animals (Heath *et al.*, 1993).
In developmental studies the maternal NOAEL value of 150 mg/kg bw/day based on clinical signs of decreased food consumption and decreased body weight gain. The developmental NOAEL 400 mg/kg bw/day derived on mean fetal weight decrease (Holland, 1991).

N-acetyl glyphosate
[^{14}C] *N*-acetyl glyphosate was rapidly and incompletely (approximately 66 %) absorbed in rats following a single oral dose of 15 mg/kg bw (Cheng and Howard, 2004). The maximum concentration of

radioactivity in plasma was reached after 2 hours, and the half-life for elimination from plasma was 15.6 hours. Elimination was mainly *via* urine (66.1 %) and, to a lesser extent, faeces (26.4 %); more than 90 % of the total radioactivity was eliminated by 48 hours post-dosing. *N*-acetyl glyphosate was metabolised to a very limited extent. One metabolite, glyphosate (< 1 % of the total radioactivity), was detected in faeces after a single oral dose of 15 mg/kg bw, whereas glyphosate and *N*-acetyl AMPA were found in urine following subchronic exposure at dose levels of 56 mg/kg bw per day and above.

N-acetyl glyphosate has of low acute oral toxicity with a median lethal dose (LD_{50}) greater than 5000 mg/kg bw in rats (Vegarra, 2004).
In a 90-day study with rats (MacKenzie, 2007) the subchronic toxicity of *N*-acetyl glyphosate was evaluated. Statistically significantly lower overall mean body weight gain (86 % of control) was observed in 18,000 ppm males but was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean food consumption or food efficiency. No test substance-related deaths occurred, and no clinical, ophthalmological, or neurobehavioral observations were attributed to exposure to the test substance. There were no adverse effects on clinical pathology parameters, organ weights, gross pathology, or microscopic pathology in male or female rats. The no-observed-adverse-effect level (NOAEL) for male and female rats was 18,000 ppm, *N*-acetylation is a common detoxification pathway of xenobiotic compounds in mammals; therefore, *N*-acetyl glyphosate is expected to be of similar toxicity to or lower toxicity than glyphosate. A structure–activity relationships analysis indicates that the *N*-acetylated group is not a structural alert for carcinogenicity, mutagenicity or endocrine effects, moreover the toxicological data for *N*-acetyl glyphosate show low acute toxicity, low sub-chronic toxicity (with no organ toxicity in rats at doses up to 1157 mg/kg bw per day) and a lack of genotoxicity.

N-methyl glyphosate (NMG)
The toxicological relevance has been evaluated in genotoxicity, carcinogenicity, developmental and reproductive toxicity, repeated-dose toxicity, skin sensitisation, eye and skin irritation/corrosion as well as in acute oral toxicity studies Further details regarding the evaluation are presented in Document J, PART A, Confidential Information.

N-acetyl AMPA
In an acute oral study with *N*-acetyl AMPA clinical signs of toxicity were observed in all rats up to 2 days after dosing, which included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyper-reactivity. No body weight loss occurred after dosing, moreover no test substance-related gross lesions were found. Under the conditions of this study, the oral LD_{50} for IN-EY252 was greater than 5000 mg/kg bw for female rats (Carpenter, 2007).
In a sub-chronic 90-day feeding study with rats (Haas, 2008) NOAEL of 18000 ppm had been derived (equivalent to 1163 and 1400 mg/kg bw/day, for males and females respectively). This NOAEL was set based on lack of adverse effects on in-life parameters, neurobehavioral evaluation, clinical and anatomic pathology in males and females at any dose level.

Based on the structural similarity of *N*-acetyl AMPA with AMPA, the following considerations can be communicated. *N*-acetyl AMPA is a charged molecule at physiological pH and is expected to be poorly absorbed from the gastrointestinal tract. Moreover, *N*-acetylation is a common detoxification pathway of xenobiotic compounds in mammals therefore, *N*-acetyl AMPA is expected to be of similar toxicity to or lower toxicity than AMPA or glyphosate. Based on structure–activity relationships related information, the *N*-acetylated group is not considered as structural alert for carcinogenicity, mutagenicity or endocrine effects.

N-methyl AMPA and methyl-phosphonic acid
Taking over the read-across and grouping evaluation (OECD QSAR Toolbox) by considering the log Pow difference and relative molecular weight difference of glyphosate and the metabolites, one can assume that general toxicity data with glyphosate and AMPA can sufficiently cover the evaluation of *N*-methyl

AMPA (M03) and methyl phosphonic acid (M08). These metabolites subjected to the current general toxicity assessment expected to be no greater toxicological concern than the parent glyphosate.

Assessment of metabolites for inclusion in the residue definition

The following major compounds were identified to be of potential relevance in food and feed of plant and animal origin and require further assessment:

Conventional crops (relevant to the representative uses)

	Major compound in food and feed items
Fruit crops (conventional):	Parent glyphosate
Root and tuber crops (conventional, CRC incl. primary):	Parent glyphosate AMPA
Leafy crops (CRC incl. primary):	Parent glyphosate AMPA
Pulses and oilseeds (conventional, CRC incl. primary):	Parent glyphosate AMPA
Cereals and grass crops (conventional, CRC incl. primary):	Parent glyphosate AMPA
Miscellaneous crops (conventional):	Parent glyphosate

For conventional crops and rotational crops, the only major compounds identified were **glyphosate and AMPA**, which should be included in the respective residue definition for risk assessment. No further assessment of the contribution of the identified metabolites to the total residues is needed.

Animal matrices - gavage of glyphosate or glyphosate + AMPA

	Major compound in food items
Poultry muscle:	Parent glyphosate AMPA (only after glyphosate + AMPA feeding)
Poultry liver:	Parent glyphosate AMPA
Poultry kidney:	Parent glyphosate AMPA (only after glyphosate + AMPA feeding)
Poultry fat:	Parent glyphosate
Poultry skin:	Parent glyphosate
Poultry eggs:	Parent glyphosate AMPA
Ruminant muscle:	Parent glyphosate
Ruminant liver:	Parent glyphosate AMPA
Ruminant kidney:	Parent glyphosate AMPA
Ruminant fat:	Parent glyphosate
Milk:	Parent glyphosate

In animal matrices after gavage of glyphosate or glyphosate + AMPA, the only major compounds identified were also **glyphosate and AMPA**, which should be included in the respective residue definition for risk assessment. No further assessment of the contribution of the identified metabolites to

the total residues is needed.

Tolerant crops (not supported in the EU and only relevant to imported commodities)

	Major compound in food and feed items
Root and tuber crops (CP4 EPSPS modified):	Parent glyphosate AMPA
Pulses and oilseeds (CP4 EPSPS and GOX modified):	Parent glyphosate AMPA *N*-methyl AMPA (but <10 % TRR) *N*-acetyl AMPA *N*-glyceryl-AMPA (but <10 % TRR) *N*-malonyl-AMPA (but <10 % TRR)
Pulses and oilseeds (GAT modified):	Parent glyphosate AMPA *N*-acetyl glyphosate *N*-acetyl AMPA
Cereals and grass crops (CP4 EPSPS and GOX modified):	Parent glyphosate AMPA *N*-glyceryl-AMPA (but <10 % TRR)
Cereals and grass crops (GAT modified):	Parent glyphosate *N*-acetyl glyphosate

For tolerant crops, besides parent glyphosate and AMPA, the metabolites *N*-methyl AMPA, *N*-glyceryl AMPA and *N*-malonyl AMPA (*via* CP4 EPSPS and CP4 EPSPS and GOX modified crops) and *N*-acetyl glyphosate and *N*-acetyl AMPA are candidates for inclusion into the risk assessment residue definition for plants, all occurring at levels ≥ 10 % TRR (and ≥ 0.01 mg/kg) (*N*-acetyl glyphosate and *N*-acetyl AMPA) or ≥ 0.05 mg/kg (*N*-methyl AMPA, *N*-glyceryl AMPA and *N*-malonyl AMPA) in terms of absolute levels in at least one food commodity (see **Error! Reference source not found.** to Table 6.7.1-21).

In the tables below, the contribution of the identified metabolites to the total identified residues is assessed. A compound or a number of compounds giving a reasonably high coverage of the percentage of identified are chosen for a possible residue definition in each single commodity. Commodities that are only feed items are also listed in the tables below to complete the picture and facilitate a decision on a residue definition for a crop/crop category although the consumer is not directly exposed to these residues.
Subsequently, the results for all commodities are combined to derive the final proposal for residue definition.

Table 6.7.1-38: Assessment of the contribution of the identified metabolites to the total residues: Food and feed of plant origin - CP4 EPSPS or CP4 EPSPS and GOX modified crops

	Root and tuber crops (primary)				Pulses and oilseeds (primary)			
	Sugar beet roots		Tops		Cotton seed		Soybean seed	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	95.31	96.2	79.65	97.7	23.7	94.4	25.2	31.5
AMPA	3.79	3.8	1.84	2.3	1.4	5.6	49.1	61.5
N-methyl AMPA	-	-	-	-	-	-	0.8	1.0
N-acetyl AMPA	-	-	-	-	-	-	1.4	1.8
N-glyceryl AMPA	-	-	-	-	-	-	1.6	2.0
N-malonyl AMPA	-		-	-	-	-	1.8	2.3
Sum	99.1	100	81.49	100	25.1	100	79.9	100
Normalisation factor	-	1.01	-	1.23	-	3.98	-	1.25
RD coverage of TRR (%)	95.31		79.65		23.7		74.3	
RD coverage of identified (%)	96.2		97.7		94.4		93.0	
Possible RD	Glyphosate		Glyphosate		Glyphosate		Glyphosate & AMPA	
	Pulses and oilseeds (primary)		Cereals and grass crops (primary)					
	Rape (canola) seed		Maize grain		Wheat grain		Wheat straw	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	-	-	7.4	10.8	72.40	86.7	69.19	97.5
AMPA	7.1	60.7	54.1	79.1	10.77	12.9	5.08	6.8
N-acetyl AMPA	0.7	6.0	-	-	-	-	-	-
N-glyceryl AMPA	3.9	33.3	6.9	10.1	0.34	0.4	-	-
Sum	11.7	100	68.4	100	83.51	100	74.27	100
Normalisation factor	-	8.55	-	1.46	-	1.20	-	1.35
RD coverage of TRR (%)	11.0		54.1		72.4		24.09	
RD coverage of identified (%)	94.0		79.1		86.7		93.2	
Possible RD	AMPA & *N*-glyceryl AMPA		AMPA		Glyphosate		Glyphosate	
	Cereals and grass crops (primary)							
	Wheat forage		Wheat hay		Maize forage		Maize silage	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	89.44	99.2	83.86	96.0	71.9	81.3	67.1	82.1
AMPA	0.76	0.8	3.45	4.0	15.9	18.0	13.1	16.0
N-glyceryl AMPA		-	-	-	0.5	0.6	1.5	1.8
Sum	90.20	100	87.31	100	88.3	100	81.7	100
Normalisation factor	-	1.11	-	1.15	-	1.13	-	1.22
RD coverage of TRR (%)	89.44		83.86		71.9		67.1	
RD coverage of identified (%)	99.2		96.0		81.3		82.1	
Possible RD	Glyphosate		Glyphosate		Glyphosate		Glyphosate	

Table 6.7.1-38: Assessment of the contribution of the identified metabolites to the total residues: Food and feed of plant origin - CP4 EPSPS or CP4 EPSPS and GOX modified crops

	Root and tuber crops (primary)				Pulses and oilseeds (primary)			
	Sugar beet roots		Tops		Cotton seed		Soybean seed	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified

	Cereals and grass crops (primary)		Pulses and oilseeds (primary)			
	Maize fodder		Soybean forage		Soybean hay	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	**74.8**	**85.4**	**89.1**	**92.3**	**53.6**	**78.2**
AMPA	**11.2**	12.8	6.8	7.0	**12.8**	18.7
N-methyl AMPA	1.6	1.8	0.6	0.6	1.3	1.9
N-glyceryl AMPA	-	-	-	-	0.8	1.2
Sum	87.6	100	96.5	100		100
Normalisation factor	-	1.14	-	1.04	-	1.46
RD coverage of TRR (%)	74.8		89.1		53.6	
RD coverage of identified (%)	85.4		92.3		78.2	
Possible RD	**Glyphosate**		**Glyphosate**		**Glyphosate**	

Grey: Food
Blue: Feed
% TRR: relative amount of given metabolite in % TRR
% of identified: relative percentage of given metabolite after normalisation of sum of all identified metabolites to 100 %
Normalisation factor: Factor for normalising sum of TRRs to 100 %
RD coverage of TRR: Percentage of TRR covered by compounds included in residue definition
RD coverage of identified: Percentage of identified compounds covered by residue definition
Major metabolites are given in **bold** (% TRR). If values <10 % are emboldened, then ≥0.05 mg/kg were detected.
Contribution of metabolites considered are given in **bold** (% of identified).

Table 6.7.1-39: Assessment of the contribution of the identified metabolites to the total residues: Food and feed of plant origin - GAT modified crops

	Pulses and oilseeds (primary)				Cereals and grass crops (primary)			
	Rape seed		Soybean seeds		Maize grain		Maize stover	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	**20.8**	**23.5**	**3.2**	3.4	0.1	0.1	**74.9**	**76.9**
AMPA	1.9	2.1	**11.2**	11.8	6.1	9.1	3.4	3.5
N-acetyl glyphosate	**51.1**	**57.7**	**56.9**	**60.0**	**51.2**	**76.6**	**17.8**	**18.3**
N-acetyl AMPA	**14.7**	**16.6**	**23.5**	**24.8**	9.4	**14.1**	1.3	1.3
Sum	88.5	100	94.8	100	66.8	100	97.4	100
Normalisation factor	-	1.13	-	1.05	-	1.50	-	1.03
RD coverage of TRR (%)	71.9		80.4		60.6		92.7	
RD coverage of identified (%)	81.2		84.8		90.7		95.2	
Possible RD	**Glyphosate & *N*-acetyl glyphosate**		***N*-acetyl glyphosate & *N*-acetyl AMPA**		***N*-acetyl glyphosate & *N*-acetyl AMPA**		**Glyphosate & *N*-acetyl glyphosate**	
	Cereals and grass crops (primary)				Cereals and grass crops (primary)			
	Maize forage		Rape immature foliage		Rape foliage		Soybean foliage	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	**58.0**	**63.9**	3.0	3.1	-	-	**53.4**	**55.1**
AMPA	**4.0**	4.4	**1.4**	1.4	-	-	**10.3**	10.6
N-acetyl glyphosate	**27.0**	**29.8**	**89.5**	**92.0**	**93.0**	**100**	**31.9**	**32.9**
N-acetyl AMPA	1.7	1.9	3.4	3.5	-	-	1.4	1.4
Sum	90.7	100	97.3	100		100	97.0	100
Normalisation factor	-	1.10	-	1.03	-	1.08	-	1.03
RD coverage of TRR (%)	81.0		89.5		93.0		85.3	
RD coverage of identified (%)	93.7		92.0		100		88.0	
Possible RD	**Glyphosate & *N*-acetyl glyphosate**		**N-acetyl glyphosate**		**Glyphosate**		**Glyphosate & *N*-acetyl glyphosate**	

Grey: Food
Blue: Feed
% TRR: relative amount of given metabolite in % TRR
% of identified: relative percentage of given metabolite after normalisation of sum of all identified metabolites to 100 %
Normalisation factor: Factor for normalising sum of TRRs to 100 %
RD coverage of TRR: Percentage of TRR covered by compounds included in residue definition
RD coverage of identified: Percentage of identified compounds covered by residue definition
Major metabolites are given in **bold** (% TRR). If values <10 % are emboldened, then ≥0.05 mg/kg were detected.
Contribution of metabolites considered are given in bold (% of identified).

For CP4 EPSPS or CP4 EPSPS and GOX modified crops, **glyphosate and AMPA** represent >79 % of the identified compounds in all food and feed commodities except rape (canola) seed (60.7 %). However, in total only 11.7 % of the TRR were identified in this matrix. Therefore, it is considered reasonable to rather rely on findings in cotton seed and soybean seed, which belong to the same crop group (oilseeds), for the purposes of residue definition. To include *N*-glyceryl AMPA in the residue definition for risk assessment due to the sole contribution in rape seed with little overall identification is not considered justified.

For GAT modified crops, **glyphosate, *N*-acetyl glyphosate** and/or ***N*-acetyl AMPA** represent >81 % of the identified compounds in all food and feed commodities. As **AMPA** was found in significant amounts of >10 % TRR in soybean seeds and foliage and is also present in almost all other food and feed commodities, it should be part of the residue definition as well.

Animal matrices - gavage of *N*-acetyl glyphosate (only relevant after feeding of GAT modified plants)

	Major compound in food items
Poultry muscle:	- (identified compounds <0.01 mg/kg)
Poultry liver:	*N*-acetyl glyphosate Glyphosate
Poultry fat:	*N*-acetyl glyphosate Glyphosate
Poultry eggs:	*N*-acetyl glyphosate
Ruminant muscle:	*N*-acetyl glyphosate
Ruminant liver:	*N*-acetyl glyphosate Glyphosate AMPA
Ruminant kidney:	*N*-acetyl glyphosate Glyphosate
Ruminant fat:	*N*-acetyl glyphosate *N*-acetyl AMPA
Milk:	- (identified compounds <0.01 mg/kg)

In animal matrices after gavage of *N*-acetyl glyphosate, *N*-acetyl glyphosate and glyphosate were the major compounds in poultry, while in ruminants AMPA and *N*-acetyl AMPA were identified as major in addition.
All animal commodities are listed in the tables below to facilitate a decision on animal matrices.

Table 6.7.1-40: Assessment of the contribution of the identified metabolites to the total residues: Food of animal origin: Poultry - Gavage of *N*-acetyl glyphosate

	Egg white		Egg yolk		Liver		Muscle	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	10.9	19.2	5.69	7.5	16.34	18.0	7.19	14.1
AMPA	-	-	0.91	1.2	6.74	7.4	16.69	32.7
N-acetyl glyphosate	41.48	73.1	68.40	89.9	63.82	70.2	25.22	49.5
N-acetyl AMPA	4.34	7.7	1.10	1.4	4.04	4.4	1.89	3.7
Sum	56.72	100	76.10	100	90.94	100	50.99	100
Normalisation factor	-	1.76	-	1.31	-	1.10	-	1.96
RD coverage of TRR (%)	52.38		68.40		80.16		41.91	
RD coverage of identified (%)	92.3		89.9		88.2		82.2	
Possible RD	***N*-acetyl glyphosate & glyphosate**		***N*-acetyl glyphosate**		***N*-acetyl glyphosate & glyphosate**		***N*-acetyl glyphosate & AMPA**	

Table 6.7.1-40: Assessment of the contribution of the identified metabolites to the total residues: Food of animal origin: Poultry - Gavage of N-acetyl glyphosate

	Fat	
	% TRR	% of identified
Glyphosate	**39.43**	**46.7**
AMPA	11.29	13.4
N-acetyl glyphosate	**23.45**	**27.8**
N-acetyl AMPA	10.18	12.1
Sum	84.35	100
Normalisation factor	-	1.19
RD coverage of TRR (%)	62.88	
RD coverage of identified (%)	74.5	
Possible RD	***N*-acetyl glyphosate & glyphosate**	

% TRR: relative amount of given metabolite in % TRR
% of identified: relative percentage of given metabolite after normalisation of sum of all identified metabolites to 100 %
Normalisation factor: Factor for normalising sum of TRRs to 100 %
RD coverage of TRR: Percentage of TRR covered by compounds included in residue definition
RD coverage of identified: Percentage of identified compounds covered by residue definition
Major metabolites are given in **bold** (% TRR).
Contribution of metabolites considered are given in **bold** (% of identified)

Table 6.7.1-41: Assessment of the contribution of the identified metabolites to the total residues: Food of animal origin: Ruminant - Gavage of *N*-acetyl glyphosate

	Milk		Liver		Kidney		Muscle	
	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified	% TRR	% of identified
Glyphosate	3.59	7.7	**14.71**	**18.7**	4.98	6.1	-	-
AMPA	3.35	7.1	8.45	10.7	-	-	-	-
N-acetyl glyphosate	39.98	**85.2**	**55.51**	**70.6**	**77.12**	**93.9**	**16.70**	**100**
Sum	46.92	100	78.67	100	82.10	100	16.70	100
Normalisation factor	-	2.13	-	1.27	-	1.22	-	5.99
RD coverage of TRR (%)	39.98		70.22		77.12		16.70	
RD coverage of identified (%)	85.2		89.3		93.9		100	
Possible RD	***N*-acetyl glyphosate**		***N*-acetyl glyphosate & glyphosate**		***N*-acetyl glyphosate**		***N*-acetyl glyphosate**	

Table 6.7.1-41: Assessment of the contribution of the identified metabolites to the total residues: Food of animal origin: Ruminant - Gavage of *N*-acetyl glyphosate

	Fat (subcutaneous)	
	% TRR	% of identified
Glyphosate	2.65	3.0
AMPA	4.77	5.5
N-acetyl glyphosate	**64.73**	**74.4**
N-acetyl AMPA	**14.86**	**17.1**
Sum	87.01	100
Normalisation factor	-	1.15
RD coverage of TRR (%)	79.59	
RD coverage of identified (%)	91.5	
Possible RD	***N*-acetyl glyphosate & *N*-acetyl AMPA**	

% TRR: relative amount of given metabolite in % TRR
% of identified: relative percentage of given metabolite after normalisation of sum of all identified metabolites to 100 %
Normalisation factor: Factor for normalising sum of TRRs to 100 %
RD coverage of TRR: Percentage of TRR covered by compounds included in residue definition
RD coverage of identified: Percentage of identified compounds covered by residue definition
Major metabolites are given in **bold** (% TRR).
Contribution of metabolites considered are given in bold (% of identified).

After gavage of *N*-acetyl glyphosate to poultry, glyphosate and *N*-acetyl glyphosate represent >74 % of the identified compounds in all food commodities except muscle (63.6 %). In muscle, AMPA represents a significant portion of the identified residue.

For ruminants, glyphosate and *N*-acetyl glyphosate represent >77 % of the identified compounds in all food commodities. In fat, *N*-acetyl AMPA was also found in significant amounts of >10 % TRR.

Combining the results for all livestock fed with *N*-acetyl glyphosate to derive a single residue definition for these commodities for reasons of simplicity, **glyphosate, AMPA, *N*-acetyl glyphosate and *N*-acetyl AMPA** should be included. This would cover 100 % of the identified compounds in all animal matrices.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

Plant matrices

For glyphosate, MRLs are established in several crops. In order to support the renewal of approval for glyphosate, new and already evaluated residue trials are presented for the representative uses. Since, based on metabolism, a zero-residue situation is expected and in accordance with Commission Regulation (EU) No 283/2013 and Guidance SANCO 7525/VI/95 Rev. 10.3, the uses may be supported by a limited set of trials (3 trials per crop). This approach was applied recently in the Reasoned Opinion on the Review of the existing maximum residue levels for glyphosate (EFSA Journal 2019;17(10):5862) where it is stated that: "*regarding the uses on conventional crops, according to the RMS, a no residue situation can be anticipated for all orchards (except olives, since the fruits can be picked from the ground) and for all soil applications done before sowing/planting or as interrow treatment or by wiping or as local treatment by rubbing and dabbing (envelope approach).*
It is noted that the envelope approach has been fully supported by EFSA and the MSs in the framework of the peer review".

The presented residue studies are supporting the critical GAP of the intended uses of this submission.

Use in fruit tree plantations

For the use of glyphosate in fruit tree plantations 31 trials (7 in the northern and 24 in the southern zone) are available for citrus, tree nuts, pome fruit, stone fruit, kiwi and banana. The tree plantations were treated once with application rates from 2.88 to 3.6 kg a.s./ha. Even though the application rates are higher than the critical GAP, no residues of glyphosate above the limit of quantitation (LOQ) of 0.05 mg/kg were found in any of the treated samples taken 0 to 7 days after the application. Therefore, sufficient trials to support the representative GAP in citrus, tree nuts, pome fruit, stone fruit, kiwi and banana (1-2 × 1.44 kg a.s./ha (max. 2.88 kg a.s./ha/season), PHI 7 days) are available.

Table 6.7.2-1: Summary of residues data from the supervised residue trials on fruit tree plantations

Region	Year	Crop	Number of trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL (mg/kg)
Citrus fruit							
S-EU	2013	Mandarin	2	2 x <0.05	<0.05	<0.05	0.05*
Tree nuts							
S-EU	2015	Hazelnut	1	<0.05	<0.05	<0.05	0.05*
S-EU	2015	Pistachio	1	<0.05			
Pome fruit							
N-EU	2013	Apple	4	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2013	Apple	2	2 x <0.05			
Stone fruit							
S-EU	2012	Apricot	1	<0.05	<0.05	<0.05	0.05*
S-EU	2015	Apricot	4	4 x <0.05			
S-EU	2013	Cherry	2	2 x <0.05			
S-EU	2012	Peaches	2	2 x <0.05			
N-EU	2012	Plum	3	3 x <0.05			
S-EU	2012	Plum	1	<0.05			
S-EU	2013	Plum	2	2 x <0.05			
Kiwi							
S-EU	2015	Kiwi	2	2 x <0.05	<0.05	<0.05	0.05*
Banana							
S-EU	2015	Banana	4	4 x <0.05	<0.05	<0.05	0.05*

Based on the trials conducted on citrus fruit, tree nuts, pome fruit, stone fruit, kiwi and banana according to the critical GAP, where residue levels were all below LOQ, an **MRL** of **0.05* mg/kg** is supported for orchard crops (citrus, tree nuts, pome and stone fruits groups).

Olives

In total 9 trials are available for table olives in Southern Europe. For table olives, which are harvested directly from the tree, residues of glyphosate were always below the LOQ of 0.05 mg/kg. Sufficient trials to support the representative GAP in table olives (1-2 × 1.44 kg a.s./ha (max. 2.88 kg a.s./ha/season), PHI 7 days) are available.

Table 6.7.2-2: Summary of residues data from the supervised residue trials on table olives

Region	Year	Crop	Number of trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL (mg/kg)
S-EU	1995	Olive	8	Fruit from tree: 8 x <0.05	<0.05	<0.05	0.05*
S-EU	1988	Olives	1	<0.05			

Based on the trials conducted on table olives, picked directly from the tree, according to the critical GAP, where residue levels were all below LOQ, an **MRL** of **0.05* mg/kg** is supported for table olives.

Grapes

In total 18 trials are available in Northern and Southern Europe. In all trials the residues of glyphosate were always below the LOQ of 0.05 mg/kg. Therefore, sufficient trials to support the representative GAP (1-2 × 1.44 kg a.s./ha (max. 2.88 kg a.s./ha/season), PHI 7 days) are available.

Table 6.7.2-3: Summary of residues data from the supervised residue trials on grapes

Region	Year	Crop	Number of trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL (mg/kg)
N-EU	2015	Grapes	2	2 x <0.05	<0.05	<0.05	0.05*
N-EU	2014	Grapes	7	7 x <0.05			
S-EU	2014	Grapes	9	9 x <0.05			

Based on the trials conducted on grapes according to the critical GAP, where residue levels were all below LOQ, an **MRL** of **0.05* mg/kg** is supported for grapes.

Use before emergence, sowing or transplanting

For the use of glyphosate in vegetables before emergence or before transplanting, 31 trials (16 trials in Northern Europe and 19 trials in Southern Europe) are available for root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica vegetables, leafy vegetables, stem vegetables and sugar beet. The fields were treated once with the maximum seasonal application rate of 2.16 kg a.s./ha. No residues of glyphosate above the limit of quantitation (LOQ) of 0.05 mg/kg, in the most cases even below the LOD of 0.015 mg/kg were found in any of the treated samples at harvest. Therefore, sufficient trials to support the representative GAP (1-2 × 1.44 kg a.s./ha (max. 2.16 kg a.s./ha/season) are available.

Table 6.7.2-4: Summary of residues data from the supervised residue trials on vegetables (pre-emergence, pre-sowing, pre-planting application)

Region	Year	Crop	Number of trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL (mg/kg)
Root and tuber vegetables							
N-EU	2011	Potato	2	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2011	Potato	2	2 x <0.05			
N-EU	2011	Carrot	2	2 x <0.05			
S-EU	2011	Carrot	2	2 x <0.05			
Bulb vegetables							
N-EU	2011	Bulb onion	2	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2011	Bulb onion	2	2 x <0.05			
Fruiting vegetables							
N-EU	2011	Tomato	2	2 x <0.05	<0.05	<0.05	0.05*
N-EU	2011	Zucchini	1	<0.05			
S-EU	2011	Zucchini	1	<0.05			
S-EU	2011	Cucumber	1	<0.05			
Brassica vegetables							
N-EU	2011	Cauliflower	2	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2011	Cauliflower	2	2 x <0.05			
N-EU	2011	Head cabbage	2	2 x <0.05			
S-EU	2011	Head cabbage	2	2 x <0.05			
Leafy vegetables							
N-EU	2011	Leaf lettuce	2	2 x <0.05	<0.05	<0.05	0.05*

Table 6.7.2-4: Summary of residues data from the supervised residue trials on vegetables (pre-emergence, pre-sowing, pre-planting application)

Region	Year	Crop	Number of trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL (mg/kg)
S-EU	2011	Head lettuce	2	2 x <0.05			
Stem vegetables							
N-EU	2011	Leek	2	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2011	Leek	2	2 x <0.05			
Sugar beet							
S-EU	2011/ 2012	Sugar beet	2	2 x <0.05	<0.05	<0.05	0.05*

Inter-row use

For inter-row use in vegetables glyphosate, 44 trials (14 trials in Northern Europe and 30 trials in Southern Europe) are available for root and tuber vegetables, bulb vegetables, fruiting vegetables, leafy vegetables and legume vegetables. The fields were treated once with the maximum seasonal application rate of 1.08 kg a.s./ha. No residues of glyphosate above the limit of detection (LOD) of 0.015 mg/kg were found in any of the treated samples at harvest. Therefore, sufficient trials to support the representative GAP (1 × 1.08 kg a.s./ha) are available.

Table 6.7.2-5: Summary of residues data from the supervised residue trials on vegetables (inter-row treatment)

Region	Year	Crop	Number of trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Proposed MRL (mg/kg)
Root and tuber vegetables							
S-EU	2015	Carrot	4	4 x <0.05	<0.05	<0.05	0.05*
S-EU	2015	Radish	2	2 x <0.05			
Bulb vegetables							
N-EU	2015	Bulb onion	2	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2015	Bulb onion	4	4 x <0.05			
Fruiting vegetables							
S-EU	2015	Tomato	4	4 x <0.05	<0.05	<0.05	0.05*
N-EU	2015	Cucumber	2	2 x <0.05			
S-EU	2015	Cucumber	2	2 x <0.05			
N-EU	2015	Courgette	2	2 x <0.05			
S-EU	2015	Courgette	2	2 x <0.05			
Leafy vegetables							
N-EU	2015	Head lettuce	2	2 x <0.05	<0.05	<0.05	0.05*
S-EU	2015	Head lettuce	4	4 x <0.05			
N-EU	2015	Parsley	2	2 x <0.05			
S-EU	2015	Parsley	2	2 x <0.05			
Legume vegetables							
N-EU	2015	Green beans	4	4 x <0.05	<0.05	<0.05	0.05*
S-EU	2015	Green beans	4	4 x <0.05			

Established EU MRLs are placed in Annex III of Regulation (EC) No 396/2005. They were recently reviewed under Article 12 of Regulation (EC) No 396/2005 (EFSA, 2019. Reasoned Opinion on the Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862) but not yet formally placed within Regulation (EC) No 396/2005. The MRLs derived for the herein supported

representative uses are equal to or below the corresponding MRLs recommended in the EFSA reasoned opinion. Therefore, the representative uses do not make it necessary to change any of the existing MRLs.

Honey

Based on the recently conducted tunnel honey residue trials an MRL of 15 mg/kg is derived for glyphosate in honey. According to the Technical Guidelines SANTE/11956/2016 rev. 9 of 14 September 2018 it is also possible to set temporary MRLs in honey on the basis of monitoring data. Using the monitoring data from 2016 and 2017 an **MRL** of **0.6 mg/kg** for **honey** can be derived. The Technical Guidelines do not explain which of the two approaches to be favoured. Ultimately the selection of a suitable MRL is up to Risk Managers. However, the value derived from monitoring seems to be more in line with the ALARA principle. For details please refer to MCA 6.10.1.

Animal matrices

The uses of glyphosate are adequately covered by the animal dietary burden calculations (please refer to MCA 6.4); as a consequence, the following MRLs for glyphosate in livestock can be proposed for animal matrices.
For lactating cows and laying hens feeding studies were conducted with either a mixture of glyphosate and AMPA or with glyphosate-trimesium. For pigs a feeding study conducted with a mixture of glyphosate and AMPA is available.

A summary of the values derived from the different livestock feeding studies is presented in the tables below.

Table 6.7.2-6: MRL calculation for animal matrices based the feeding studies conducted with glyphosate and AMPA

Animal commodity	Residues at the closest feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	STMR (mg/kg)	HR (mg/kg)
	Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
Cattle (all diets)								
Closest feeding level:	1.64 mg/kg bw	53.4 N Dairy cattle (highest diet)						
Muscle	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Fat	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Liver	0.13	0.15	0.00	0.00	**0.06***	n.c.	0.002	0.003
Kidney	0.35	0.44	0.006	0.008	**0.06***	n.c.	0.006	0.008
Cattle (dairy only)								
Closest feeding level:	1.64 mg/kg bw	53.4 N Dairy cattle						
Milk	0.06	0.06	0.06	0.06	**0.07**	n.c.	0.06	0.06
Sheep (all diets)								
Closest feeding level:	1.64 mg/kg bw	53.2 N Ram/Ewe (highest diet)						
Muscle	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Fat	0.13	0.13	0.13	0.126	**0.15**	n.c.	0.13	0.13
Liver	0.13	0.15	0.00	0.00	**0.06***	n.c.	0.00	0.00
Kidney	0.35	0.44	0.006	0.008	**0.06***	n.c.	0.006	0.008
Sheep (dairy only)								
Closest feeding level:	1.64 mg/kg bw	53.2 N Ewe						
Milk	0.06	0.06	0.06	0.06	**0.07**	n.c.	0.06	0.06
Swine								
Closest feeding level:	1.08 mg/kg bw	55.7 N Breeding (highest diet)						
Muscle	0.05	0.05	0.05	0.05	**0.06***			
Fat	0.05	0.05	0.05	0.05	**0.06***			
Liver	0.05	0.05	0.05	0.05	**0.06***	1.9	0.09	0.09
Kidney	0.37	0.61	0.007	0.011	**0.06***	1.2	0.008	0.013
Poultry (all diets)								

Table 6.7.2-6: MRL calculation for animal matrices based the feeding studies conducted with glyphosate and AMPA

Animal commodity	Residues at the closest feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	STMR (mg/kg)	HR (mg/kg)
	Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
Closest feeding level:	2.78 mg/kg bw 214.4 N Layer (highest diet)							
Muscle	0.13	0.13	0.13	0.13	**0.15**	1.0	0.12	0.12
Fat	0.13	0.13	0.13	0.13	**0.15**	1.1	0.13	0.13
Liver	0.13	0.14	0.0006	0.0006	**0.06***	1.3	0.0008	0.0008
Kidney								
Poultry (layer only)								
Closest feeding level:	2.78 mg/kg bw 214.4 N Layer							
Eggs	0.06	0.06	0.06	0.06	**0.07**	1.0	0.06	0.06

Table 6.7.2-7: MRL calculation for animal matrices based the feeding studies conducted with glyphosate-trimesium

Animal commodity	Residues at the closest feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	STMR (mg/kg)	HR (mg/kg)
	Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
Cattle (all diets)								
Closest feeding level:	0.012 mg/kg bw 0.4 N Dairy cattle (highest diet)							
Muscle	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Fat	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Liver	0.50	0.50	0.50	0.50	**0.5**	n.c.	0.50	0.50
Kidney	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Cattle (dairy only)								
Closest feeding level:	0.012 mg/kg bw 0.4 N Dairy cattle							
Milk	0.05	0.05	0.05	0.05	**0.06***	n.c.	0.05	0.05
Sheep (all diets)								
Closest feeding level:	0.012 mg/kg bw 0.4 N Ram/Ewe (highest diet)							
Muscle	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Fat	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Liver	0.50	0.50	0.50	0.50	**0.5**	n.c.	0.50	0.50
Kidney	0.13	0.13	0.13	0.13	**0.15**	n.c.	0.13	0.13
Sheep (dairy only)								
Closest feeding level:	0.012 mg/kg bw 0.4 N Ewe							
Milk	0.05	0.05	0.05	0.05	**0.06***	n.c.	0.05	0.05
Swine								
Closest feeding level:	1.08 mg/kg bw 55.7 N Breeding (highest diet)							
Muscle	0.05	0.05	0.05	0.05	**0.06***	n.c.	0.05	0.05
Fat	0.05	0.05	0.05	0.05	**0.06***	n.c.	0.05	0.05
Liver	0.05	0.05	0.05	0.05	**0.06***	1.9	0.09	0.09
Kidney	0.37	0.61	0.61	0.61	**0.7**	1.2	0.71	0.71
Poultry (all diets)								
Closest feeding level:	0.025 mg/kg bw 1.9 N Layer (highest diet)							
Muscle	0.13	0.13	0.13	0.13	**0.15**	#DIV/0!	#DIV/0!	#DIV/0!
Fat	0.13	0.13	0.13	0.13	**0.15**	#DIV/0!	#DIV/0!	#DIV/0!
Liver	0.13	0.13	0.13	0.13	**0.15**	#DIV/0!	#DIV/0!	#DIV/0!
Kidney								
Poultry (layer only)								
Closest feeding level:	0.025 mg/kg bw 1.9 N Layer							

Table 6.7.2-7: MRL calculation for animal matrices based the feeding studies conducted with glyphosate-trimesium

Animal commodity	Residues at the closest feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	STMR (mg/kg)	HR (mg/kg)
	Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
Eggs	0.04	0.04	0.04	0.04	**0.06***	#DIV/0!	#DIV/0!	#DIV/0!

Since the feeding level of the study conducted with glyphosate-trimesium is closer to the calculated feed burden these derived MRLs, STMRs and HRs will be considered for the risk assessment.

Established EU MRLs are placed in Annex III of Regulation (EC) No 396/2005. They were recently reviewed under Article 12 of Regulation (EC) No 396/2005 in 2018 (EFSA, 2019. Reasoned Opinion on the Review of the existing maximum residue levels for glyphosate according to Article 12 of Regulation (EC) No 396/2005 – revised version to take into account omitted data. EFSA Journal 2019;17(10):5862) but not yet formally placed within Regulation (EC) No 396/2005.
The dietary burdens for the representative uses are far below the burdens estimated in the reasoned opinion. Therefore the residues in food of animal origin resulting from the representative uses are necessarily below the MRLs recommended by EFSA in the reasoned opinion (for the sum of glyphosate, AMPA, N-acetyl glyphosate, and N-acetyl AMPA).

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

Not relevant.

CA 6.8 Proposed Safety Intervals

Pre-harvest interval for the representative uses

Not relevant for vegetables (root & tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, sugar beet), application on bare soil before planting or sowing or on bare soil after harvest.
The pre-harvest intervals for post-emergence of weeds in orchard crops (stone and pome fruits, kiwi, tree nuts, banana, and table olives) and vines (table and wine grape, leaves not intended for human consumption) are 7 days, respectively.
The pre-harvest intervals for vegetables (root & tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, sugar beet) for an inter-row application (ground directed, shielded spray application) on bare soil are 60 days.

Re-entry period for livestock

Not relevant. Glyphosate is used on bare soil before planting or sowing and not on areas to be grazed.

Re-entry period for man to crops

Re-entry assessments are given for the representative uses in the supplemental product dossiers (MCP chapter 7.2).

Withholding period for animal feeding stuffs

Feed items of the target crops are side products of food products. Feed items proposed for feeding-stuffs will therefore be harvested at or beyond the pre-harvest interval.

Waiting period between application and crop sowing or planting the crop to be protected

Not relevant for permanent crops like orchard crops (stone and pome fruits, kiwi, tree nuts, banana, and table olives) and vines (table and wine grape, leaves not intended for human consumption).
For the pre-emergence uses the waiting period between treatment and planting or sowing of the new crop depends on the temperature, the moisture, the structure and texture of the soil. According to results of confined rotational crop studies a planting of various primary crops was possible after 3 days.

Waiting period between application and handling

This is not relevant here since a post-harvest treatment is not intended for the representative uses.

Waiting period between last application and sowing or planting succeeding crops

The results of the rotational crop studies show that glyphosate residues in emergency replant and rotational crops will be less than those found in the primary crop. Therefore, no limitation concerning the succeeding crops is necessary.

CA 6.9 Estimation of the Potential and Actual Exposure through Diet and other Sources

Assessments of the potential chronic and acute dietary consumer risk due to exposure to residues of glyphosate were performed using the EFSA Pesticide Residue Intake Model for chronic and acute risk assessment - rev. 3.1 (PRIMo).

The ADI and ARfD for the active substance glyphosate are summarised in the table below.

Table 6.9-1: Toxicological endpoints – glyphosate

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.5 mg/kg bw/d	Developmental toxicity, rabbit	100	
Acute Reference Dose (ARfD)	0.5 mg/kg bw/d	Developmental toxicity, rabbit	100	

Chronic consumer risk assessment

The chronic consumer risk assessment was conducted based on the intended uses of this renewal. To take AMPA into account for the risk assessment the total residues for risk assessment were calculated as:

mg/kg glyphosate + mg/kg AMPA x 1.5 (equivalency factor).

The input values are presented in the Table 6.9-2.

The summary of the calculation using PRIMo rev. 3.1 is presented in Table 6.9-3. For the assessment, the ADI of 0.5 mg/kg bw/day was used. According to the EFSA model the chronic exposure estimate has been simultaneously calculated for adults, children, toddlers and infants (different age groups), vegetarian and elderly in different EU countries.

Table 6.9-2: Glyphosate - Input values used for risk assessment

Code number	Commodity	Proposed MRLs (mg/kg)	Input values (mg/kg)	
			Chronic RA (STMR$_{RA}$)	Acute RA (HR$_{RA}$)
0100000	FRUITS, FRESH or FROZEN; TREE NUTS			
0110000	Citrus fruits			
0110010	Grapefruits	0.05*	0.125	0.125
0110020	Oranges	0.05*	0.125	0.125
0110030	Lemons	0.05*	0.125	0.125
0110040	Limes	0.05*	0.125	0.125
0110050	Mandarins	0.05*	0.125	0.125
0110990	Others	0.05*	0.125	0.125
0120000	Tree nuts			
0120010	Almonds	0.05*	0.125	0.125
0120020	Brazil nuts	0.05*	0.125	0.125
0120030	Cashew nuts	0.05*	0.125	0.125
0120040	Chestnuts	0.05*	0.125	0.125
0120050	Coconuts	0.05*	0.125	0.125
0120060	Hazelnuts/cobnuts	0.05*	0.125	0.125
0120070	Macadamias	0.05*	0.125	0.125
0120080	Pecans	0.05*	0.125	0.125
0120090	Pine nut kernels	0.05*	0.125	0.125
0120100	Pistachios	0.05*	0.125	0.125
0120110	Walnuts	0.05*	0.125	0.125
0120990	Others	0.05*	0.125	0.125
0130000	Pome fruits			
0130010	Apples	0.05*	0.125	0.125
0130020	Pears	0.05*	0.125	0.125
0130030	Quinces	0.05*	0.125	0.125
0130040	Medlars	0.05*	0.125	0.125
0130050	Loquats/Japanese medlars	0.05*	0.125	0.125
0130990	Others	0.05*	0.125	0.125
0140000	Stone fruits			
0140010	Apricots	0.05*	0.125	0.125
0140020	Cherries (sweet)	0.05*	0.125	0.125
0140030	Peaches	0.05*	0.125	0.125
0140040	Plums	0.05*	0.125	0.125
0140990	Others	0.05*	0.125	0.125
0150000	Berries and small fruits			
0151000	(a) grapes			
0151010	Table grapes	0.05*	0.125	0.125
0151020	Wine grapes	0.05*	0.125	0.125
0160000	Miscellaneous fruits with			
0161000	(a) edible peel			
0161010	Dates	0.05*	0.125	0.125
0161020	Figs	0.05*	0.125	0.125
0161030	Table olives	0.05*	0.125	0.125
0161040	Kumquats	0.05*	0.125	0.125
0161050	Carambolas	0.05*	0.125	0.125

Table 6.9-2: Glyphosate - Input values used for risk assessment

Code number	Commodity	Proposed MRLs (mg/kg)	Input values (mg/kg)	
			Chronic RA ($STMR_{RA}$)	Acute RA (HR_{RA})
0161060	Kaki/Japanese persimmons	0.05*	0.125	0.125
0161070	Jambuls/jambolans	0.05*	0.125	0.125
0161990	Others	0.05*	0.125	0.125
0162000	(b) inedible peel, small			
0162010	Kiwi fruits (green, red, yellow)	0.05*	0.125	0.125
0162020	Litchis/lychees	0.05*	0.125	0.125
0162030	Passionfruits/maracujas	0.05*	0.125	0.125
0162040	Prickly pears/cactus fruits	0.05*	0.125	0.125
0162050	Star apples/cainitos	0.05*	0.125	0.125
0162060	American persimmons/Virginia kaki	0.05*	0.125	0.125
0162990	Others	0.05*	0.125	0.125
0163000	(c) inedible peel, large			
0163020	Bananas	0.05*	0.125	0.125
0200000	VEGETABLES, FRESH or FROZEN			
0210000	Root and tuber vegetables			
0211000	(a) potatoes	0.05*	0.125	0.125
0212000	(b) tropical root and tuber vegetables			
0212010	Cassava roots/manioc	0.05*	0.125	0.125
0212020	Sweet potatoes	0.05*	0.125	0.125
0212030	Yams	0.05*	0.125	0.125
0212040	Arrowroots	0.05*	0.125	0.125
0212990	Others	0.05*	0.125	0.125
0213000	(c) other root and tuber vegetables except sugar beets			
0213010	Beetroots	0.05*	0.125	0.125
0213020	Carrots	0.05*	0.125	0.125
0213030	Celeriacs/turnip rooted celeries	0.05*	0.125	0.125
0213040	Horseradishes	0.05*	0.125	0.125
0213050	Jerusalem artichokes	0.05*	0.125	0.125
0213060	Parsnips	0.05*	0.125	0.125
0213070	Parsley roots/Hamburg roots parsley	0.05*	0.125	0.125
0213080	Radishes	0.05*	0.125	0.125
0213090	Salsifies	0.05*	0.125	0.125
0213100	Swedes/rutabagas	0.05*	0.125	0.125
0213110	Turnips	0.05*	0.125	0.125
0213990	Others	0.05*	0.125	0.125
0220000	Bulb vegetables			
0220010	Garlic	0.05*	0.125	0.125
0220020	Onions	0.05*	0.125	0.125
0220030	Shallots	0.05*	0.125	0.125
0220040	Spring onions/green onions and Welsh onions	0.05*	0.125	0.125
0220990	Others	0.05*	0.125	0.125
0230000	Fruiting vegetables			
0231000	(a) solanacea			
0231010	Tomatoes	0.05*	0.125	0.125

Table 6.9-2: Glyphosate - Input values used for risk assessment

Code number	Commodity	Proposed MRLs (mg/kg)	Input values (mg/kg)	
			Chronic RA ($STMR_{RA}$)	Acute RA (HR_{RA})
0231020	Sweet peppers/bell peppers	0.05*	0.125	0.125
0231030	Aubergines/eggplants	0.05*	0.125	0.125
0231040	Okra/lady's fingers	0.05*	0.125	0.125
0231990	Others	0.05*	0.125	0.125
0232000	(b) cucurbits with edible peel			
0232010	Cucumbers	0.05*	0.125	0.125
0232020	Gherkins	0.05*	0.125	0.125
0232030	Courgettes	0.05*	0.125	0.125
0232990	Others	0.05*	0.125	0.125
0233000	(c) cucurbits with inedible peel			
0233010	Melons	0.05*	0.125	0.125
0233020	Pumpkins	0.05*	0.125	0.125
0233030	Watermelons	0.05*	0.125	0.125
0233990	Others	0.05*	0.125	0.125
0240000	Brassica vegetables (excluding brassica roots and brassica baby leaf crops)			
0241000	(a) flowering brassica			
0241010	Broccoli	0.05*	0.125	0.125
0241020	Cauliflowers	0.05*	0.125	0.125
0241990	Others	0.05*	0.125	0.125
0242000	(b) head brassica			
0242010	Brussels sprouts	0.05*	0.125	0.125
0242020	Head cabbages	0.05*	0.125	0.125
0242990	Others	0.05*	0.125	0.125
0243000	(c) leafy brassica			
0243010	Chinese cabbages/pe-tsai	0.05*	0.125	0.125
0243020	Kales	0.05*	0.125	0.125
0243990	Others	0.05*	0.125	0.125
0250000	Leaf vegetables, herbs and edible flowers			
0251000	(a) lettuces and salad plants			
0251010	Lamb's lettuces/corn salads	0.05*	0.125	0.125
0251020	Lettuces	0.05*	0.125	0.125
0251030	Escaroles/broad-leaved endives	0.05*	0.125	0.125
0251040	Cresses and other sprouts and shoots	0.05*	0.125	0.125
0251050	Land cresses	0.05*	0.125	0.125
0251060	Roman rocket/rucola	0.05*	0.125	0.125
0251070	Red mustards	0.05*	0.125	0.125
0251080	Baby leaf crops (including brassica species)	0.05*	0.125	0.125
0251990	Others	0.05*	0.125	0.125
0252000	(b) spinaches and similar leaves			
0252010	Spinaches	0.05*	0.125	0.125
0252020	Purslanes	0.05*	0.125	0.125
0252030	Chards/beet leaves	0.05*	0.125	0.125
0252990	Others	0.05*	0.125	0.125

Table 6.9-2: Glyphosate - Input values used for risk assessment

Code number	Commodity	Proposed MRLs (mg/kg)	Input values (mg/kg)	
			Chronic RA ($STMR_{RA}$)	Acute RA (HR_{RA})
0256000	(f) herbs and edible flowers			
0256010	Chervil	0.05*	0.125	0.125
0256020	Chives	0.05*	0.125	0.125
0256030	Celery leaves	0.05*	0.125	0.125
0256040	Parsley	0.05*	0.125	0.125
0256050	Sage	0.05*	0.125	0.125
0256060	Rosemary	0.05*	0.125	0.125
0256070	Thyme	0.05*	0.125	0.125
0256080	Basil and edible flowers	0.05*	0.125	0.125
0256090	Laurel/bay leave	0.05*	0.125	0.125
0256100	Tarragon	0.05*	0.125	0.125
0256990	Others	0.05*	0.125	0.125
0260000	Legume vegetables			
0260010	Beans (with pods)	0.05*	0.125	0.125
0260020	Beans (without pods)	0.05*	0.125	0.125
0260030	Peas (with pods)	0.05*	0.125	0.125
0260040	Peas (without pods)	0.05*	0.125	0.125
0260050	Lentils	0.05*	0.125	0.125
0260990	Others	0.05*	0.125	0.125
0270000	Stem vegetables			
0270060	Leeks	0.05*	0.125	0.125
0400000	OILSEEDS AND OIL FRUITS			
0402000	Oil fruits			
0402010	Olives for oil production	0.05*	0.125	0.125
0600000	TEAS, COFFEE, HERBAL INFUSIONS, COCOA AND CAROBS			
0630000	Herbal infusions from			
0632000	(b) leaves and herbs			
0632010	Strawberry	0.05*	0.125	0.125
0632020	Rooibos	0.05*	0.125	0.125
0632030	Mate/maté	0.05*	0.125	0.125
0632990	Others	0.05*	0.125	0.125
0900000	SUGAR PLANTS			
0900010	Sugar beet roots	0.05*	0.125	0.125
0900030	Chicory roots	0.05*	0.125	0.125
1000000	PRODUCTS OF ANIMAL ORIGIN - TERRESTRIAL ANIMALS			
1010000	Tissues from			
1011000	(a) swine			
1011010	Muscle	0.060	0.050	0.050
1011020	Fat tissue	0.060	0.050	0.050
1011030	Liver	0.060	0.090	0.090
1011040	Kidney	0.700	0.710	0.710
1011050	Edible offals (other than liver and kidney)			
1011990	Others			

Table 6.9-2: Glyphosate - Input values used for risk assessment

Code number	Commodity	Proposed MRLs (mg/kg)	Input values (mg/kg)	
			Chronic RA ($STMR_{RA}$)	Acute RA (HR_{RA})
1012000	(b) bovine			
1012010	Muscle	0.150	0.130	0.130
1012020	Fat tissue	0.150	0.130	0.130
1012030	Liver	0.500	0.500	0.500
1012040	Kidney	0.150	0.130	0.130
1012050	Edible offals (other than liver and kidney)			
1012990	Others			
1013000	(c) sheep			
1013010	Muscle	0.150	0.130	0.130
1013020	Fat tissue	0.150	0.130	0.130
1013030	Liver	0.500	0.500	0.500
1013040	Kidney	0.150	0.130	0.130
1013050	Edible offals (other than liver and kidney)			
1013990	Others			
1014000	d) goat			
1014010	Muscle	0.150	0.130	0.130
1014020	Fat tissue	0.150	0.130	0.130
1014030	Liver	0.500	0.500	0.500
1014040	Kidney	0.150	0.130	0.130
1015000	(e) equine			
1015010	Muscle	0.060	0.050	0.050
1015020	Fat tissue	0.060	0.050	0.050
1015030	Liver	0.060	0.090	0.090
1015040	Kidney	0.700	0.710	0.710
1016000	(f) poultry			
1016010	Muscle	0.150	Proposed MRL	Proposed MRL
1016020	Fat tissue	0.150	Proposed MRL	Proposed MRL
1016030	Liver	0.150	Proposed MRL	Proposed MRL
1020000	Milk			
1020010	Cattle	0.06*	Proposed MRL	Proposed MRL
1020020	Sheep	0.06*	Proposed MRL	Proposed MRL
1020030	Goat	0.06*	Proposed MRL	Proposed MRL
1020040	Horse	0.06*	Proposed MRL	Proposed MRL
1020990	Others	0.06*	Proposed MRL	Proposed MRL
1030000	Birds eggs			
1030010	Chicken	0.06*	Proposed MRL	Proposed MRL
1030020	Duck	0.06*	Proposed MRL	Proposed MRL

Table 6.9-2: Glyphosate - Input values used for risk assessment

Code number	Commodity	Proposed MRLs (mg/kg)	Input values (mg/kg)	
			Chronic RA ($STMR_{RA}$)	Acute RA (HR_{RA})
1030030	Geese	0.06*	Proposed MRL	Proposed MRL
1030040	Quail	0.06*	Proposed MRL	Proposed MRL
1030990	Others	0.06*	Proposed MRL	Proposed MRL
1040000	Honey and other apiculture products	0.60	0.6	0.60

RA Residue definition for risk assessment

With the current EFSA model the chronic risk assessment ranges from 0.1 to 2 % of the ADI (see Table 6.9-3). The diet with the highest TMDI is "NL toddler" with 1 % of the ADI. For this diet, the highest contributors are milk (cattle) with 0.6 % of the ADI.

Since the ADI utilisation does not exceeds 100 %, a refinement was not performed.

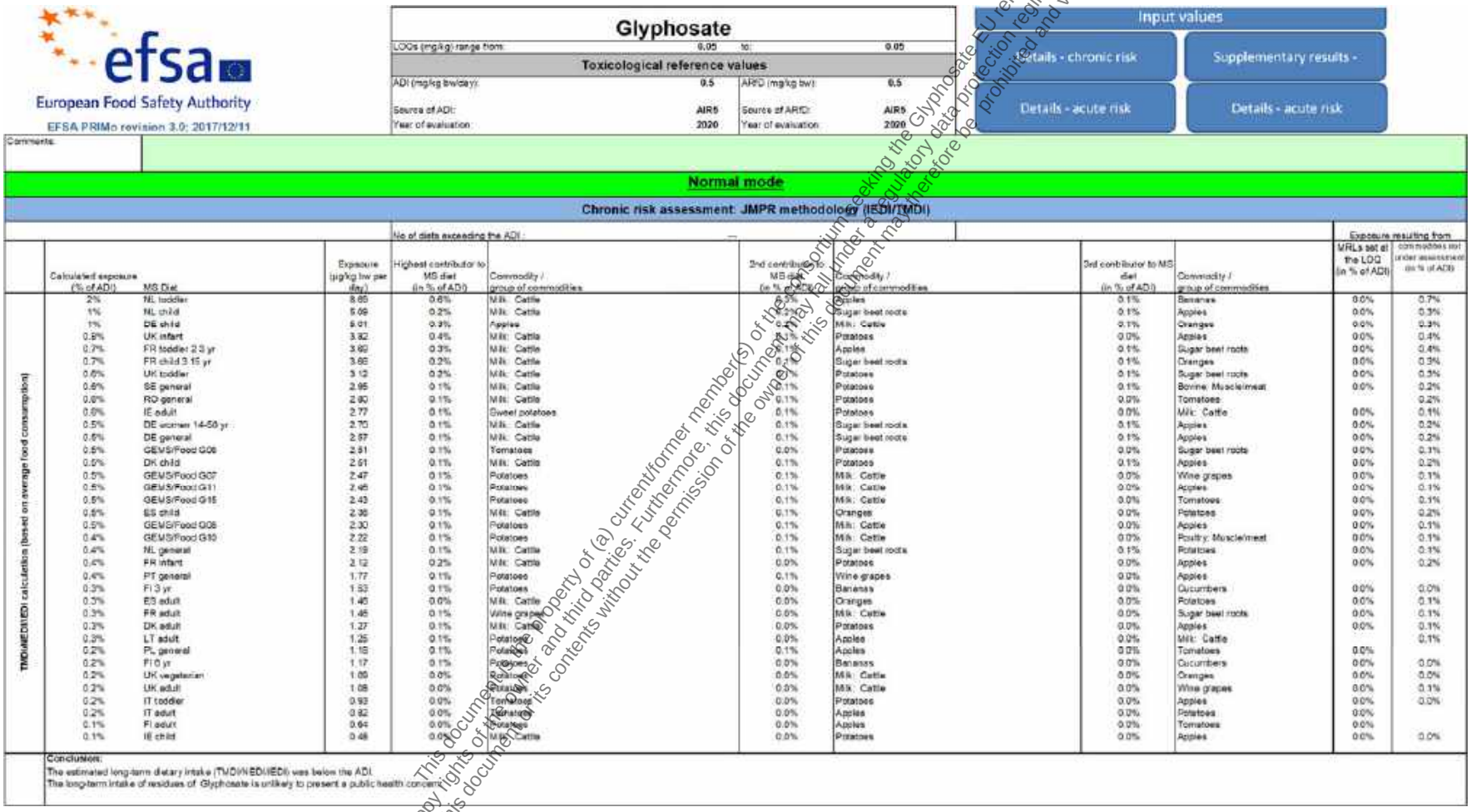
Acute consumer risk assessment

A revised acute exposure assessment was performed using representative crops only, as well as animal matrices. The input values are given in Table 6.9-2.
The summary of the calculation using PRIMo rev. 3.1 is presented in Table 6.9-4. For the assessment, the ARfD of 0.5 mg/kg bw was used.

With the current EFSA model the acute risk assessment is 4 % of the ARfD for children and 3 % for adults due to consumption of potatoes and sugar beets, respectively.

The ARfD utilisation does not exceed 100 %. Thus, a risk for the consumer is not expected.

Table 6.9-3: EFSA Pesticide Residue Intake Model (PRIMo rev. 3.1) – Chronic risk assessment calculation for glyphosate



European Food Safety Authority

EFSA PRIMo revision 3.0; 2017/12/11

Glyphosate

LOQs (mg/kg) range from: 0.05 to: 0.05

Toxicological reference values

ADI (mg/kg bw/day):	0.5	ARfD (mg/kg bw):	0.5
Source of ADI:	AIR5	Source of ARfD:	AIR5
Year of evaluation:	2020	Year of evaluation:	2020

Input values

Details - chronic risk | Supplementary results - | Details - acute risk | Details - acute risk

Comments:

Normal mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI: –

	Calculated exposure (% of ADI)	MS Diet	Exposure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from MRLs set at the LOQ (in % of ADI)	Exposure resulting from commodities not under assessment (in % of ADI)
TMDI/MEDI/IEDI calculations (based on average food consumption)	2%	NL toddler	8.85	0.6%	Milk: Cattle	0.3%	Apples	0.1%	Bananas	0.0%	0.7%
	1%	NL child	5.09	0.2%	Milk: Cattle	0.1%	Sugar beet roots	0.1%	Apples	0.0%	0.3%
	1%	DE child	5.01	0.3%	Apples	0.1%	Milk: Cattle	0.1%	Oranges	0.0%	0.3%
	0.8%	UK infant	3.82	0.4%	Milk: Cattle	0.1%	Potatoes	0.0%	Apples	0.0%	0.4%
	0.7%	FR toddler 2 3 yr	3.69	0.3%	Milk: Cattle	0.1%	Apples	0.1%	Sugar beet roots	0.0%	0.4%
	0.7%	FR child 3 15 yr	3.65	0.2%	Milk: Cattle	0.1%	Sugar beet roots	0.1%	Oranges	0.0%	0.3%
	0.6%	UK toddler	3.12	0.2%	Milk: Cattle	0.1%	Potatoes	0.1%	Sugar beet roots	0.0%	0.3%
	0.6%	SE general	2.95	0.1%	Milk: Cattle	0.1%	Potatoes	0.1%	Bovine: Muscle/meat	0.0%	0.2%
	0.6%	RO general	2.80	0.1%	Milk: Cattle	0.1%	Potatoes	0.0%	Tomatoes		0.2%
	0.6%	IE adult	2.77	0.1%	Sweet potatoes	0.1%	Potatoes	0.0%	Milk: Cattle	0.0%	0.1%
	0.5%	DE women 14-50 yr	2.70	0.1%	Milk: Cattle	0.1%	Sugar beet roots	0.1%	Apples	0.0%	0.2%
	0.5%	DE general	2.57	0.1%	Milk: Cattle	0.1%	Sugar beet roots	0.1%	Apples	0.0%	0.2%
	0.5%	GEMS/Food G06	2.51	0.1%	Tomatoes	0.0%	Potatoes	0.0%	Sugar beet roots	0.0%	0.1%
	0.5%	DK child	2.51	0.1%	Milk: Cattle	0.1%	Potatoes	0.1%	Apples	0.0%	0.2%
	0.5%	GEMS/Food G07	2.47	0.1%	Potatoes	0.1%	Milk: Cattle	0.0%	Wine grapes	0.0%	0.1%
	0.5%	GEMS/Food G11	2.46	0.1%	Potatoes	0.1%	Milk: Cattle	0.0%	Apples	0.0%	0.1%
	0.5%	GEMS/Food G15	2.43	0.1%	Potatoes	0.1%	Milk: Cattle	0.0%	Tomatoes	0.0%	0.1%
	0.5%	ES child	2.38	0.1%	Milk: Cattle	0.1%	Oranges	0.0%	Potatoes	0.0%	0.2%
	0.5%	GEMS/Food G08	2.30	0.1%	Potatoes	0.1%	Milk: Cattle	0.0%	Apples	0.0%	0.1%
	0.4%	GEMS/Food G10	2.22	0.1%	Potatoes	0.1%	Milk: Cattle	0.0%	Poultry: Muscle/meat	0.0%	0.1%
	0.4%	NL general	2.19	0.1%	Milk: Cattle	0.1%	Sugar beet roots	0.1%	Potatoes	0.0%	0.1%
	0.4%	FR infant	2.12	0.2%	Milk: Cattle	0.0%	Potatoes	0.0%	Apples	0.0%	0.2%
	0.4%	PT general	1.77	0.1%	Potatoes	0.1%	Wine grapes	0.0%	Apples		
	0.3%	FI 3 yr	1.53	0.1%	Potatoes	0.0%	Bananas	0.0%	Cucumbers	0.0%	0.0%
	0.3%	ES adult	1.46	0.0%	Milk: Cattle	0.0%	Oranges	0.0%	Potatoes	0.0%	0.1%
	0.3%	FR adult	1.46	0.1%	Wine grapes	0.0%	Milk: Cattle	0.0%	Sugar beet roots	0.0%	0.1%
	0.3%	DK adult	1.27	0.1%	Milk: Cattle	0.0%	Potatoes	0.0%	Apples	0.0%	0.1%
	0.3%	LT adult	1.25	0.1%	Potatoes	0.0%	Apples	0.0%	Milk: Cattle		0.1%
	0.2%	PL general	1.15	0.1%	Potatoes	0.1%	Apples	0.0%	Tomatoes	0.0%	
	0.2%	FI 0 yr	1.17	0.1%	Potatoes	0.0%	Bananas	0.0%	Cucumbers	0.0%	0.0%
	0.2%	UK vegetarian	1.09	0.0%	Potatoes	0.0%	Milk: Cattle	0.0%	Oranges	0.0%	0.0%
	0.2%	UK adult	1.08	0.0%	Potatoes	0.0%	Milk: Cattle	0.0%	Wine grapes	0.0%	0.1%
	0.2%	IT toddler	0.93	0.0%	Tomatoes	0.0%	Potatoes	0.0%	Apples	0.0%	0.0%
	0.2%	IT adult	0.82	0.0%	Tomatoes	0.0%	Apples	0.0%	Potatoes	0.0%	
	0.1%	FI adult	0.64	0.0%	Potatoes	0.0%	Apples	0.0%	Tomatoes	0.0%	
	0.1%	IE child	0.48	0.0%	Milk: Cattle	0.0%	Potatoes	0.0%	Apples	0.0%	0.0%

Conclusion:
The estimated long-term dietary intake (TMDI/MEDI/IEDI) was below the ADI.
The long-term intake of residues of Glyphosate is unlikely to present a public health concern.

Table 6.9-4: EFSA Pesticide Residue Intake Model (PRIMo rev. 3.1) – Acute risk assessment calculation for glyphosate

Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

Show results of IESTI calculation only for crops with GAPs under assessment

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): —

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
4%	Potatoes	0.05 / 0.13	19
4%	Melons	0.05 / 0.13	19
3%	Pears	0.05 / 0.13	17
3%	Oranges	0.05 / 0.13	17
3%	Watermelons	0.05 / 0.13	15
3%	Apples	0.05 / 0.13	13
2%	Bananas	0.05 / 0.13	12
2%	Peaches	0.05 / 0.13	12
2%	Grapefruits	0.05 / 0.13	9.8
2%	Table grapes	0.05 / 0.13	9.1
2%	Cucumbers	0.05 / 0.13	8.2
2%	Carrots	0.05 / 0.13	7.9
2%	Kiwi fruits (green, red,	0.05 / 0.13	7.8
1%	Sweet peppers/bell peppers	0.05 / 0.13	7.4
1%	Mandarins	0.05 / 0.13	7.3

Expand/collapse list

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): —

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
1%	Head cabbages	0.05 / 0.13	5.3
1%	Watermelons	0.05 / 0.13	5.1
1.0%	Melons	0.05 / 0.13	4.9
0.9%	Swedes/rutabagas	0.05 / 0.13	4.3
0.8%	Table grapes	0.05 / 0.13	4.2
0.8%	Oranges	0.05 / 0.13	3.8
0.8%	Pears	0.05 / 0.13	3.8
0.7%	Potatoes	0.05 / 0.13	3.7
0.7%	Okras	0.05 / 0.13	3.5
0.7%	Apples	0.05 / 0.13	3.5
0.7%	Cucumbers	0.05 / 0.13	3.5
0.6%	Chinese cabbages/pe-tsai	0.05 / 0.13	3.2
0.6%	Broccoli	0.05 / 0.13	3.0
0.6%	Wine grapes	0.05 / 0.13	3.0
0.6%	Courgettes	0.05 / 0.13	2.9

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI):

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
3%	Sugar beets (root) / sugar	0.05 / 1.5	14
2%	Potatoes / fried	0.05 / 0.13	12
2%	Pumpkins / boiled	0.05 / 0.13	11
2%	Broccoli / boiled	0.05 / 0.13	9.8
2%	Cauliflowers / boiled	0.05 / 0.13	8.7
2%	Escaroles/broad-leaved end	0.05 / 0.13	8.3
2%	Head cabbages / boiled	0.05 / 0.13	7.9
1%	Potatoes / dried (flakes)	0.05 / 0.56	7.4
1%	Apples / juice	0.05 / 0.13	6.8
1%	Oranges / juice	0.05 / 0.13	6.6
1%	Parsnips / boiled	0.05 / 0.13	6.3
1%	Sweet potatoes / boiled	0.05 / 0.13	6.3
1%	Witloofs / boiled	0.05 / 0.13	6.1
1%	Beetroots / boiled	0.05 / 0.13	5.5
1%	Wine grapes / juice	0.05 / 0.13	5.5

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): —

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
1%	Pumpkins / boiled	0.05 / 0.13	6.9
1%	Sugar beets (root) / sugar	0.05 / 1.5	5.5
1%	Cauliflowers / boiled	0.05 / 0.13	5.2
0.6%	Apples / juice	0.05 / 0.13	4.2
0.6%	Broccoli / boiled	0.05 / 0.13	3.0
0.6%	Courgettes / boiled	0.05 / 0.13	2.9
0.6%	Beetroots / boiled	0.05 / 0.13	2.8
0.5%	Kohlrabies / boiled	0.05 / 0.13	2.7
0.5%	Wine grapes / juice	0.05 / 0.13	2.6
0.5%	Escaroles/broad-leaved	0.05 / 0.13	2.6
0.5%	Turnips / boiled	0.05 / 0.13	2.4
0.5%	Cassava roots / boiled	0.05 / 0.13	2.4
0.5%	Witloofs / boiled	0.05 / 0.13	2.3
0.5%	Celeriacs / boiled	0.05 / 0.13	2.3
0.4%	Sweet potatoes / boiled	0.05 / 0.13	1.9

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity. A short term intake of residues of Glyphosate is unlikely to pres
For processed commodities, no exceedance of the ARfD/ADI was identified.

Relevant published articles from Literature Search Report

1. Information on the study

Data point	CA 6.9/001
Report author	Zoller, O. *et al.*
Report year	2018
Report title	Glyphosate residues in Swiss market foods: monitoring and risk evaluation
Document No.	DOI 10.1080/19393210.2017.1419509 E-ISSN 1939-3229
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Uncertain reliability

2. Full summary of the study according to OECD format

Executive Summary

A total of 243 samples of diverse foodstuffs were analysed for glyphosate and aminomethylphosphonic acid (AMPA) using a liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) method with a relatively low limit of quantification in the range of 0.0005 – 0.0025 mg/kg. Main contributors for dietary glyphosate and AMPA intake were cereals and pulses. The results suggest that pasta is a very important foodstuff for dietary glyphosate residue intake in Switzerland. Interestingly all samples of wine, fruit juice and nearly all samples of honey tested positive for glyphosate although at very low levels. A dietary risk assessment was conducted. Food products for analysis were not selected purely at random, rather products were selected for which high levels of glyphosate residues were suspected. However, even in samples where high residue levels were expected, no exceedances of maximum residue levels were found. Consequently, human exposure did not exceed neither acceptable daily intake nor acute reference dose. Therefore, glyphosate residues found in the sampled foodstuffs from the Swiss market were of no concern for human health.

Materials and Methods

Samples
In total, 243 samples were analysed. All samples were bought in retail stores with the aim to represent a wide range of food products. Usually a single consumer package of 500 – 2000 g was sampled, irrespective of the lot size. When necessary, samples were homogenised using different mills and mixing devices to a particle size of about 0.1 mm before further processing.

Chemicals, reagents, and consumables
All solvents were obtained in LC-MS grade (Chromasolv ®) from Sigma-Aldrich (Buchs, Switzerland), as well as formic acid. Ultrapure water, further referred to as water, was obtained from an Elga Purelab ultra-water purification system (Labtec Services, Villmergen, Switzerland). Glyphosate standards and AMPA were obtained from Sigma-Aldrich; glyphosate internal standard (IS) $^{13}C_3$-D_2-Glyphosate from Alsachim (Illkirch-Graffenstaden, France); AMPA IS ^{13}C-^{15}N-AMPA from Dr. Ehrenstorfer (LGC Standards, Teddington, UK). All dilutions of standard solutions were prepared in water except the last dilution for standards ready for injection where dilution solvent was used. These dilutions were made in 20 mL vials, which were rinsed with water and methanol before use.
The extraction solvent was a water/methanol 1/1 (v/v) mixture with 0.5 % formic acid; the dilution solvent was a water/acetonitrile 1/1 (v/v) mixture with 0.2 % formic acid; the glyphosate IS and the

AMPA IS solutions were 5000 ng/mL in water; the glyphosate and the AMPA stock solutions were 250 ng/mL in water; the calibration working solutions were 0.004 mL each of glyphosate IS and of AMPA IS solutions, ranging 0 – 0.060 mL of both stock solutions, respectively and extraction solvent up to 0.500 mL. The calibration injection solutions for solid samples were 0.100 mL of calibration working solutions diluted with 0.400 mL of dilution solvent. Similar for liquid samples, but dilution with 0.200 mL of dilution solvent.
The applied consumables were 2 and 50 mL centrifuge vials, polypropylene (PP) tubes, high density polyethylene (PE) screw caps (Eppendorf, Hamburg, Germany); 20 mL super PE vials for liquid scintillation (PerkinElmer, Waltham, MA, USA); 0.6 mL PE autosampler vials (06-PESV, Chromacol, Thermo Fisher Scientific Inc., Waltham, MA, USA); PP pipet tips for microman (Gilson Inc., Middleton, WI, USA); solid-phase extraction (SPE) cartridges Oasis HLB, 3 cc, 60 mg sorbent (Waters, Milford, MA, USA).

Sample preparation
Solid samples
Five gram of the homogenous or homogenised sample was weighed (rounded to the next 10 mg) into a 50 mL centrifuge vial and 20 mL of extraction solvent and 0.160 mL each of IS solutions were added. The tube was vigorously shaken by hand, then treated for 10 min in an ultrasound bath and shaken for 30 min on a shaker (Innova 2000, Eppendorf, Hamburg, Germany) at 400 rpm. The mixture was then centrifuged for 10 min at 2500 relative centrifugal force (RCF) and 10°C. Two times 1.5 mL of the supernatant was transferred into a 2 mL centrifuge vial and centrifuged for 10 min at 20,000 RCF. The combined supernatants were the final extract. Clean-up was performed on a SPE cartridge, which was first activated with 2 mL of methanol, conditioned with 2 mL of extraction solvent and pre-rinsed with 0.5 mL of extract. The eluate was discarded up to this step. A further 0.4 mL of extract was loaded onto the cartridge, the eluate collected in a 2 mL centrifuge vial and 0.100 mL of this eluate was diluted with 0.400 mL of dilution solvent in an autosampler vial.
Liquid samples
Five millitre of degassed (20 s in an ultrasound bath) beverage was transferred into a 50 mL centrifuge vial and 5 mL of extraction solvent and 0.080 mL each of IS solutions were added. The tube was shaken by hand. The SPE cartridge clean-up was performed as described above, only differing in the last step where 0.100 mL of the final eluate was diluted with 0.200 mL of dilution solvent in an autosampler vial.

Calibration
A 6-point calibration curve, corresponding to a range of 0 – 0.120 mg/kg for solid samples and a range of 0 – 0.060 mg/L for liquid samples, was constructed. If a sample contained a higher concentration, an extract using a lower amount of sample was prepared or further calibration points were introduced..

LC/MS/MS conditions
LC-system and conditions
A Symbiosis-System (Spark Holland B.V., Emmen, The Netherlands) was used with the following parameters: injection volume 10 µL; column BioRad Micro-Guard Cation H Refill Cartridge 30 × 4.6 mm (BioRad, Hercules, CA, USA); column oven at 40°C; elution solvent A: water; elution solvent B: acetonitrile with 0.2 % formic acid; program: 0:00 flow rate 0.5 mL/min 60 % A; 1:00 flow rate 0.5 mL/min 60 % A; 1:30 flow rate 0.5 mL/min 99 % A; 3:30 flow rate 0.5 mL/min 99 % A; 3:35 flow rate 0.8 mL/min 99 % A; 7:50 flow rate 0.8 mL/min 99 % A; 8:00 flow rate 0.8 mL/min 60 % A; 10:00 flow rate 0.5 mL/min 60 % A; 10:10 flow rate 0.5 mL/min 60 % A. The use of a specific rinsing procedure was important to minimise carryover and contamination. Needle rinsing was performed as follows: 500 µl water/methanol/acetonitrile 8/1/1 (v/v) followed by 700 µl water/methanol 1/1 (v/v) with 0.1 % phosphoric acid 85 % and finishing with 500 µl water/acetonitrile 6/4 (v/v) with 0.1 % formic acid. After each sample, a blank run was carried out.
MS/MS-system and conditions
An API 5000 (AB Sciex Netherlands B.V., Nieuwerkerk aan den Ijssel, The Netherlands) with electrospray ionisation in negative mode was used and scheduled multiple reaction monitoring was applied. The eluent in the first 1.5 min was diverted into waste. The optimised ionisation source

parameters were source temperature, 650°C; ionisation voltage − 4500 V; curtain gas, 25 units; collision gas, 5 units; gas 1, 60 units; gas 2, 50 units; Dwell time, 50 ms. The transitions measured were the following (quantifier in bold): glyphosate, 168 → 150, 168 → 124, 168 → 79, **168 → 63**; glyphosate IS, 173 → 128, 173 → 81, **173 → 63**; AMPA, 110 → 81, 110 → 79, **110 → 63**; AMPA IS, 112 → 81, 112 → 79, **112 → 63**..

Method validation

The applied anion exchange method was based on the methods published by Guo *et al.* (2016) and Jensen *et al.* (2016). Validation of the analytical method was based on repeated experiments verifying limit of detection (LOD), LOQ, repeatability, and recovery in different matrices. Internal reference materials were used in each run. For the LOQ, the signal-to-noise threshold was set at 10 for the quantifier and at 7 for the two qualifiers. In addition, two external reference materials of wheat flour and rapeseed and the respective blank materials were analysed on a regular basis: reference material P1601-RMWh, wheat flour spiked with glyphosate, AMPA, glufosinate; blank material P1601-BLWh, wheat flour; reference material P1601-RMRape, rapeseed spiked with glyphosate, AMPA, glufosinate; blank material P1601-BLRape, rapeseed; all from PROOF-ACS GmbH (Hamburg, Germany). Further details of these reference materials are given in the explanation to **Table 1**. A Food Analysis Performance Assessment Scheme (FAPAS 2017) proficiency test on oat test material with chlormequat, mepiquat, and glyphosate was also completed, of which only glyphosate was analysed.

Results and Discussion

Method quality assurance

The method showed to be very robust and can be applied for nearly all kind of foodstuffs. It turned out that it is not necessary to use matrix-matched calibration. The absolute recovery was estimated using the absolute peak area of the IS. The absolute recovery was always better than 70 % for liquid samples and for solid samples it was always better than 50 % and in most cases also better than 70 %. Dilution experiments with naturally contaminated samples with concentrations above 0.05 mg/kg showed identical quantitative results. There was no indication for disturbing matrix effects in the undiluted sample. The LOQ for solid samples was generally 0.001 and 0.0025 mg/kg for glyphosate and AMPA, respectively. For liquid samples (i.e. beverages like wine and beer), the LOQ was 0.0005 mg/kg for glyphosate and 0.0005 – 0.001 mg/kg for AMPA. Details of the performance data of the method are given in **Table 1**. The FAPAS proficiency test (2017) was successfully passed with a z-score of 0.9 at the assigned value for glyphosate of 0.483 mg/kg. This level was appropriate for the validation of the higher levels that were measured, for instance in durum wheat and pasta, but not optimal for the lower levels around and below 0.05 mg/kg. For these levels, the wheat and rapeseed reference materials (PROOF-ACS GmbH) with assigned values for glyphosate of 0.034 and 0.086 mg/kg, respectively, were more appropriate. In **Table 1** it is shown that our measurements were in good agreement with the assigned values and also with the spiked values. In the FAPAS 09109b, oats blank material, 0.0057 mg/kg of glyphosate was measured.
The measurement uncertainty which is indicated in the supporting information is an estimate for the expanded uncertainty with a confidence level of 95 %. The values are roughly estimated with the help of the method performance data given in **Table 1**. Twenty percent is set as minimum value for the uncertainty. A more conservative approach would be to take the uncertainty from the proficiency tests of the mentioned FAPAS test and PROOF-ACS reference materials. The range of ±2 for z-scores is a good estimate for the confidence interval of 95 %. In this case, the uncertainty would generally be set at 45 % as the uncertainty for all values from the PROOF-ACS materials were between 43.3 % and 44.7 %. The respective uncertainty for glyphosate in the FAPAS test was 35.6 %.
In a few cases where it was suspected that the sample might not be sufficiently homogeneous, another two subsamples were analysed. In all cases, the difference to the first result was well below 10 %. In the case of the gram flour with a concentration of 2.756 mg/kg of glyphosate, which is discussed further down in the text, a package of the same lot could be purchased 6 months later. The measured concentration in the second package differed less than 2 % from the first result.

Table 1: Method performance data.

Analyte	Matrix	LOD [mg kg^{-1}]	LOQ [mg kg^{-1}]	concentration [mg kg^{-1}]	Repetitions (n)	Recovery (%)	RSD (%)	Comments and applied reference materials
Glyphosate	Wheat, white flour	0.0003	0.001	0.001	5	94	9.5	s, st
AMPA	Wheat, white flour	0.001	0.0025	0.005	5	101	6.5	s, st
Glyphosate	Beer	0.0002	0.0005	0.001	5	103	2.2	s, st
AMPA	Beer	0.0005	0.001	0.001	5	97	6.6	s, st
Glyphosate	Beer	0.0002	0.0005	0.010	3	98	7.1	s, st, d
AMPA	Beer	0.0005	0.001	0.010	3	102	0.6	s, st, d
Glyphosate	Wine	0.0002	0.0005	0.010	2	92	9.2	s, st, d
AMPA	Wine	0.0005	0.001	0.010	2	99	5.0	s, st, d
Glyphosate	Milk	0.0002	0.0005	0.004	2	96	1.8	s, st, d
AMPA	Milk	0.0005	0.001	0.004	2	111	1.6	s, st, d
Glyphosate	Honey	0.0003	0.001	0.005	5	92	13.9	s, st, d
AMPA	Honey	0.001	0.0025	0.005	5	115	3.5	s, st, d
Glyphosate	Vegetable oil	0.0004	0.001	0.010	2	102	2.8	s, st, d
AMPA	Vegetable oil	0.001	0.0025	0.010	2	92	6.1	s, st, d
Glyphosate	Smoked salmon	0.0004	0.001	0.010	1	95	N/A	s
AMPA	Smoked salmon	0.001	0.0025	0.010	1	97	N/A	s
Glyphosate	Poultry meat	0.0003	0.001	0.050	3	102	1.3	s, st, d
AMPA	Poultry meat	0.001	0.0025	0.050	3	100	1.3	s, st, d
Glyphosate	Red wine	0.0002	0.0005	0.0132	7	N/A	5.6	nc, lt
AMPA	Red wine	0.0005	0.001	<0.001	7	N/A	N/A	nc, lt
Glyphosate	Whole meal flour	0.0003	0.001	0.051	5	N/A	10.7	nc, st
AMPA	Whole meal flour	0.001	0.0025	0.0036	5	N/A	8.4	nc, st
Glyphosate	Whole meal flour	0.0003	0.001	0.051	22	N/A	10.4	nc, lt
AMPA	Whole meal flour	0.001	0.0025	0.0024	22	N/A	17.5	nc, lt
Glyphosate	Wheat	0.0003	0.001	<0.001	19	N/A	N/A	P1601-BLWh, lt
AMPA	Wheat	0.001	0.0025	<0.0025	19	N/A	N/A	P1601-BLWh, lt
Glyphosate	Wheat	0.0003	0.001	0.0376	21	N/A	8.4	P1601-RMWh, lt
AMPA	Wheat	0.001	0.0025	0.0577	21	N/A	9.1	P1601-RMWh, lt
Glyphosate	Rapeseed	0.0003	0.001	<0.001	3	N/A	N/A	P1601-BLRape, lt
AMPA	Rapeseed	0.001	0.0025	<0.0025	3	N/A	N/A	P1601-BLRape, lt
Glyphosate	Rapeseed	0.0003	0.001	0.0925	3	N/A	2.2	P1601-RMRape, lt
AMPA	Rapeseed	0.001	0.0025	0.0778	3	N/A	3.1	P1601-RMRape, lt

N/A: not applicable; s: spiked; nc: naturally contaminated; st: repetitions within 1 day; lt: repetitions over a time period of 7 months; d: different products; P1601-BLWh: wheat blank material; P1601-RMWh: wheat reference material, spiked level for glyphosate 0.037 mg kg^{-1} and assigned value by proficiency test 0.034 mg kg^{-1}, spiked level for AMPA 0.055 mg kg^{-1} and assigned value by proficiency test 0.050 mg kg^{-1}; P1601-BLRape: rapeseed blank material; P1601-RMRape: rapeseed reference material, spiked level for glyphosate 0.084 mg kg^{-1} and assigned value by proficiency test 0.0859 mg kg^{-1}, spiked level for AMPA 0.088 mg kg^{-1} and assigned value by proficiency test 0.0739 mg kg^{-1}.

Another peak showing quite similar ion transitions as glyphosate, eluting just after glyphosate, was often observed. This peak was identified as 2-amino-3-phosphonopropionic acid, a substance with identical sum formula and similar functional groups as glyphosate. This compound seems to occur in many products in the range of 0.001 – 0.5 mg/kg. For this reason, it can be recommended to check if 2-amino-3-phosphonopropionic acid is properly distinguished from glyphosate in the chromatograms, as to avoid the risk of too high results when analysing glyphosate. 2-Amino-3-phosphonopropionic acid was analysed semi-quantitatively and seems to occur in many products, especially in cereals, in the range of 0.001 – 0.9 mg/kg. There was no correlation between the concentration of 2-amino-3-phosphono-propionic acid and glyphosate. From the chemical structure point of view, it seems unlikely that 2-amino-3-phosphonopropionic acid is a metabolite of glyphosate. 2-Amino-3-phosphonopropionic acid may be a natural compound. Its occurrence in the ciliate *Tetrahymena pyriformis* is described by Horsman and Zechel (2017); however, no reference on the occurrence in higher plants is available. This issue will be examined in more detail in the context of another project.

Table 2: Concentrations of glyphosate and AMPA in different food categories.

Food category	Glyphosate: Number of samples	Glyphosate: Number of samples above the LOQ	Glyphosate: Proportion of samples above the LOQ	Glyphosate: LOQ (mg kg⁻¹)	Glyphosate: Min (mg kg⁻¹)	Glyphosate: Median (mg kg⁻¹)	Glyphosate: Arithmetic mean (mg kg⁻¹)	Glyphosate: Max (mg kg⁻¹)	AMPA: Number of samples above the LOQ	AMPA: Proportion of samples above the LOQ	AMPA: LOQ (mg kg⁻¹)	AMPA: Min (mg kg⁻¹)	AMPA: Median (mg kg⁻¹)	AMPA: Arithmetic mean (mg kg⁻¹)	AMPA: Max (mg kg⁻¹)
Beer	15	2	13%	0.0005	<0.0005	<0.0005	0.0006	0.0068	0	0%	0.001	<0.001	<0.001	<0.001	<0.001
Wine	21	21	100%	0.0005	0.0006	0.0031	0.0048	0.0189	4	19%	0.0007	<0.0007	<0.0007	0.0005	0.0034
Mineral water	2	0	0%	0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0	0%	0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Milk	3	0	0%	0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Fruit juice	11	11	100%	0.0005	0.0005	0.0016	0.0019	0.0035	2	18%	0.0006	<0.0006	<0.0006	0.0002	0.0006
Baby food	11	0	0%	0.001	<0.001	<0.001	<0.001	<0.001	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Potatoes and vegetables	10	3	30%	0.001	<0.001	<0.001	0.0013	0.0077	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Honey	16	15	94%	0.001	<0.001	0.0030	0.0046	0.0159	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Eggs	1	0	0%	0.001	<0.001	<0.001	<0.001	<0.001	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Meat and fish	13	3	23%	0.001	<0.001	<0.001	0.0008	0.0049	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Pulses	41	21	51%	0.001	<0.001	0.0012	0.1733	2.948	10	24%	0.0025	<0.0025	<0.0025	0.0031	0.025
Oilseeds and vegetable oil	6	0	0%	0.001	<0.001	<0.001	<0.001	<0.001	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Pseudo cereals	3	0	0%	0.001	<0.001	<0.001	<0.001	<0.001	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Breakfast cereals	10	8	80%	0.001	<0.001	0.0036	0.0508	0.291	3	30%	0.0025	<0.0025	<0.0025	0.0025	0.010
Durum wheat	18	16	89%	0.001	<0.001	0.139	0.0349	0.421	15	83%	0.0025	<0.0025	0.0107	0.0110	0.0247
Pastry and snacks	11	4	36%	0.001	<0.001	<0.001	0.0027	0.0179	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Bread	10	7	70%	0.001	<0.001	0.0019	0.0069	0.0458	0	0%	0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Flour and baking mixtures	28	8	29%	0.001	<0.001	<0.001	0.0106	0.133	2	7%	0.0025	<0.0025	<0.0025	0.0007	0.0027
Other cereal products	13	2	15%	0.001	<0.001	<0.001	0.0012	0.0124	1	8%	0.0025	<0.0025	<0.0025	0.0007	0.0052

Description of categories: *pulses*: including products thereof like tofu and soy sauce, etc.; *breakfast cereals*: processed breakfast cereals like cornflakes, pops, etc. Rolled oats are placed in the category of other cereal products; *durum wheat*: all products with durum wheat as main ingredient as for instance pasta; *pastry and snacks*: all dry bake goods, sweet or salty, and also tortilla chips and potato chips (crisps); *bread*: also special bread that may contain minor amounts of oilseeds or pulses; *flour and baking mixtures*: flour and baking mixtures for bread making, the main ingredients are bread cereals like wheat, rye, and spelt, but they may also contain minor amounts of other cereals, oilseeds, and pulses; *other cereal products*: category with a wide variety of products like rolled oats, popcorn, semolina of maize (polenta), pasta with wheat instead of durum wheat, etc.; for beverages as beer, wine, milk, fruit juices, and mineral water, the measurement unit is mg L^{-1} instead of mg kg^{-1}. For the calculation of the arithmetic mean, all samples below the LOD were taken as zero; for samples between the LOQ and the LOD, the estimated value was used.

Concentrations in foodstuffs

Food products were sampled with the aim to determine the relevant foodstuffs for glyphosate intake. Samples with higher residue concentrations are probably over-represented to some extent, because categories like pulses and durum wheat were more frequently sampled, since these were suspect to reveal more glyphosate positive results. Additionally, every time when food samples turned out to contain more than 0.01 mg/kg, a few similar food items were collected. All together survey results are probably not representative for the residue levels in all foodstuffs on the market, as to achieve this goal analysis of a few thousand samples would have been necessary. The results for glyphosate and AMPA are summarised in **Table 2** and grouped into different food categories. Detailed data is available as supporting information.

For cereals and pulses, the contamination rate for glyphosate on the level above 0.1 mg/kg is comparable with data from Germany (Scherbaum *et al.* 2012) and a bit lower as in the United Kingdom (Stephenson and Harris 2016). The two samples with the highest glyphosate concentration were chickpeas originating from Canada with 2.948 mg/kg and gram flour (chickpea flour) with 2.756 mg/kg produced in the United Kingdom with unknown origin of the processed chickpeas. In 24 samples, glyphosate was measured above 0.1 mg/kg, but all AMPA values were below 0.1 mg/kg and usually much lower than the respective glyphosate values. Thirteen of 24 samples were durum wheat products like pasta and semolina, 8 samples were pulses and products thereof, 2 further samples were breakfast cereals and the last product was a bread baking mix containing seeds. It could be shown that the main contributor for glyphosate residue in this mix was linseed. There was no hint that 1 of these 24 products contained relevant ingredients of Swiss origin. Pulses are not consumed very often in Switzerland; however, pasta is an important dish of the regional diet. As nearly 100 % of durum wheat for the production of pasta is imported, this might be an important commodity regarding glyphosate residues. All samples of wine and fruit juice and all except one sample of honey were positive for glyphosate but all in the low ng/g range.

Of all analysed samples, 38 were clearly indicated as made of Swiss ingredients. The product with the highest glyphosate concentration of this category was a red wine containing 0.0132 mg/kg. All cereal products of this category contained undetectable or low amounts. The highest value found was 0.0025 mg/kg glyphosate in a wholegrain wheat flour. The number of 38 samples with ingredients of Swiss origin is not large enough as to guarantee that Swiss regulations on the use of glyphosate in agricultural practice are not violated, but at least do not indicate unregistered use of glyphosate, since not one single high contamination was found in food items containing raw products originating from Switzerland.

Also, all products labelled as organic had no or only low residues. In 37 of totally 43 organic samples, the concentration was below the LOQ and only 6 samples showed quantifiable amounts. In three of these six samples the concentration was just above the LOQ and only one sample showed a concentration above 0.01 mg/kg. This organic sample with the highest glyphosate concentration was a pasta product (spaghetti) containing 0.0123 mg/kg of glyphosate and 0.0024 mg/kg of AMPA. On the label, it was indicated that the durum wheat originated from North America, Europe and the eggs from Europe. Carryover during transport and production is conceivable. No detailed data are available to what extent such a contamination is avoidable by using adequate practices. As far as we know there is not yet a binding agreement on how low the residues in organic products should be, but a value of 0.01 mg/kg is at least under discussion or maybe already partially implemented.

Risk assessment

Based on the measured residues (**Table 2**), simple exposure estimates were derived (**Table 3**) and compared to the ARfD and the ADI, both amounting to 0.5 mg/kg bw/day, as recently established by EFSA's revaluation (EFSA 2015). Food consumption values applied in the exposure estimation were chosen at a level to overestimate actual daily average consumption. It seems plausible that these amounts of the respective food items are consumed at least occasionally during a single day. Risk assessments, i.e. comparison of estimated residue intake with the ADI and ARfD, were conducted for both the measured median and MRLs found per food item.

None of the median residues found in any food item resulted in an exposure greater than 0.5 % of the ADI/ARfD and virtually all are significantly below 0.5 % of the ADI/ARfD. If measured MRLs were applied, substantial exposures (ca. 5 % of ADI/ARfD in adults and ca. 10 % of ADI/ARfD in children)

resulted for pulses, exclusively. All other MRLs resulted in exposures that were mostly significantly lower than 1 % of the ADI/ARfD. It is concluded that none of the residue levels identified in any of the food categories are of any health concern. This is not surprising, as none of the measured residue levels exceeded the legally tolerated MRL.

Table 3: Exposure to median and maximum glyphosate residue levels and expected urine glyphosate concentrations (nr: not relevant).

	Child of 15 kg body weight				Adult of 60 kg body weight			
		Exposure as % of ADI or ARfD				Exposure of % of ADI or ARfD		
Food category	Consumption (kg or L/day)	At median residue level	At maximum residue level	Expected urine concentration (µg L^{-1})	Consumption (kg or L per day)	At median residue level	At maximum residue level	Expected urine concentration (µg L^{-1})
Beer	nr	nr	nr	nr	0.50	0.0008	0.0013	0.340
Wine	nr	nr	nr	nr	0.25	0.0026	0.0158	0.473
Mineral water	1.00	0.0067	0.0067	0.067	2.00	0.0033	0.0033	0.100
Milk	0.50	0.0033	0.0033	0.033	1.00	0.0017	0.0017	0.050
Fruit juice	0.50	0.0107	0.0233	0.233	1.00	0.0033	0.0117	0.350
Potatoes and vegetables	0.25	0.0033	0.0257	0.257	0.50	0.0017	0.0128	0.385
Honey	0.03	0.0010	0.0053	0.053	0.05	0.0005	0.0027	0.080
Eggs	0.10	0.0013	0.0013	0.013	0.20	0.0007	0.0007	0.020
Meat and fish	0.25	0.0033	0.0163	0.163	0.50	0.0017	0.0082	0.245
Pulses	0.25	0.0033	9.8267	98.27	0.50	0.0017	4.9133	147.4
Oilseeds	0.05	0.0007	0.0007	0.007	0.10	0.0003	0.0003	0.010
Pseudo cereals	0.10	0.0013	0.0013	0.013	0.20	0.0007	0.0007	0.020
Breakfast cereals	0.10	0.0048	0.3880	3.880	0.28	0.0024	0.1940	5.820
Durum wheat	0.25	0.4633	1.4033	14.03	0.50	0.2317	0.7017	21.05
Pastry and snacks	0.05	0.0007	0.0119	0.119	0.10	0.0003	0.0060	0.179
Bread	0.25	0.0063	0.1527	1.527	0.30	0.0032	0.0763	2.290
Flour and baking mixtures	0.25	0.0033	0.4433	4.433	0.50	0.0017	0.2217	6.650
Other cereal products	0.10	0.0013	0.0165	0.165	0.20	0.0007	0.0083	0.248

Exposure per kg body weight is calculated by multiplying the residue concentration in food by the assumed food consumption and dividing the result by body weight (15 kg for children and 60 kg for adults). Risk is expressed by calculating exposure as per cent ADI or ARfD (both amounting to 0.5 mg kg^{-1} bw). Maximally expected urine concentrations are calculated by multiplying maximum residue concentrations in food by the assumed consumption and by the fraction of orally ingested glyphosate excreted by the urine (20 %). The obtained result is divided by an assumed daily urine volume of 1.5 L for a child and 2 L for an adult. If residues were below LOQ, the LOQ value was used for risk assessment.

The exposure estimates for maximum residues derived as described above were also used to predict probable urine concentrations. It was assumed that the amount indicated in Table 3 of the respective food item was ingested and this food item contained the measured MRL of glyphosate (**Table 2**). Based on toxicokinetic studies, the amount of an orally ingested single dose of glyphosate excreted with the urine was assumed to equal 20 % (EFSA 2015). Further, it was assumed that daily urine volumes of 1.5 and 2.0 L are excreted by children and adults, respectively. For glyphosate residues at the maximally measured levels, predicted urine concentrations would be greater than 0.5 µg/L only for a few commodities. Again, only for the maximum residues found in pulses substantial amounts were predicted in urine of adults (ca. 147 µg/L). Overall, the predicted urine concentrations correspond very well with actually measured glyphosate urine levels in samples of the human population: Conrad *et al.* (2017) reported median levels well below 0.5 µg/L in samples of the German population, while maximum values slightly exceeded 0.5 µg/L. Also Niemann *et al.* (2015) concluded that urine concentrations of glyphosate corresponded well with levels in food; however, urine levels of AMPA were somewhat too high and not in good agreement with reported levels in foodstuffs. In a report of glyphosate urine levels in a small, not representative survey of the Swiss population, values in the range of 0.1 – 1.5 µg/L (RTS 2015) were measured.

Conclusion

In this market survey, food products for analysis were not selected purely randomly, rather products were selected for which measurable levels of glyphosate residues were suspected. However, even in samples where high residues were expected, no exceedances of MRLs were detected. Consequently, exposures did not exceed neither ADI nor ARfD. Therefore, glyphosate residues found in the sampled foodstuffs from the Swiss market are of no health concern for the consumer. This conclusion may be valid for all food

products on the Swiss food market, considering that products for which high residue levels were suspected were over-represented in this survey.

Chromatographic conditions

Chromatograph:	Symbiosis
Column:	BioRad Micro-Guard Cation H Refill Cartridge (30 mm x 4.6 mm)
Column oven temperature:	40°C
Injection volume:	10 µL
Mobile phases:	(A) Water (B) Acetonitrile with 0.2 % formic acid

Gradient:

Time (Min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)
0:00	60	40	0.5
1:00	60	40	0.5
1:30	99	1	0.5
3:30	99	1	0.5
3:35	99	1	0.8
7:50	99	1	0.8
8:00	60	40	0.8
10:00	60	40	0.5
10:10	60	40	0.5

Retention time:	Not provided		
Detector:	Sciex API 5000 triple quadrupole mass spectrometer		
Scan type:	MRM		
Ion source:	ESI negative		
Source gas 1:	60 units	Source gas 2:	50 units
Collision gas:	5 units	Source temperature:	650°C
Curtain gas:	25 units	Source voltage:	−4500 V

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering potential (V)	Collision energy (eV)	Scan time (ms)
Primary transition (quantification)					
Glyphosate	168	63	–	–	50
Glyphosate (IS)	173	63	–	–	50
AMPA	110	63	–	–	50
AMPA (IS)	112	63	–	–	50
Secondary transition (confirmation)					
Glyphosate	168	79	–	–	50
Glyphosate (IS)	173	81	–	–	50
AMPA	110	79	–	–	50
AMPA (IS)	112	79	–	–	50

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the results of monitoring analyses for residues of glyphosate and AMPA in food conducted by Swiss authorities between 2012 and 2017. A total of 243 samples of diverse food commodities were analysed for glyphosate and AMPA using an LC-MS/MS method that was developed specifically by the Swiss monitoring laboratory. According to the authors the method has a limit of quantification of 0.001 mg/kg for parent glyphosate and 0.0025 mg/kg for AMPA in solid matrices and 0.0005 mg/kg and 0.001 mg/kg, respectively, in liquid matrices (beer, fruit juice, wine). While it seems that these LOQs were established according to recognised procedures, details are missing and it is, therefore, difficult to evaluate the reliability of the provided analytical results. This would be especially important since the reported LOQs are far below the LOQs achieved by most of the other official monitoring laboratories.

As stated by the authors the publication is not intended to provide a representative picture of the residues of glyphosate and AMPA in food commodities placed on the market in Switzerland since the commodities showing high residues were over-represented. In spite of that, the samples relevant to the uses supported in the renewal dossier (e.g. fruits, vegetables, fruit juice, wine, food of animal origin) all showed residues of glyphosate and AMPA far below 0.05 mg/kg (LOQ of most enforcement method so far).

In total, 16 honey samples from Europe and the Americas were analysed. They showed residues of parent glyphosate between < 0.001 mg/kg and 0.0159 mg/kg while the residues of AMPA were always < 0.0025 mg/kg (details are provided as supplementary data). Since according to SANTE/11956/2016 rev. 9 it is possible to derive EU MRLs in honey based on monitoring data and since honey marketed in Switzerland is likely to be also marketed in the EU, these results are deemed relevant to the setting of an EU MRL for glyphosate in honey. The fact that all the samples showed residues of AMPA < 0.0025 mg/kg is in contrast to another publication in which the analyses were also conducted with a very sensitive analytical method and where the residues of AMPA were often found at levels comparable to or even greater than the levels of parent glyphosate residues.

CA 6.10 Other Studies

CA 6.10.1 Effect on the residue level in pollen and bee products

Residue study in Honey

Study submitted to the EU for the first time

1. Information on the study

Data point:	CA 6.10.1/001
Report author	[redacted]
Report year	2020
Report title	Determination of residues of glyphosate in honey after one application in *Phacelia tanacetifolia* at 4 sites in Germany 2019
Report No	S19-04329
Document No	--
Guidelines followed in study	OECD Guideline for the Testing of Chemicals on Crop Field Trial (TG 509 published in September 2009) EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) Commission Regulation (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 (Oct. 2009)

Deviations from current test guideline	According to SANTE/11956/2016 rev. 9, for one trial no replicate sample could be taken due to the low amount of food stores available in the hives.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Valid
Category study in AIR 5 dossier (L docs)	Category 1

2. Full summary of the study according to OECD format

Executive Summary

The objective of the study was to collect honey samples from *Phacelia tanacetifolia* after one application of MON52276 under semi-field conditions in order to measure the residues of glyphosate. MON52276 is a soluble concentrate formulation with a nominal content of 360 g/L of glyphosate as its isopropylammonium salt.

The study included 4 field trials in Germany during the 2019 season. The Phacelia fields were treated once, at a target rate of 2.16 kg glyphosate per hectare. Samples of honey were taken from the bee combs for analysis 8-9 days after application. Residues of glyphosate and AMPA in honey ranged from <0.025 mg/kg to 6.9 mg/kg and from <0.025 mg/kg to 0.028 mg/kg, respectively.

I. Materials and Methods

A. Materials

1. Test material

Description:	MON52276 Roundup BIO, Glyphosate 360 g/L
Batch number:	AJC070810A
EAS test item code:	M-00022865
Active ingredient(s):	Glyphosate (s/f IPA)
CAS number:	38641-94-0
Content of a.s. nominal:	41.5 % w/v 360 g/L
Content of a.s. analysed:	31.1 %
Formulation type:	SL
Appearance/colour:	yellow brownish liquid
Certificate of analysis:	30.08.2019
Expiry date:	07.03.2024

Test commodities

Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S19-03987-01	Phacelia	*Phacelia tanacetifolia*	Balo	Honey	58.36 g
S19-03987-02	Phacelia	*Phacelia tanacetifolia*	Balo	Honey	99.12 g
S19-03987-03	Phacelia	*Phacelia tanacetifolia*	Stala	Honey	47.33 g
S19-03987-04	Phacelia	*Phacelia tanacetifolia*	Stala	Honey	5.983 g

Test facilities

Study directory:	Eurofins Agroscience Services Ecotox GmbH, 75223 Niefern-Öschelbronn / Germany
Field phase (S19-03987-01, S19-03987-02, S19-03987-03 and S19-03987-04):	Eurofins Agroscience Services Ecotox GmbH, 75223 Niefern-Öschelbronn / Germany
Analytical phase:	Eurofins Agroscience Services Ecotox GmbH, 75223 Niefern-Öschelbronn / Germany

B. Methods

1. Field phase

Four semi-field trials were conducted to collect honey samples from Phacelia (outdoor) during 2019 in Germany (S19-03987-01, S19-03987-02, S19-03987-03 and S19-03987-04). One application of MON52276 (360 g/L glyphosate) was performed to phacelia at BBCH 63-65 at a target rate of 6 L product/ha. The volume of water used to prepare the spray solution was in the range of 392-413 L/ha. The main application parameters are outlined in the table below.

Table 6.10.1-1: Application information

Trial no.	Application code	Growth stage (BBCH)	Application rate kg a.s./ha	Water volume L/ha
S19-04329-01	T	64-65	2.202	408
S19-04329-02	T	64-65	2.232	413
S19-04329-03	T	63-65	2.182	404
S19-04329-04	T	63-65	2.118	392

On each trial site one tunnel (5 m x 40 m) confining the bees was established on the control and the treated plot. One bee hive was set up per tunnel for the control and treated plot each.

Application was performed with boom sprayer equipped with flat fan nozzles, which were duly calibrated before use. The actual applied amount was calculated by measuring the remaining spray solution after application.

Honeybee colonies *(Apis mellifera* L.) were used as sampling device and for honey production (i.e. collection of nectar and processing to honey). Approx. 2 weeks before set-up all hives were equipped with a queen exclusion chamber to decrease brood production and decrease the amount of nectar needed by bees for feeding the brood. Thus, the chance of getting honey for sampling is increased. For all hives a colony assessment was performed prior set-up (0 to 5 days before setup of hives) and 3 to 5 days after sampling of honey.
Shortly before set-up of hives in the tunnels 3 empty combs were marked and placed in the brood body. The details are given in the table below.

Table 6.10.1-2: Set-up and preparation of colonies

Trial no.	Set-up of colonies	No of empty combs added
S19-04329-01	28.06.2019 / 1DBA / BBCH 64-65	3 in C / 3 in T
S19-04329-02	14.07.2019 / 1DBA / BBCH 64-65	3 in C / 3 in T
S19-04329-03	17.072019 / 1DBA / BBCH 63-65	3 in C / 3 in T
S19-04329-04	02.09.2019 / 1DBA / BBCH 63-65	3 in C / 3 in T

Since glyphosate is an herbicide, the treated Phacelia quickly decayed and became unattractive to the bees. Therefore, 3-4 days after application the colonies were moved to a monitoring site where they were allowed to freely fly in the surroundings. Honey was collected 2-5 days later, once mature (i.e. at comb closure and sugar content ≥ 80 %). Honey was collected from initially empty combs which were introduced in the hive the evening before the application performed the following day.

2. Sampling

Honey was sampled as separate sample from each tunnel of each trial. Honey was collected from initially empty combs which were introduced in the hive shortly before the application. Honey was collected once mature after comb closure for subsequent residue analysis.

Untreated control specimens were taken before treated specimens. Each field specimen (original field sample, as well as field retain sample) was taken from several spots across the combs.
Honey was collected by gently pushing a spoon into the walls of storage cells, allowing the honey to flow onto the spoon, or with a syringe or with a plastic pipette, extracting the honey from single cells. Sampling was done by collecting honey from several combs or spots within a comb.

Table 6.10.1-3: Sampling information

Trial	Commodity	DALA[1]	Subsample[2]	Quantity g	Sugar content %	Date of sampling
S19-04329-01	Honey	9	A	58.36	80.4	05.07.2019
			R	20.27		
S19-04329-02	Honey	8	A	99.12	81.0	23.07.2019
			R	67.94		
S19-04329-03	Honey	8	A	47.33	81.1	26.07.2019
			R	11.54		
S19-04329-04	Honey	8	A	5.98[3]	82.0	11.09.2019
			R	0.0		

1 Days after last application.
2 A = analysed sample; R = retain sample
3 Not enough food stores were present in the hive in order to collect at least 10 g in the A-samples nor to collect R-samples.

3. Analytical phase

Residue analysis was conducted according to Monsanto method ME-2220-01. The residues of glyphosate and AMPA were extracted from the samples by high-speed blending using 0.1 % formic acid in water. Following centrifugation and filtration the analytes were determined by LC-MS/MS using internal standards. The limit of quantitation (LOQ) for glyphosate and AMPA was 0.025 mg/kg with a limit of detection (LOD) of 0.0075 mg/kg for each analyte expressed as itself.

Treated and untreated specimens were maintained deep frozen and adequately separated during storage and shipment. The maximum sample storage interval from sampling to extraction was 83 days, and the maximum interval from extraction to analysis was 2 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.

The method validation was done with a set of recoveries at the LOQ (5 x 0.025 mg/kg for each analyte) and 10 x LOQ (5 x 0.25 mg/kg for each analyte) level. Additional concurrent recoveries were performed at 10 x LOQ (1 x 0.25 mg/kg for each analyte) and 320 x LOQ (3 x 8.0 mg/kg for each analyte). The results are summarised in the table below.

Table 6.10.1-4: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Honey	Glyphosate	Quantification transition 168 > 63 m/z					
		0.025	108, 109, 112, 101, 101	106	5.0	4.7	5
		0.5	91, 96, 89, 98, 91, 96	94	3.6	3.9	6
		8.0	91, 91, 90	91	0.58	0.64	3
		Overall	**89-112**	**97**	**7.7**	**7.9**	**10**
	AMPA	Quantification transition 110 > 63 m/z					
		0.05	100, 96, 101, 98, 92	97	3.6	3.7	5
		0.5	98, 98, 96, 103, 99, 102	99	2.7	2.7	6
		8.0	83, 82, 100	88	10	11	3
		Overall	**82-103**	**96**	**6.5**	**6.7**	**14**

1 Residues of glyphosate and AMPA in blank matrix were below the limit of detection (< 0.0075 mg/kg).

II. Results and Discussion

The test item was applied and specimens were generated and analysed according to the study objectives. The results of the analyses, therefore, allow to evaluate the residue behaviour of glyphosate and its metabolite AMPA after usage of MON52276 when applied to Phacelia plant.

Residues of glyphosate and AMPA in honey ranged from <0.025 mg/kg to 6.9 mg/kg and from <0.025 mg/kg to 0.028 mg/kg, respectively.

No residues of glyphosate and AMPA above the LOQ (0.025 mg/kg) were found in any untreated specimens of honey samples.

Detailed residue levels are shown in the table below.

Table 6.10.1-5: Residue levels of glyphosate and AMPA in honey after one application of MON52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S19-04329-01 / 75177, Pforzheim, Baden-Württemberg, Germany/ NEU / 2019	Phacelia / Balo	64-65	Honey	6.9	0.028	9	20.09.2019-27.09.2019
S19-04329-02 / 76297, Stutensee, Baden-Württemberg, Germany/ NEU / 2019	Phacelia / Balo	64-65	Honey	0.87	n.d.	8	20.09.2019-27.09.2019

Table 6.10.1-5: Residue levels of glyphosate and AMPA in honey after one application of MON52276 (360 g/L glyphosate)

Trial No. / Location / EU zone / Year	Crop/ Variety	Growth stage[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)	Date of analysis
				Glypho-sate	AMPA		
S19-04329-03 / 74081, Heilbronn, Baden-Württemberg, Germany/ NEU / 2019	Phacelia / Stala	63-65	Honey	3.2	n.d.	8	20.09.2019-27.09.2019
S19-04329-04 / 76681, Kraichtal , Baden-Württemberg, Germany/ SEU / 2019	Phacelia / Stala	63-65	Honey	n.d.	n.d.	8	20.09.2019-27.09.2019

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.025 mg/kg
3 n.d. (not detected): < 0.0075 mg/kg
4 Days after last application

III. Conclusion

Residues of glyphosate and AMPA in honey, sampled 8-9 days after application of glyphosate at the rate of 2.12-2.23 kg a.s./ha. ranged from <0.025 mg/kg to 6.9 mg/kg and from <0.025 mg/kg to 0.028 mg/kg, respectively

No residues of glyphosate and AMPA above the LOQ (0.025 mg/kg) were found in any untreated specimens of honey samples.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The study was previously not evaluated at EU level. It was performed under GLP and is considered to be reliable and acceptable. The study is deemed to comply with current requirements as laid down in Reg. (EU) No 283/2013, OECD Guideline for the Testing of Chemicals, 509 and SANTE/11956/2016 rev. 9. It adequately supports the use for glyphosate.

Assessment and conclusion by RMS:

Based on the available residue data in honey presented above an MRL is calculated for honey.

Region	Residue trials	Individual residue values (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Calculated MRL[1] (mg/kg)
Northern Europe	n = 4	<0.025, 0.87, 3.2, 6.9	2.04	6.9	15

1 MRL_Calculator EU-OECD 2015

The MRL was calculated using the sum of the mean and 4 * SD. The available data set is very divers and therefore, the calculated MRL very high.

To get a more realistic MRL it is proposed to use the EU monitoring data for setting the MRL in honey.

MRL setting on the basis of monitoring data

According to the Technical Guidelines SANTE/11956/2016 rev. 9 of 14 September 2018 it is possible to set temporary MRLs in honey on the basis of monitoring data.

The detailed results of the EU pesticide residue monitoring were downloaded from the Zenodo website. The search gives a list of references with pesticide residue monitoring data for individual EU and EEA Member States. For each State the monitoring data for 2016 and 2017 were provided. At the time of when this evaluation was conducted, the detailed monitoring data for 2018 were not published yet.

For each year, the monitoring data from all States were grouped to obtain a single data set of EU monitoring data (one for 2016 and one for 2017).
The residue data for parent glyphosate and AMPA in honey were extracted from these EU monitoring databases for 2016 and 2017 and grouped with all the glyphosate and AMPA residues in honey from the 2016 and 2017 monitoring.

The dataset includes a total of 618 analytical results, which were provided by Germany (n = 512), Austria (n = 104) and the Netherlands (n = 2). While most of the analysed samples originate from Germany and Austria, the dataset also includes samples from other European countries as well as samples from Latin America.
A total of 406 samples were analysed for parent glyphosate. Out of these 212 samples were also analysed for AMPA. There are no duplicate results in the table (sample analysed more than once). The LOQs were variable and ranged between 0.01 and 0.05 mg/kg for parent glyphosate (except for one sample for which the reported LOQ was 0.14 mg/kg) and between 0.01 and 0.03 mg/kg for AMPA.
Measurable residues of glyphosate (i.e. residues ≥ LOQ) were found in 42 samples and these residues ranged between 0.01 mg/kg and 0.61 mg/kg. The remaining 364 samples showed residues of glyphosate < LOQ.
The residues of AMPA were always < LOQ (n = 212).

In line with the monitoring residue definition for plant commodities it is proposed to set the monitoring residue definition for honey as parent glyphosate only. Therefore, for the derivation of the MRL in honey, only the monitoring results for glyphosate are considered (n = 406).
The above mentioned Technical Guidelines describe two possible approaches to derive an MRL in honey based on monitoring data.

According to the FAO spice method the MRL is set at the upper 95th confidence limit for the 95th percentile, considering the samples with measurable residues only (i.e. ≥ LOQ). A minimum of 58 samples with measurable residues is required. Since so far (i.e. based on the 2016 and 2017 monitoring data) only 42 results ≥ LOQ are available, this method is not applicable.

According to the FAO extraneous MRL approach the MRL is estimated based on the 99th or 99.5th percentile of the entire dataset (including results < LOQ). Based on the EU monitoring data for 2016-2017 the 99th and 99.5th percentile residue levels for glyphosate in honey are 0.310 and 0.584 mg/kg, respectively. Therefore, it seems appropriate to set **the MRL** for **glyphosate in honey** at **0.6 mg/kg**.

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
0010ED3D84B350ECF74D	Germany	2017	0.03	-	0.03
0139B97862BA5A133890	Germany	2016	0.02	-	0.02
01B1A9E9326C9FCDE411	Germany	2016	0.03	-	0.03
01B39E812B5FB3261DC9	Germany	2016	0.05	-	0.05
0503A2D246277166756C	Germany	2016	0.02	-	0.02
057C97A7C7F6F2952058	Germany	2017	0.03	-	0.03
05C6160133CF5F6DAB87	Germany	2017	0.03	-	0.03
0633B4DFC18BCBD64290	Austria	2017	0.01	-	0.01
06C32351DB221A730F26	Germany	2017	0.03	-	0.03
0717DDA152B12B92B311	Germany	2016	0.02	-	0.02
07C84539F8F83FAE7146	Austria	2017	0.01	-	0.01
0887729C9A2AB715FB2F	Germany	2017	0.03	-	0.03
097FDE14EBBAAEEC62D6	Germany	2017	0.03	-	0.03
0D4FE46805C2637FF9B1	Germany	2016	0.02	-	0.02
0E12C4BF67F647DB3A62	Germany	2016	0.02	0.039	0.039
0F6059D1E9FFFDB5A6FE	Germany	2016	0.02	-	0.02
0FC307488DD4E5DB847A	Germany	2016	0.03	-	0.03
101EF1A53C9A42809065	Austria	2017	0.01	-	0.01
10D6CE6863BD0D413F81	Germany	2016	0.02	-	0.02
11759E9F1EA6144BA097	Germany	2017	0.02	-	0.02
11943BFBE808F91EAA5C	Germany	2016	0.01	0.04	0.04
11F8D8A76B8B5B12F541	Austria	2017	0.01	-	0.01
12193F6A7928C444C735	Germany	2016	0.02	-	0.02
12CFBDC62782BF4FD3C2	Austria	2017	0.01	-	0.01
12EA985B7A3319C65D22	Germany	2016	0.045	-	0.045
12FC6919E5CD60595B0F	Germany	2017	0.03	-	0.03
15407597C3646034426B	Austria	2017	0.01	-	0.01
16D1A64965D1356D8A52	Germany	2017	0.02	-	0.02
188483C2611E27D6DD8A	Germany	2017	0.03	-	0.03
18CBF05125D206688A56	Germany	2016	0.02	-	0.02
18CE6F2E873044814C07	Germany	2016	0.02	0.091	0.091
1A4AA6A322C37D80AC06	Germany	2016	0.02	-	0.02
1AB5B63CB7C4DFD1DFD1	Germany	2016	0.05	-	0.05
1B7CB38A2EFE60B3D76F	Germany	2016	0.02	-	0.02
1CC90E11ABCB676FE3DA	Germany	2017	0.025	-	0.025
1DEFE2D9459BFE12E150	Germany	2016	0.02	-	0.02
1DFADE970058F772BF37	Germany	2016	0.01	-	0.01
1E201548E13A920CB49E	Germany	2017	0.03	-	0.03
1E85298720AE8F17C375	Germany	2016	0.02	-	0.02
1F705A6DB45B56859ABD	Germany	2016	0.02	-	0.02
1F93306032396BED9DDF	Germany	2016	0.03	-	0.03
1FB0734D1518C8EB8BCE	Germany	2017	0.03	-	0.03
1FDC0C1FDF56820DB16D	Germany	2016	0.02	-	0.02
1FFBC8FF62766BA50AB1	Germany	2017	0.03	-	0.03
20F0B5CD960405C4B79A	Germany	2017	0.03	0.051	0.051
214222EB6A951D1006BA	Germany	2017	0.03	-	0.03
23ABCDF6E0AEF4719392	Germany	2017	0.02	-	0.02
2581CBFE79C09DE183FF	Germany	2017	0.03	-	0.03
25CD1BFE952C39C45286	Germany	2016	0.02	-	0.02
25F6862ED207250A2773	Germany	2016	0.02	-	0.02
260CD96BEB85DEC45563	Germany	2016	0.02	-	0.02
2646B98CAC2FF9C5CA3E	Germany	2016	0.02	-	0.02

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
2740B1FE8E07DE01DF75	Austria	2017	0.01	-	0.01
27B44AA63B641BD37AEF	Austria	2017	0.01	-	0.01
28A4789EBF15D16B60DE	Germany	2016	0.02	-	0.02
2A23AE492E0622B69A8C	Germany	2016	0.01	0.013	0.013
2A2BBBC787A4AE7ED848	Germany	2016	0.03	-	0.03
2A6BFD3D4295B356F79B	Germany	2017	0.03	-	0.03
2AA7FA4EC495B1A6AB2F	Germany	2016	0.02	-	0.02
2AB2C24ED88E08B00775	Austria	2017	0.01	-	0.01
2AB5141C16E6C86B9B36	Germany	2017	0.02	-	0.02
2C33CF35A2EDD8B6B779	Germany	2016	0.05	-	0.05
2CDD41800D588146C083	Germany	2017	0.03	-	0.03
2EEADF8C7E215C858A59	Germany	2016	0.02	-	0.02
30C3C2489A8E5C4CA0D9	Germany	2017	0.03	-	0.03
30C71A645F5A30C81780	Germany	2016	0.02	-	0.02
3191CBB51C0254AB128B	Germany	2017	0.03	-	0.03
321D2553924907BD9DF2	Germany	2016	0.02	-	0.02
32214A96B487A91DA34E	Germany	2017	0.03	-	0.03
3289C64E15640DF73954	Germany	2016	0.02	-	0.02
33C8D2BBF4495ED96154	Germany	2017	0.03	-	0.03
342A760D3A4CE0DA1AFA	Austria	2017	0.01	-	0.01
34414278279F114B54FD	Germany	2016	0.02	-	0.02
3473529106345353AF55	Germany	2016	0.02	-	0.02
388249F2BDA5FCB552F3	Germany	2016	0.02	-	0.02
38C25CC208381E225204	Germany	2016	0.02	-	0.02
38F68E544DC89304F3E8	Germany	2016	0.02	-	0.02
393193522867AC20A19B	Germany	2016	0.05	0.61	0.61
39E7E78714FFB3976937	Germany	2017	0.03	-	0.03
3A47D2A16AC24D44FF61	Austria	2017	0.01	-	0.01
3B720900FBD7FF145596	Germany	2017	0.03	0.042	0.042
3C2875B84DDE93100B4E	Germany	2017	0.03	-	0.03
3C313C0DE51C180890C7	Germany	2016	0.03	-	0.03
41760C123C2EA0E4510F	Germany	2016	0.02	0.029	0.029
423DACAA846163EA81CF	Germany	2016	0.01	-	0.01
4242EA0EF05C605DDBE2	Germany	2016	0.02	-	0.02
4282828ABDABC787CFAF	Germany	2016	0.02	-	0.02
42CC9DF136D74AC5B792	Austria	2017	0.01	-	0.01
44020748C94D04CADD7E	Germany	2017	0.03	-	0.03
44AC8C8359C94865988D	Austria	2017	0.01	-	0.01
44CCD06B79EA3ADB4EDF	Germany	2016	0.02	0.15	0.15
4529C978DCB4E26D9EED	Germany	2016	0.02	-	0.02
45CE0A626FC46E639429	Germany	2016	0.02	-	0.02
46A746BE890A09A2AF21	Germany	2016	0.02	-	0.02
46CBA2F1C0BCE89B2143	Germany	2016	0.03	-	0.03
47AAC86ABDDDB87F093C	Germany	2016	0.01	-	0.01
486975036EFAEEAF179C	Germany	2017	0.03	-	0.03
4878E99BD7F07629350F	Germany	2017	0.03	-	0.03
487AC4D0AFC731CA986C	Austria	2017	0.01	-	0.01
48AAEFE4D7CCF96FF500	Germany	2016	0.03	-	0.03
48BFBF166B927A33C17D	Germany	2016	0.05	-	0.05
495BA8FE03CD26C08E47	Germany	2016	0.02	-	0.02
4A73A4A9D00407844CD0	Austria	2017	0.01	0.039	0.039
4B3FD64CF7C523DBA5E0	Germany	2016	0.03	-	0.03

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
4B7DEB85CCA5096E0118	Austria	2017	0.01	-	0.01
4D020978622AFA18B985	Germany	2016	0.02	-	0.02
4D3C7CD8B668E76932AF	Germany	2017	0.03	-	0.03
4DB6B6CB7BC747C1B119	Germany	2017	0.03	-	0.03
4DE2B90E8D9AD8E06756	Germany	2016	0.02	-	0.02
4DF9AAE785BA401CD4EF	Germany	2016	0.01	-	0.01
4E6E38E37B09EF0B80E5	Germany	2017	0.02	-	0.02
4F83B45B6EC13E3EEAC0	Germany	2016	0.03	-	0.03
4FE15900E93329E73C1D	Germany	2016	0.02	-	0.02
5054E648EDB7D3740644	Germany	2016	0.02	-	0.02
513EA638CA004626143B	Germany	2017	0.03	-	0.03
51795F0563C9DE14B587	Netherlands	2016	0.01	0.013	0.013
52F290B09E3513B05144	Germany	2016	0.02	-	0.02
54A0E676967DF6ED4B55	Germany	2016	0.02	-	0.02
59BD36F5CD277DB7202C	Germany	2017	0.03	-	0.03
5A2AABD2C17D892D535F	Germany	2016	0.03	-	0.03
5BC029017EBFD93C62E3	Germany	2017	0.03	-	0.03
5C78CA52BE27D0A871EC	Germany	2017	0.025	-	0.025
5CBCF8F8F66E1EC4B7D3	Germany	2016	0.03	-	0.03
5CC7B8B02966E78285CD	Germany	2016	0.02	-	0.02
5D9E6817321530CAC0A7	Germany	2017	0.03	-	0.03
5F0D71DFD634A1AEA34E	Germany	2016	0.03	-	0.03
5F19CAB8B080C9C0C237	Germany	2016	0.02	-	0.02
5F6A274B5D8ED470AA1F	Germany	2017	0.02	0.03	0.03
605ABB7E3E6C418E3D0F	Germany	2016	0.02	-	0.02
6066D5410103FB4386FB	Germany	2017	0.02	-	0.02
60B66A6CD7E000FCB80F	Germany	2017	0.03	-	0.03
61B0EDB0F314231E779B	Germany	2016	0.02	-	0.02
6219B8DCA44B5E25A35A	Germany	2016	0.045	-	0.045
62217BE09E16AA467886	Germany	2017	0.03	-	0.03
62387794DEEFB01F8AC5	Germany	2016	0.045	-	0.045
6243009C246101281CE8	Germany	2016	0.02	-	0.02
62DE55243930B2D55C12	Germany	2017	0.025	-	0.025
637CBB069F3D4F20EBB2	Germany	2016	0.02	-	0.02
63C68F8C0BF2B39EB09C	Germany	2016	0.02	-	0.02
643A89CE323EF5D96C56	Germany	2016	0.02	-	0.02
64426E1786100DFB4888	Germany	2016	0.03	-	0.03
64D28634DAD724369545	Germany	2016	0.02	-	0.02
64D55C5728DC6506A10E	Germany	2016	0.02	-	0.02
64F1AC5A9205672004D6	Germany	2016	0.02	-	0.02
658866EAFF21B81A9BA4	Germany	2017	0.02	-	0.02
65A3D15F13EDB36A6A1F	Germany	2016	0.02	-	0.02
65A56F427442A3254001	Germany	2017	0.03	-	0.03
65EDEA3058473329EF4E	Germany	2016	0.02	-	0.02
66002C08C86459DC70F5	Austria	2017	0.01	-	0.01
667257925AFDDFFBD9A0	Germany	2016	0.03	-	0.03
6781060F25763395DCA9	Germany	2017	0.03	0.059	0.059
68E2D1E508A54156880F	Austria	2017	0.01	-	0.01
690402318DD79EB45D6B	Germany	2017	0.03	-	0.03
6AA1126BFDA72AD35C6A	Germany	2016	0.02	-	0.02
6AEA0B3013DBBB9AD4B0	Germany	2016	0.02	0.049	0.049
6B7455B698C56B950332	Germany	2016	0.05	-	0.05

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
6DD754DB8A72216E9628	Germany	2016	0.02	-	0.02
6E0D7E8F55011C6F6884	Germany	2016	0.01	-	0.01
6E281500C78CADCF81EB	Germany	2017	0.03	-	0.03
6E4DCBF4A853B782AFB9	Germany	2017	0.02	0.028	0.028
6EEB7B270A390B63A884	Germany	2016	0.02	-	0.02
6FB36DEDDFE9FF1B07D5	Germany	2017	0.03	-	0.03
6FCB7C0A20C806F807D0	Germany	2016	0.02	-	0.02
70E092E686211202B76A	Germany	2016	0.02	-	0.02
71F9C40A55C1B8C27DCE	Germany	2017	0.025	0.037	0.037
72028AA18DB700B7479C	Germany	2016	0.02	-	0.02
7385E46D5C48B705A884	Austria	2017	0.01	-	0.01
73DA08BC994B498166F8	Germany	2016	0.02	-	0.02
7408243461552D27BD8A	Germany	2016	0.05	-	0.05
746F904E020CE7F977C7	Germany	2017	0.03	-	0.03
748C07B17EC1EC926F60	Germany	2017	0.03	-	0.03
74EE34AF14AF18731007	Germany	2016	0.01	0.05	0.05
75C835AC94B1A874EC77	Germany	2017	0.03	-	0.03
77883D04E27FAFFFFBE3	Germany	2016	0.03	-	0.03
77BC5B40E088F5B2C14C	Germany	2017	0.03	-	0.03
791D7294497EAF736B88	Germany	2017	0.03	-	0.03
7A97ED6B2CFD0CB09576	Germany	2016	0.02	-	0.02
7AB317EC4C8E147B6F34	Germany	2016	0.02	-	0.02
7AF617F2746972E24F98	Germany	2016	0.02	-	0.02
7B073031A2C7B9AE004F	Germany	2016	0.045	0.05	0.05
7B1CFD9517F01C5462D9	Germany	2016	0.02	-	0.02
7C2BA778A06B200645DB	Austria	2017	0.01	-	0.01
7C686718B15A83364FCE	Germany	2016	0.02	-	0.02
7CC0A6DE017923926360	Germany	2017	0.025	-	0.025
7CF1B73EAF8EAB46F0D6	Germany	2017	0.03	-	0.03
7E5B4C26A9891A7A1963	Austria	2017	0.01	-	0.01
7EBF9A9C78DE4200101C	Germany	2016	0.05	-	0.05
7F0ACF5986386F80DB24	Germany	2017	0.03	-	0.03
7F8D2C4FB16595E24DB2	Germany	2017	0.03	-	0.03
7F990F82279647A4809A	Germany	2016	0.03	0.126	0.126
7FE34A18FA42FF2926FB	Germany	2017	0.03	0.046	0.046
7FFD7589381A7C36920B	Austria	2017	0.01	-	0.01
82919908D160246B643F	Germany	2016	0.02	-	0.02
82CA0D9CED533D7C885A	Germany	2016	0.03	-	0.03
837A8F467399711107D3B	Germany	2016	0.03	-	0.03
839E1517A8A5979FC3EE	Germany	2017	0.03	-	0.03
846D8D3BA11481B67991	Germany	2017	0.03	-	0.03
84E760EEAB4F104EE3DC	Germany	2016	0.03	-	0.03
852778757F48751D8283	Germany	2017	0.03	-	0.03
855810F24EA90D0880F5	Austria	2017	0.01	-	0.01
85F192460FA7D05E3EF6	Austria	2017	0.01	-	0.01
86169E970E6C34770300	Germany	2016	0.02	-	0.02
86EB68CD1256D6D08739	Germany	2017	0.02	-	0.02
877DE720BD4DC5273712	Germany	2016	0.03	0.031	0.031
878D7E5E57F5A72426D6	Germany	2017	0.025	-	0.025
881B6F0D2252C73591CB	Austria	2017	0.01	-	0.01
88CF10FA329617BE76E0	Germany	2017	0.03	-	0.03
88E5BBDEDA780177D926	Germany	2016	0.02	-	0.02

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
892B0203DEEE9A2DD84D	Germany	2017	0.03	-	0.03
8946EDA67A062F5A347F	Germany	2016	0.01	-	0.01
89937F111AF5CED421AC	Germany	2017	0.02	-	0.02
8A821ABF479E66E54FA0	Germany	2017	0.02	-	0.02
8AE56183FCACBCA987D2	Germany	2017	0.03	-	0.03
8B46DF802EA714C223F5	Germany	2017	0.02	-	0.02
8CC15A0BBD8CEB0B03F6	Germany	2016	0.02	-	0.02
8DAD3995A288FF698F55	Austria	2017	0.01	-	0.01
8DCDAE0B9F5BDADD731F	Germany	2017	0.03	0.03	0.03
8E9470BF16C8CD0BC89C	Austria	2017	0.01	-	0.01
8F8DE319E61CEF845564	Germany	2016	0.02	-	0.02
9012CA697981F2A9A7D3	Austria	2017	0.01	0.01	0.01
90ADC7A926E55FFC38EF	Netherlands	2016	0.01	0.022	0.022
90D4DD17562036C7F563	Germany	2016	0.02	-	0.02
90DF2B617D0310B4AD8B	Germany	2016	0.14	-	0.14
90F9C2FE1F7BAEFFE8B4	Germany	2016	0.02	-	0.02
9287FDD6D32321E8D3AA	Germany	2017	0.03	-	0.03
92EE01423B11081FEFB3	Germany	2016	0.02	-	0.02
93C764B99CF2F37D3E72	Germany	2016	0.02	-	0.02
93CA47D9B2100DE89A54	Germany	2016	0.02	-	0.02
954E769D06495BA182AD	Germany	2016	0.03	-	0.03
95A50E491F8077509CFF	Germany	2016	0.01	-	0.01
95D24B73862C787DC13A	Germany	2017	0.03	-	0.03
95ED3E9F7FFC0115FE57	Germany	2016	0.02	-	0.02
961551D857666A6B66A8	Germany	2016	0.02	-	0.02
96922889702907E7919A	Germany	2016	0.02	-	0.02
96F6D0C0CC5DFCD31C20	Germany	2016	0.05	-	0.05
97A8BFA408FC5B301596	Germany	2017	0.03	-	0.03
97AD960AAE4EF0304890	Germany	2016	0.05	-	0.05
97B71DF07283BFCDDCCA	Germany	2017	0.03	-	0.03
98231E2F6D9D7AAD9B98	Germany	2017	0.03	-	0.03
9964AECFE8B2E828AA35	Germany	2017	0.03	-	0.03
99842C278092E0104EFB	Germany	2016	0.02	-	0.02
9A0D2843BEEC87415E2B	Germany	2017	0.03	0.311	0.311
9A6221E9B9A7DD927D7C	Germany	2017	0.025	-	0.025
9A93D43A4F95629ABE81	Germany	2017	0.03	-	0.03
9AB19FFCA6F4C3932FC4	Germany	2017	0.02	-	0.02
9B49313EB40080ADF356	Germany	2016	0.05	-	0.05
9B770194405680A0D76B	Germany	2016	0.02	-	0.02
9C5EAFBA15AA8D5A2EBB	Germany	2016	0.02	-	0.02
9EF21FCD9303483E33FA	Austria	2017	0.01	-	0.01
9F05D4166763FBCF8095	Germany	2016	0.02	-	0.02
A000208E3D07C779C7FE	Germany	2016	0.02	-	0.02
A0695F8162886F2801E1	Germany	2017	0.01	-	0.01
A157B37DD3B41572AAB5	Germany	2017	0.03	-	0.03
A1F135E9640C92FBB15C	Austria	2017	0.01	-	0.01
A2013A9A0321483F7C55	Germany	2016	0.02	-	0.02
A25D28E66AE0B51F0B6B	Germany	2016	0.02	-	0.02
A29D34B2BF87772601DF	Germany	2016	0.02	-	0.02
A325137A7789F3E47B09	Germany	2017	0.03	-	0.03
A339EBF43CCEA2D2C346	Germany	2017	0.03	-	0.03
A37869A69CF3EBEE119C	Austria	2017	0.01	-	0.01

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
A3D2F40FC8E589723C73	Germany	2017	0.03	-	0.03
A44A8DEC050F2D478BDA	Germany	2016	0.02	-	0.02
A45AF66A9A9D7CAA1EF9	Germany	2016	0.02	0.292	0.292
A45E457268AE1E02A21F	Germany	2016	0.03	-	0.03
A59D1D7BED4504B489AC	Germany	2016	0.02	-	0.02
A5C922F50F56C65FBB67	Germany	2016	0.01	-	0.01
A72519BCF55A13D14C8A	Germany	2016	0.02	-	0.02
A7486C8A00117A0465FB	Germany	2016	0.02	-	0.02
A81C688CE444C3785C58	Germany	2016	0.03	-	0.03
A920D3838E5205CEED0B	Germany	2017	0.03	0.058	0.058
A9C5887600B5F3E8DEA2	Germany	2017	0.03	-	0.03
A9E73876895057652367	Germany	2017	0.025	0.033	0.033
AAC30AD4E617AAEE884B	Germany	2016	0.02	-	0.02
AB29B109B10D3734310C	Germany	2016	0.02	-	0.02
AB570BA7752BCA2D1693	Austria	2017	0.01	-	0.01
AD5DA660200DD26F65DE	Austria	2017	0.01	0.034	0.034
AD6A7B83E3FCB7579677	Germany	2016	0.02	-	0.02
ADA9DA84F2464166CB4A	Germany	2017	0.03	-	0.03
ADCA1D77EEDEA8265243	Germany	2016	0.01	-	0.01
ADF00296009BC18D4675	Germany	2016	0.03	-	0.03
ADF7EAE1A4ECEE680256	Germany	2016	0.02	-	0.02
AF4B389238385E64BFE3	Germany	2016	0.02	-	0.02
AF8AD3DE1DC339807E5A	Germany	2016	0.01	-	0.01
AFA4A9E051C237454E4B	Austria	2017	0.01	-	0.01
AFFB00C2536FCF43CEFA	Germany	2017	0.03	-	0.03
B0E14E2221220E5E7539	Austria	2017	0.01	-	0.01
B17C7439B96CC06FD1A7	Germany	2016	0.02	-	0.02
B1975E497178DF460D6E	Germany	2017	0.03	-	0.03
B363422F69706107DD08	Germany	2016	0.02	-	0.02
B3753717657628146F90	Germany	2017	0.03	-	0.03
B3FE5F1619947FD45CDD	Germany	2017	0.02	-	0.02
B42144744411C4C83C6C	Germany	2016	0.02	-	0.02
B64A4E5388A08EE72831	Germany	2016	0.02	-	0.02
B68679EE3A8DC13B10DA	Germany	2016	0.03	-	0.03
B7FBFACE636DE4CD49C6	Germany	2017	0.03	-	0.03
B822FCEBA5BCA7FEFE3D	Germany	2016	0.02	-	0.02
B868F3D46C4CD577BA4A	Germany	2016	0.02	-	0.02
B898683CAD6FE28C1403	Germany	2016	0.02	-	0.02
B93879A14E3EFC1AE541	Germany	2017	0.03	-	0.03
BAEE814948E33DDE10A0	Germany	2017	0.02	-	0.02
BB6C0DD739666471C56D	Germany	2017	0.03	-	0.03
BB760C7CCADA32BB431A	Germany	2017	0.03	-	0.03
BB80E95621623AD63458	Germany	2016	0.02	-	0.02
BDE0B73281472655E22C	Germany	2016	0.02	-	0.02
BE089A03791315608770	Germany	2016	0.02	-	0.02
BEB90B122ED9B0BBA34E	Germany	2017	0.03	-	0.03
BEFB4B93051DBD2438FC	Germany	2017	0.025	-	0.025
BF04CA6ED38FD9D23881	Germany	2016	0.03	-	0.03
BF9F5F7FBADBEA16A0BB	Germany	2016	0.045	-	0.045
C1EE3741CDF2ED3F69C7	Germany	2016	0.05	-	0.05
C20156F798B7F354D580	Germany	2016	0.01	0.015	0.015
C26D440A42F1170CE04C	Germany	2016	0.02	-	0.02

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
C2F24DF38FEF17D4DA2B	Germany	2017	0.02	0.023	0.023
C367435C05506E0D830D	Germany	2017	0.03	0.031	0.031
C39F9455B0255D2F1C17	Austria	2017	0.01	-	0.01
C3A8DCE57204E9DA311A	Germany	2016	0.03	-	0.03
C5D1FBE239926E08B835	Germany	2016	0.02	-	0.02
C68DFF922A94923A9BA1	Germany	2017	0.03	-	0.03
C84CF939C8D6F8AB33AA	Germany	2016	0.01	-	0.01
C8C2E1CEB4D1543838CF	Germany	2016	0.03	-	0.03
C8F2755E1C71DB9F17F6	Austria	2017	0.01	-	0.01
C99D668DD376CBA3CABC	Austria	2017	0.01	-	0.01
CA00C043F01A351D96C4	Germany	2016	0.045	-	0.045
CA91122F45DA93989F2E	Germany	2016	0.045	-	0.045
CAC41083439CD59E7156	Germany	2016	0.02	-	0.02
CC25AE5423AFEB7EC89F	Austria	2017	0.01	-	0.01
CC2A2F023F610FECE56E	Germany	2017	0.025	-	0.025
CD3E0739BB7186FA5532	Germany	2016	0.02	-	0.02
CDD0F99BE95E9D7FDEBF	Germany	2016	0.02	-	0.02
CEC1B2EF316FD564D5DC	Germany	2016	0.05	-	0.05
CEE198D5A0D0E14CCC1A	Germany	2017	0.03	-	0.03
D0736FD96750CC0A7598	Germany	2016	0.02	-	0.02
D3D29BD805141B05D060	Germany	2017	0.03	-	0.03
D49BC68BDC78A091CDA3	Austria	2017	0.01	0.03	0.03
D5166581735E53BDBA8E	Germany	2016	0.02	-	0.02
D556BE14786DE73E2003	Germany	2016	0.01	0.019	0.019
D61B3B904EE1BD9AE9F3	Germany	2016	0.02	-	0.02
D80331C0851A1D4FCB1A	Germany	2017	0.02	-	0.02
D9917CF5BC392DC998A4	Germany	2017	0.025	-	0.025
D9A047B3A0B233CCD7E0	Germany	2016	0.02	-	0.02
DC2C50F94F5C3EB5C584	Germany	2016	0.05	-	0.05
DC38BABCAA1913D6437E	Germany	2017	0.02	0.033	0.033
DC509DF3112A0561C57C	Germany	2016	0.045	-	0.045
DCFA16220FEFA4845608	Germany	2016	0.02	-	0.02
DD3E6F215FF02BABF2AC	Germany	2016	0.02	-	0.02
DE2F17301202AE9AF32E	Germany	2017	0.03	-	0.03
DF33C6138094C8C2DFC2	Germany	2017	0.03	-	0.03
DF7243519491F1181D1F	Germany	2017	0.03	-	0.03
DF8557DFDD6F148C40C3	Germany	2016	0.02	-	0.02
E2BF651257CBADE20ED0	Austria	2017	0.01	-	0.01
E33FE7559CB6FFEA670E	Austria	2017	0.01	-	0.01
E3B33D9160E1B8F4DB87	Germany	2017	0.025	-	0.025
E581F039DF143AAEA426	Germany	2016	0.05	-	0.05
E5C63D90CBE6AA2B1F80	Germany	2017	0.03	-	0.03
E5EF8A339197FEA5ECA9	Germany	2017	0.03	-	0.03
E633B924DDB649EA419A	Germany	2017	0.025	-	0.025
E654C5D87E32DD7F48AD	Germany	2016	0.02	-	0.02
E66A3DE4E2B1C258A4B3	Germany	2016	0.045	-	0.045
E6B184DEA62165EBF504	Germany	2016	0.02	-	0.02
E73157D382C23458E23C	Germany	2016	0.02	-	0.02
E7486E8A9BC2E2AE020B	Germany	2016	0.02	-	0.02
E810BE286CEE91929046	Germany	2017	0.02	0.025	0.025
E8DE46276D310F03A6BC	Germany	2016	0.045	-	0.045
E982457A41D556D26D0E	Austria	2017	0.01	0.41	0.41

Table 6.10.1-6: Monitoring data of glyphosate in honey for the years 2016 and 2017

Lab Sample Code	Country	Year	LOQ	Residues above LOQ	Residue level for MRL calculation
EA29D5D3A2EE8197E523	Germany	2016	0.02	-	0.02
EA65D60DC17A6538EF0F	Germany	2017	0.03	-	0.03
EA6F3BFAD79EA0F734D4	Germany	2017	0.03	-	0.03
EA8A9339449D8B962895	Germany	2016	0.03	-	0.03
EB2F50944825B563EAAE	Germany	2017	0.025	0.064	0.064
EE0B1BBB97B0F1039BF9	Germany	2016	0.02	-	0.02
EF3CA64C715700C3ECA6	Germany	2017	0.03	-	0.03
EF85A2C4C178429F88B6	Germany	2016	0.03	-	0.03
EFADDA98DEFF9CAE58F8	Germany	2017	0.03	-	0.03
F0087893E88FA02195A0	Germany	2017	0.03	-	0.03
F0431E8CD382485CD547	Germany	2016	0.02	-	0.02
F12F2AF558BE5B2F5C92	Germany	2016	0.02	-	0.02
F1B508B77AC9289D02C3	Austria	2017	0.01	-	0.01
F23E30FAFF38138EB1B4	Germany	2017	0.03	-	0.03
F363151E1BD978A959D0	Germany	2016	0.02	-	0.02
F3667D783AB7ADCDCEFE	Germany	2016	0.02	-	0.02
F38F7D4DCA8E06800571	Germany	2016	0.02	-	0.02
F3BD3D55F4F18ED9185A	Austria	2017	0.01	-	0.01
F421BE2D2835630E6A3F	Germany	2016	0.02	-	0.02
F4DE407DC9CC9F0A0814	Germany	2017	0.025	0.025	0.025
F4F2F82EB796855208AB	Germany	2016	0.02	-	0.02
F57B6F34570EA2B74759	Austria	2017	0.01	-	0.01
F615D99119B67D45F3D2	Germany	2016	0.02	-	0.02
F62AD440B1C6DB155635	Germany	2016	0.02	0.59	0.59
F64403227BA2B94BDD15	Germany	2016	0.02	-	0.02
F6682ECD61DAFE127725	Germany	2017	0.03	0.078	0.078
F67C565C212495E9B53A	Austria	2017	0.01	-	0.01
F6A3F8A8B425ADC1D0F0	Austria	2017	0.01	-	0.01
F7CF571768FBC5F1CF11	Germany	2016	0.02	-	0.02
F8550393D66EC2DD2E83	Germany	2016	0.02	-	0.02
F870F98259404A3F9A54	Germany	2017	0.03	0.128	0.128
F8E03379888F179A694D	Germany	2016	0.02	-	0.02
F9BE9B523D4C2502B44F	Austria	2017	0.01	-	0.01
F9D7FE4DCCDBDCAC8A7C	Germany	2017	0.03	-	0.03
FAC2237E071EB2421EBA	Germany	2016	0.02	-	0.02
FBF17D16F61505DA65EC	Germany	2017	0.03	-	0.03
FC3B945F03225273931D	Germany	2016	0.03	-	0.03
FC8C4970BC9A0BAE1B84	Austria	2017	0.01	-	0.01
FCF467699E0ABA1F91F4	Austria	2017	0.01	-	0.01
FD635ED7BABF88BB940BA	Germany	2016	0.03	-	0.03
FDB5C5E0A7443C968820	Germany	2017	0.03	-	0.03
FEA36AAA7CF9DABEE139	Austria	2017	0.01	-	0.01

Data point	CA 6.10.1/002
Report author	[redacted] *et al.*
Report year	2020
Report title	Residues of Glyphosate in Food, Feed and Urine
Report No	TRR0000298
Guidelines followed in study	Not applicable
Deviations from current test guideline	None
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No applicable
Acceptability/Reliability	Valid
Category study in AIR 5 dossier (L docs)	Category 1

The objectives of this whitepaper are to:
1) review the assays available for measuring glyphosate in food, water and beverages, urine and other substances;
2) review reports of testing glyphosate in food or urine and other consumer items, assess the plausibility of these findings and convert these values to estimates of exposure; and
3) put these exposure estimates into context by comparing them to health-based guidance values.

This paper will show that
1) glyphosate residues are neither unexpected nor ubiquitous as media reports have implied;
2) residue information can be equivocal based on mostly media results from assays that were not validated and did not properly utilize experimentally-derived limits;
3) concentrations of residues in agricultural commodities/food demonstrate a high level of compliance because they are at or below expected amounts; and
4) modeled or empirical exposure of glyphosate to consumers is low compared to the allowable intakes.

For more details, please refer to the KCA 6.10.1/002.

Relevant published articles from Literature Search Report

1. Information on the study

Data point	CA 6.10.1/003
Report author	El Agrebi, N. *et al.*
Report year	2020
Report title	Honeybee and consumer's exposure and risk characterisation to glyphosate-based herbicide (GBH) and its degradation product (AMPA): Residues in beebread, wax, and honey
Document No.	DOI 10.1016/j.scitotenv.2019.135312 E-ISSN 1879-1026
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable

Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

In order to assess bee and human exposure to residues of glyphosate-based herbicide (GBH) and its main degradation products aminomethylphosphonic acid (AMPA) and to characterise the risk posed by these substances, we analysed 3 different bee matrices; beebread (N = 81), wax (N = 100) and 10-paired samples of wax/honey collected in 2016/2017 from 379 Belgian apiaries. A high-performance liquid chromatography-electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS-MS) was used as analytical method. Limit of quantification and detection (LOQ and LOD) for GBH residues and AMPA in the 3 matrices was respectively of 10 ng/g and 1 ng/g. In beebread, 81.5 % of the samples showed a residue concentration > LOQ and 9.9 % of the samples a residue concentration < LOQ (detection without quantification); no significant difference in detection rate was found between the north and the south of the country. Glyphosate was detected in beeswax less frequently than in beebread (i.e. 26 % >LOQ versus 81.5 % >LOQ). The maximum GBH residues and AMPA concentration found in beebread (respectively 700 ng/g and 250 ng/g) led to sub-lethal exposure to bees. The Hazard Quotient (HQ) for beebread and beeswax (7 and 3.2, respectively) were far below the "safety" oral and contact thresholds for bees. For human health, the highest exposure to GBH residues in pollen corresponded to 0.312 % and 0.187 % of the ADI and of the ARfD respectively and, to 0.002 % and to 0.001 % for beeswax. No transfer of glyphosate from wax to honey was detected. Considering our results and the available regulatory data on the glyphosate molecule considered solely, not including the adjuvants in GBH formulation, the consumption of these three contaminated matrices would not be a food safety issue. Nonetheless, caution should be taken in the interpretation of the results as new studies indicate possible glyphosate/GBH residues toxicity below regulatory limits and at chronic sub-lethal doses.

Materials and Methods

Study areas

Three different bee matrices were sampled for the analysis of GBH residues and AMPA: (i) beebread (N = 179), (ii) wax from the brood chamber (N = 100) and additionally (iii) a combination of wax from the honey super and corresponding extracted honey (N = 10). We used 379 non-professional apiary sites located in Belgium, including 2,997 colonies of *Apis mellifera*. For beebread and wax sampling, apiaries were selected (193 for beebread, 186 for wax and honey) from the Federal Agency for the Safety of the Food Chain (FASFC) apiaries database that included 4,949 registered beekeepers in 2015. The apiaries were stratified by province (N = 20/province and 10 provinces in Belgium) and randomly distributed in Flanders (northern Belgium) and Wallonia (southern Belgium). All sampled bee colonies seemed healthy, with no clinical signs of infectious diseases or acute intoxication (Ravoet *et al.*, 2015). Quantum GIS (QGIS Development Team, 2009; http://qgis.osgeo.org) was used to create the maps in **Figure 1** and **Figure 2**.

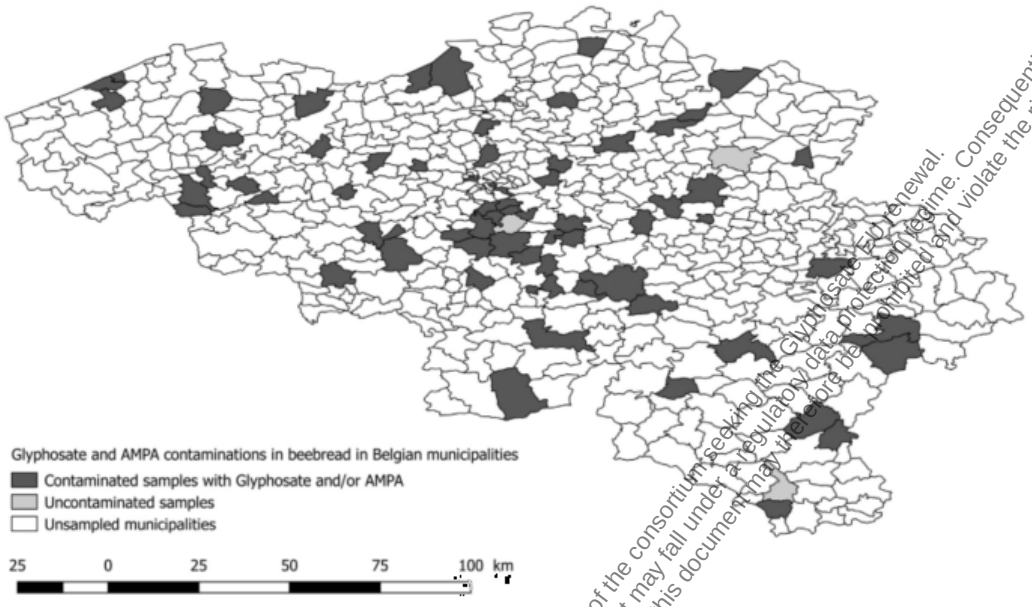
The risks posed by formulated products in the present study are restricted to the active ingredient glyphosate plus AMPA and the total risk of commercial products utilised by farmers is not the subject of this study.

Beebread collection

Beebread sampling (N = 179) was carried out by FASFC beekeepers and apiary technicians (Healthy Bee national monitoring program) between September and October 2016 from 193 apiaries including 865 colonies, out of 75 municipalities covering the entire Belgian territory (**Figure 1**). The samples were provided with a protocol defining sampling collection details and were personally instructed by expert beekeepers to improve the harmonisation of the procedure across apiaries. At each apiary, one hive was sampled randomly by cutting a comb portion of 8 by 8 cm filled with beebread. The coded samples were kept in hermetic plastic bags and stored at -20°C the same day in order to be processed. A cool-box was

used for shipment of samples from FASFC to Liège University to ensure that samples were maintained frozen (Tosi *et al.*, 2018) until processing.

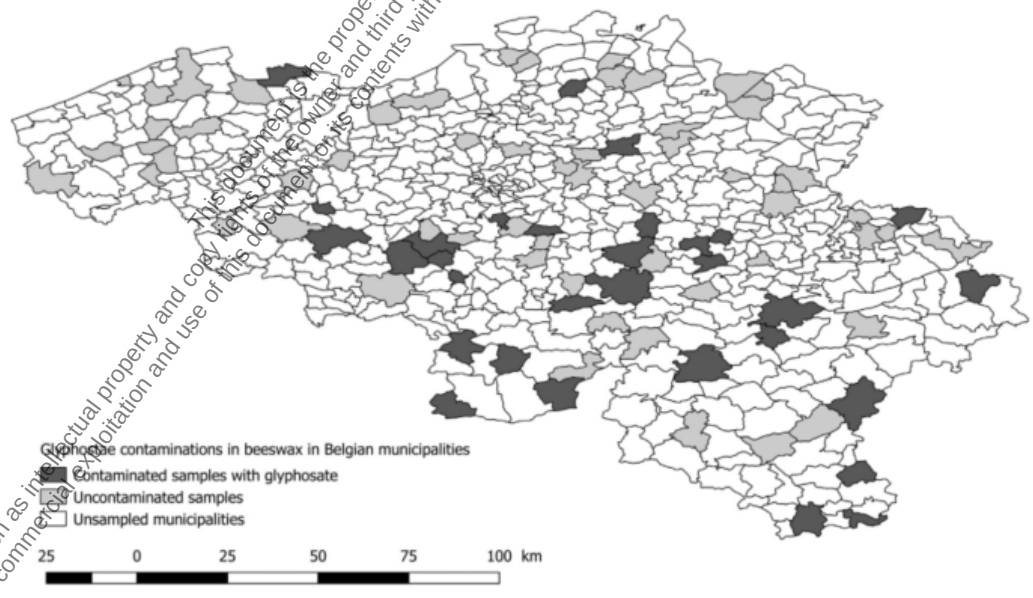
Figure 1: Glyphosate residues and AMPA contaminations in beebread across Belgium, in 2016.



Beebread extraction

For analyse purpose, 20 g of beebread were extracted manually from each comb sample using a disposable surgical blade (1 blade per sample). Cleaned beebread samples were stored in a 60 mL marked sterile polycarbonate containers with screw cap. Only 81 samples of beebread could be extracted from the 179 comb samples in adequate amounts for analysis.

Figure 2: Glyphosate residues and AMPA contaminations in beeswax across Belgium, in 2016.



Wax collection

Twenty grams (20 g) of wax from the brood chamber were sampled during spring 2016. Together with sampling, wax renewal rates were registered in a questionnaire (<50 % and ≥50 %). The coded samples were kept in hermetic plastic bags and stored the same day at -20°C until analysis. Financial limitations allowed us to randomly select only 100 wax samples out of the 186 original samples (2132 hives). These 100 samples were equally distributed between Flanders and Wallonia in 89 municipalities (**Figure 2**).

Honey/wax sampling

After wax analysis, out of the 32 beekeepers with the highest GBH residues contaminations in wax from the brood chamber, 10 beekeepers were randomly selected. Among these beekeepers, samples of 20 g of wax and of 50 g of honey harvested in summer 2017 were extracted both from the honey super (pairwise samples). The coded wax samples were kept in hermetic plastic bags, honey in polypropylene disposable containers and shipped the same day to the laboratory. Sampling and analysis of honey for GBH residues and AMPA were performed in September 2017 in the same laboratory and according to a similar method as for beebread and beeswax. Concentrations of GBH residues measured in honey were compared to the Maximum Residue Level (MRL) for human consumption (50 ng/g) (Regulation (EC) No 396/2005).

Glyphosate-based herbicide residues and AMPA detection

The GBH residues and AMPA analyses were carried out between May and June 2017 (September 2017 for the 10-paired samples of wax/honey) by the Phytocontrol laboratory (France) ISO 17,025 accredited under the number No 1–1904 for the analysis of bee products by the French competent authority. The analysis method used for the targeted matrices (beebread, beeswax, and honey) was a high-performance liquid chromatography-electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS-MS). The analytes were extracted using an aqueous solution followed by a simple clean up with a C18 solid-phase extraction (SPE) cartridge, and then glyphosate and AMPA were derivatised using 9-fluorenyl-methoxycarbonyl (FMOC-Cl) in borate buffer. For beeswax, an additional hexane treatment was used in order to defat the extract. The derivatives of glyphosate and AMPA were separated on a C18 column (105 x 4.6 mm; 5 µm) with gradient elution with the mobile phase of acetonitrile and 5 mmol/L ammonium acetate (pH 9), and finally detected with negative ion electrospray ionisation-mass spectrometry (ESI-MS) in multiple reaction monitoring (MRM) mode (drying gas flow at 15 mL/min, nebulizing gas flow at 3 L/min). Limits of quantification (LOQ) for both glyphosate and AMPA in the 3 matrices were 10 ng/g, while limits of detection (LOD) were 1 ng/g. Matrix effects were compensated by the addition of ^{13}C labeled glyphosate (used as internal standard) to the sample prior extraction, as well as in spiked samples used to set up the calibration curve. Three levels of spiking, including the LOQ, were performed on several matrices of different categories, which were analysed in condition of repeatability and intermediate fidelity. The mean spiked recoveries of glyphosate and AMPA at 3 spiked levels ranged from 72.2 % to 112.9 % with the relative standard deviations (RSD, n = 5) of 0.1 % – 4.5 %. The tolerance interval was plotted with a beta probability of 80 %, which represents the proportion of future values that the routine method will produce over the entire field of application. This allows to ensure that the molecule of glyphosate is extracted correctly and to correct any matrix effects.

Exposure assessment and risk characterisation to honeybee health

We estimated the Hazard Quotient (HQ) for honeybees using the method described by (Stoner and Eitzer, 2013). The HQ is calculated as the exposure divided by the toxicity expressed, in this study, as the maximum residue concentration (ng/g or ppb) in beebread samples divided by the oral acute LD 50 (ng/bee) and multiplyed by 100. An adult bee that consumed 100 mg pollen with an HQ of 1000 would have consumed approximately 10 % of the LD_{50} for the pesticide during this development stage (=10 days as nurse bee) (Calatayud-Vernich *et al.*, 2018). Assuming that 10 % of the LD_{50} should never be exceeded (Atkins *et al.*, 1981), the HQ value of 1000 would correspond to the limit of concern for bee health (Stoner *et al.*, 2013; Traynor *et al.*, 2016). For beeswax, we used a contact HQ of 5000 as threshold safety value, since residue concentrations are significantly higher in wax, and contact exposure routes are poorly understood in this matrix (Traynor *et al.*, 2016).

Then, we also assessed the risk posed by GBH residues and AMPA in beebread to honeybee health through the assessment of the honeybee exposure to these compounds through beebread consumption. To estimate the beebread consumption, we used published pollen consumption values. A nurse bee consumes between 13 and 120 mg of pollen during its first 10 days of life (OECD, 1998; Rortais *et al.*, 2005) with a mean value equal to 65 mg (Chauzat and Faucon, 2007). As a worst-case scenario, we took into account the maximum consumption level of 12 mg of pollen per day. Then, we multiplied this highest level of consumption with the highest GBH residues and AMPA concentrations. Finally, we compared the exposure levels with the oral acute LD_{50} of these compounds.

Until very recently, risk assessment procedures did not implement yet the side-effects of pesticides on developing brood and the chronic effects in general (OECD, 2017). We could only assess the acute risk for adult bees since the possible toxicity of GBH residues on bee larvae is currently not sufficiently characterised.

Risk to consumer's health

For human health, GBH residues toxicity has been redefined in 2015 (European Food Safety Authority, 2015); an acceptable daily intake (ADI) for consumers has been set to 0.3 mg/kg body weight/day and the acute reference dose (ARfD) at 0.5 mg/kg body weight/day. Concerning AMPA residues, only the ADI value is available (0.3 mg/kg body weight/day). ADI is the quantity of a chemical that can be ingested daily for a lifetime causing no harm (on the basis of all known facts) (Renwick, 2002). ARfD is the quantity of a chemical that can be ingested by a person at a single time causing no harm. MRL is the maximum concentration of pesticide residue legally permitted in or on food commodities or animal feeds (Food and Authority, 2017).

Then, we assessed the risk posed by GBH residues and AMPA in beebread and beeswax to consumer's health through the assessment of the consumer exposure to these compounds through pollen and beeswax consumption. Thus, we assumed that beebread contamination levels correspond to pollen contamination levels. To estimate the pollen and beeswax consumption, we used published consumption data. According to EFSA (EFSA, 2007), the 95th percentile of the daily consumption of beeswax corresponds to 1.29 g/person, which is 0.022 g/kg b.w. for a 60 kg individual. Concerning the daily consumption of pollen, the highest 95th percentile value recorded in the EFSA Comprehensive European Food Consumption Database (EFSA, 2018) corresponds to 69.55 g/person, that is 1.35 g/kg b.w. for a 52 kg individual, in France (according to the second version of the FoodEx food classification system). Then, as a worst-case scenario, we multiplied these high levels of consumption with the highest GBH residues and AMPA concentrations. Finally, we compared the exposure levels with the reference toxicological values of these compounds (above mentioned) to characterise the risk.

Statistical analysis

Yearly wax renewal rates were divided into 2 categories: <50 % and ≥50 % of wax frames changed per year in the brood chamber. A Fisher's exact test was used to compare the annual renewal rate of wax frames between regions (Flanders versus Wallonia).

A Fisher's exact test was used for each pairwise comparison of frequency of detection of GBH residues and AMPA depending on the region/country and the matrix for GBH residues only (beebread versus beeswax). A two-sample Wilcoxon rank-sum (Mann-Whitney) (i.e. non-parametric test) test was used for each pairwise comparison of concentration of GBH residues and AMPA depending on the region/country and the matrix for GBH residues only (bee-bread versus beeswax).

A logistic regression (odds ratio's (OR) with 95 % confidence intervals (95 % CI)) was used to test a possible risk factor of GBH residues detection in beeswax and regions (Stata SE 14.1®, Stata-Corp LP, College Station, TX, USA). For all tests, a level of significance of 5 % was used and divided, if needed, by the number of comparisons performed for the Bonferroni correction.

Results

Glyphosate-based herbicide residues and AMPA in beebread

In beebread, a high detection of GBH residues was registered (91.4 % of positive samples overall) and AMPA (25.9 % positive samples) in both Belgian regions. Glyphosate LOQ value (10 ng/g) was lower than the glyphosate median lethal doses LD_{50} for bees (10^6 ng/g). No significant difference of contamination prevalence in beebread between regions was confirmed by a one-tailed Fisher's exact test (1 degree of freedom; α =0.05) (N = 81; $p > 0.20$) (**Table 1**). GBH residues and AMPA were not detected in only 6 samples (7.4 %), coming from 3 of the 75 sampled municipalities (**Figure 1**). Only 2 samples contained AMPA without GBH residue.

Table 1: Glyphosate and AMPA detection, residue levels and hazard quotient to bees in beebread, beeswax and honey samples in Flanders (North Belgium), Wallonia (South Belgium) and Belgium.

Matrix	Region	Sampling period	Nb. analysed samples	Nb. samples > LOQ	Nb. samples < LOQ	Nb. samples detected	% samples > LOQ	% samples < LOQ	% samples detected	Multi-test for detection	Mean [] ng g^{-1}	SD [] ng g^{-1}	Multi-test for []	Max [] ng g^{-1}	Median [] ng g^{-1}	Max HQ
Beebread	**GBH**															
	Flanders	Fall 2016	39	34	3	37	87.2%	7.7%	94.9%	aa	58.44	133.28	aa	700	23	7
	Wallonia		42	32	5	37	76.2%	11.9%	88.1%	aa	32.41	39.70	aa	160	49.5	1.6
	Belgium		81	66	8	74	81.5%	9.9%	91.4%	a	44.52	98.89	aa	700	26	7
	AMPA															
	Flanders	Fall 2016	39	5	3	8	12.8%	7.69%	20.5%	a	39.8	25.16	a-	77	38	0.8
	Wallonia		42	10	3	13	23.8%	7.14%	30.9%	a	80.8	78.09	a-	250	58.5	2.5
	Belgium		81	15	6	21	18.5%	7.4%	25.9%	a	67.13	67.09	a-	250	44	2.5
	GBH															
Beeswax	Flanders	Spring	48	3	1	4	6.3%	2.08%	8.3%	a	28.33	22.90	aa	54	21	0.5
	Wallonia	2016	52	23	5	28	44.2%	9.62%	53.8%	b	66.43	84.01	aa	320	40	3.2
	Belgium		100	26	6	32	26%	6%	32%	b	62.04	80.05	aa	320	36	3.2
Honey	Flanders	Summer	2	0	1	1	0%	50%	50%	/	/	/	/	/	/	/
	Wallonia	2017	8	1	0	1	12.5%	0%	12%	/	11	/	/	11	11	/
	Belgium		10	1	1	2	10%	10%	20%	/	11	/	/	11	11	/

GBH: Glyphosate based herbicide, Nb.: number; > LOQ: detection with quantification, <LOQ: detection without quantification; []: concentration; AMPA: aminomethylphosphonic acid; HQ beebread (oral) threshold value = 1000; HQ wax (contact) threshold value = 5000, + detection is the sum of samples > LOQ and < LOD; S.D.: standard deviation; /: not determined. Multi-testing: a Fisher's exact test and a two-sample Wilcoxon rank-sum (Mann-Whitney) test were respectively used for each pairwise comparison of frequency of detection and mean concentration of the compound. Different letters were used for significant differences. The first position letter corresponds to the comparison of regions for a same matrix; the second position letter corresponds to the comparison of breebread and beeswax for the mean concentration of GBH. A level of significance of 5% was used, divided by the number of tests performed for the Bonferroni correction.

Exposure assessment and risk characterisation of GBH residues in beebread for honey bees

Based on the honeybee oral acute LD_{50} (48 h) of glyphosate (100 mg/bee = moderate toxicity for adult bees) (Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate 2015; Lewis *et al.*, 2016) and on the maximum concentration of GBH residues detected in beebread (700 ng/g), the estimated maximum HQ (oral) of GBH residues for beebread found in Belgium is equal to 7 (=700/100). Because the honeybee oral acute LD_{50} (48 h) of AMPA is currently unknown in published data, it was impossible to estimate its corresponding HQ.

Considering the maximum consumption level of 12 mg of pollen per day (Rortais *et al.*, 2005) (worst-case) and the maximum concentration of GBH residues detected in beebread (700 ng/g), this would correspond to a dose of 84 ng of GBH residues ingested per nurse bee over 10 days (0.012 g x 700 ng/g x 10 days). This exposure level corresponds to about 0.08 % of the oral glyphosate LD_{50}. As mentioned, in the open literature, no oral acute LD_{50} (48 h) for AMPA is available. To assess the risk of AMPA to bees, we used, therefore, the parent compound glyphosate LD_{50} (Traynor *et al.*, 2016). AMPA detection in beebread (250 ng/g) would correspond to about 0.03 % of the oral glyphosate LD_{50}. Cumulatively, GBH and AMPA maximal concentration would correspond to about 0.12 % of oral glyphosate LD_{50}.

Glyphosate-based herbicide residues and AMPA in beeswax

GBH residues were found in 32 % of Belgian beeswax samples (N = 100, T1). A significantly higher GBH residues prevalence was found in Wallonia (53.8 % positive sample, **Figure 2**), as compared to Flanders (8.3 % positive samples, one-tailed Fisher's exact test (1 degree of freedom; α = 0.05), $p < 0.001$); confirmed by a logistic regression comparing contaminations in both regions (with Flanders as a reference): OR = 18.4, 95 % CI = 4.66–72.60, $p < 0.001$). A two-sample Wilcoxon rank-sum (Mann-Whitney) test showed that the average GBH residue concentration observed in Wallonia is not significantly higher than in Flanders (p = 0.33) (**Table 1**).

Exposure assessment of GBH residues in beeswax

No trace of AMPA has been detected in beeswax. HQ (contact) of beeswax for the maximum GBH residues concentration in Belgium is equal to 3.2 (= 320/100).

Wax renewal rate in Flanders and Wallonia

Beekeepers should renew the wax foundation of their bee colonies periodically. This improves bee health reducing the disease and chemical load of beeswax and allowing bees to rear their brood in a freshly built environment.

Flemish beekeepers had a significant higher wax renewal rate (≥ 50 % per year) as compared to Walloon ones (N = 98, one-tailed Fisher's exact test (1 degree of freedom; α =0.05) , p = 0.017) (data not shown).

Risk assessment for the consumer of contaminated beebread and beeswax

As shown in **Table 1**, GBH residues contaminated significantly more frequently beebread (87.2 % >LOQ) than beeswax (26 % >LOQ) (one-tailed Fisher's exact test (1 degree of freedom; a =0.05), N = 181; p < 0.001) but the average concentration found in beebread (55.52 ng/g) and wax (51.3 ng/g) were statistically comparable (two-sample Wilcoxon rank-sum (Mann-Whitney) test; p > 0.05).

A high consumption level (95th percentile) of the most contaminated pollen and beeswax by GBH residues, according to our results, leads to an exposure of respectively 0.936 and 0.007 mg GBH residues/kg b.w./day through beeswax and pollen consumption. Concerning AMPA, the highest exposure corresponds to 0.334 mg AMPA/kg b.w./day through pollen consumption).

Transfer of GBH residues and AMPA from beeswax to honey

We wondered if a transfer of GBH residues and AMPA from beeswax to honey was possible. Thus, to further test this hypothesis, we concomitantly collected both wax and honey from the bee colony honey supers of 10 apiaries out of the 32 beekeepers with the highest GBH residues contaminations in wax from the brood chamber. We found 1 out of 10 wax samples (10 %) contaminated with GBH residues (concentration: 48 ng/g). In honey, 2 out of 10 samples were contaminated by GBH residues (20 %; 11 ng/g for the first sample and a detection lower than the quantification limit [LOQ] < 10 ng/g for the second sample). These 3 positive GBH residues samples came from different bee colonies. No trace of AMPA was detected in any of the matrices. The highest GBH residues concentration detected in honey was about 5 times lower than the MRL (50 ng/g).

Discussion

Beebread

Our study showed an extended presence of GBH residues in beebread (81.5 % positive samples at the national level) in both Belgian regions. AMPA was found in 18.5 % of beebread samples at the national level. Only 2 samples contained AMPA without GBH residue. The LOQ values for glyphosate and AMPA are of 10 ng/g, which makes the analysis method very sensitive. Simultaneous AMPA/GBH residues detection in beebread could be explained by the GBH residues degradation in the matrix or by their simultaneous occurrence in the environment. In soil, the primary pathway degradation of glyphosate residues is microbial action, which yields AMPA and glyoxylic acid (Roberts *et al.*, 1999). The maximum GBH residues concentration found (700 ng/g) led to sublethal exposure (not acutely toxic to bees), corresponding to a dose of 84 ng/bee (0.08 % of its LD_{50}), ingested over the first 10 days of life of a nurse bee. AMPA dose in beebread also corresponded to a sub-lethal exposure (to about 0.03 % of oral glyphosate LD_{50}) alone or cumulated with GBH residues (about 0.12 % of oral glyphosate LD_{50}). However, while the LD_{50} is measured as a one-time dose, bees could be exposed to GBH residues contaminated beebread for a longer period, when re-contamination occurs, since glyphosate degradation time DT_{50} ranges between 1.0 and 67.7 days. Therefore, the use of the LD_{50} as a single benchmark could underestimate the exposure risk to bees.

Bee and bee colony health is significantly impaired by doses that are lower than those we found through sub-lethal effects. Helmer *et al.* (Helmer *et al.*, 2015) orally exposed bees to sub-lethal field-realistic

doses of GBH residues (1.25, 2.50, and 5.00 ng/bee) and showed a significant decrease ($p < 0.05$; $n = 40$) of beta-carotene and protein levels in their bodies after 10 days. Our results confirm Helmer's field-realistic doses (lower than 700 ppb, corresponding to 84 ng/bee). Other studies (Herbert *et al.*, 2014), showed that adult *A. mellifera* workers exposed orally to 2.5 and 5 mg/L of GBH residues (field-realistic doses equivalent) presented reduced sucrose sensitivity leading to loss and difficulty in establishing associative memories, which, in turn, could cause inefficient collection of nectar and pollen for the colony and, finally, compromise its survival. Oral exposure to GBH residues concentrations (2.5, 5.0, and 10.0 mg/L, corresponding to a dose of 0.125, 0.25, and 0.5 µg/bee) affects honeybee cognitive abilities, with potential long-term negative consequences for colony foraging success (Balbuena *et al.*, 2015). Exposures to 5 and 10 mg/L of GBH residues (dose of 0.25 and 0.5 µg/bee) perturb the gut microbiota of honeybees. Bee gut symbionts influence bee development, nutrition, and defence against natural enemies (Motta *et al.*, 2018). Perturbations of these gut communities may affect bee susceptibility to environmental stressors, including poor nutrition (Tosi *et al.*, 2017) and pathogens (Motta *et al.*, 2018). Moreover, in evaluating the effect of Roundup® on the royal jelly-producing glands, Faita *et al.* (2018) showed that exposure to GBH residues resulted in the alteration of these glands that can trigger damage to the development and survival of bee colonies.

Regarding AMPA, no trace was found in honey and beeswax. In beebread, the maximum AMPA concentration was 250 ng/g. Because no information on AMPA toxicity to bees is available yet in the open literature, we were not able to assess its risks to bees. Nevertheless, Blot *et al.* (2019) confirmed that glyphosate have sub-lethal effects on the honeybee microbiota, while AMPA did not induce any significant change.

Beeswax

Measured GBH residues concentrations should not cause acute lethal effects since the estimated HQ for beebread and beeswax (7 and 3.2, respectively) were far below the "safety" oral and contact thresholds (1000 and 5000, respectively). Since beebread can be stored in the hive for months after collection in the field, glyphosate degradation have likely reduced its concentration over time. Furthermore, bees typically collect multiple chemicals simultaneously (Tosi *et al.*, 2018). Because bees are bio-indicators of environmental health and pollution, residues found in bee products provide valuable information on environmental punctual contamination or accumulation which, nevertheless, might be underestimated (i.e. residue degradation, dilution of highly-concentrated samples, technical limitations such as LOD) or overestimated (i.e. accumulation of contaminated pollen) (Tosi *et al.*, 2018).

Due to glyphosate high water solubility and a very low octanol/water partition coefficient (Log P (= Log Kow) at pH 7 and at 20°C = -3.2), GBH residues were expected to be found only in beebread but not in wax (a very hydrophobic matrix). Beeswax samples contamination rate was of 26 % at the national level. The addition of surfactant in the formulation of end-use pesticide products is at the origin of the phenomenon allowing glyphosate, which is water-soluble, to penetrate lipid-based structures (Shokri *et al.*, 2001). Nevertheless, the risk assessment for honey bees and the consumer has been evaluated for glyphosate molecule solely without the concomitant formulation ingredients and adjuvants, nor other possibly concurring pesticides (Tosi *et al.*, 2018). The use of the glyphosate/AMPA molecule solely does not render the combined toxic effects of the formulation constituents nor the synergetic potential effects of pesticide combinations.

Wallonia had both a higher GBH residue detection rate (53.8 %) and a significantly lower rate of wax foundation renewal rate, as compared to Flanders ($p = 0.017$). This supports our hypothesis that the beekeeping management practice of renewing wax foundation can protect bees from the accumulation of pesticide residues inside the hive. No trace of AMPA could be detected in beeswax, probably because the matrix is not suitable for microorganism growth due to its rich hydrophobic protective properties (Fratini *et al.*, 2016), resulting in no degradation of glyphosate in AMPA. Beeswax's conservative properties for pesticide residues combined with the beekeeping practice of wax recycling (Perugini *et al.*, 2018), may be at the origin of the unequal detection of GBH residues in Flanders and Wallonia. This result highlights the importance of replacing at least 50 % of wax frames per year, the current recommendation being the yearly replacement of 25 to 33 % of the wax from the brood chamber (ITSAP, 2017; Vergaert, 2017).

For human health, the highest exposure to GBH residues in pollen corresponds to 0.312 % and 0.187 % respectively of the ADI and of the ARfD, and this through the pollen consumption (69.55 g/day/person of contaminated pollen with 700 ng of GBH residues/g). The exposure to GBH residues through the beeswax consumption (1.29 g/day/person of contaminated beeswax with 320 ng of GBH residues/g) corresponds to only 0.002 % and 0.001 % respectively of the ADI and of the ARfD. Concerning AMPA, the highest exposure to this compound corresponds to 0.111 % of the ADI, and this through the pollen consumption (69.55 g/day/person of contaminated pollen with 250 ng of AMPA/g).

Honey

The honey analysis resulted in a maximum GBH residues concentration of 11 ng/g, not exceeding the EU MRL (50 ng/g) for honey and theoretically meaning no risk for the consumer. In a survey on GBH residues in honey samples originating from different countries (Brazil, Canada, China, Germany, Greece, Hungary, India, Korea, Mexico, Uruguay, New Zealand, Spain, Taiwan, Ukraine, Vietnam and USA), GBH residues were found in fifty nine percent (59 %) of analysed samples, with concentrations ranging between 17 and 163 ng/g (mean = 64 ng/g) (Rubio *et al.*, 2014).

Our concomitant analyses of wax and honey in samples (N = 10) from honey supers resulted in one wax sample being contaminated (48 ng/g). The low contamination in honey supers suggests that GBH residues are mostly stored in the brood chamber, where pollen and nectar are stored and where most bee activity occurs. This preliminary study showed no transfer from wax to honey. Because our results on the concomitant honey/wax contamination are based on limited data (N = 10), they should be confirmed with further studies.

For human health, considering our results and the assumptions we made with the available regulatory data, the consumption of these three contaminated food matrices (pollen, beeswax, and honey) would not be a food safety issue, nonetheless, caution should be taken in the interpretation the results as new studies confirmed glyphosate toxicity below regulatory limits (Mesnage *et al.*, 2015), and the genotoxicity of AMPA (Mañas *et al.*, 2009).

Bees are major pollinators in agricultural systems. Beebread, beeswax, and honey pesticide residue contamination can impact the viability of a colony when larvae develop on highly contaminated beeswax and feed with contaminated food (Orantes-Bermejo *et al.*, 2010). Even a low concentration of pesticide residues can have amplified toxic effects on animals, including bees, through interactions with other chemicals (Zhu *et al.*, 2017) or environmental stressors. The pesticide risk to bees can synergistically amplify the adverse effect of non-chemical stressors too and conversely, nutritional stress can synergistically increase the toxicity of pesticides (Tosi *et al.*, 2017).

Conclusions

Our study gives a glimpse of bees and human exposures to GBH residues. At this stage, glyphosate is analysed alone, even though it is never used in this form but only as part of a mixture with adjuvants in commercial formulations. Clarifications and further research are needed to estimate the risk of the herbicide alone and in formulations (i.e. with the adjuvants), especially at levels below the regulatory safe limits and over longer durations. More studies are needed to assess synergies with other pesticides, and longer term exposures at sub-lethal doses. More transparency is needed regarding the commercial formulation products.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a survey of pesticide residues (glyphosate/AMPA) in various bee-related matrices (beebread, wax, honey) from Belgium. While the representativeness of the sampling procedures may be questioned and although the results of the analytical method validations are not provided in a high level of details, the results are considered reliable. A considerable number of samples of beebread/pollen (n = 82) and beeswax (n = 100) were analysed for parent glyphosate

and its metabolite AMPA. However, according to the guideline SANTE/11956/2016 rev. 9 the intake of pollen and wax by consumers is negligible and, therefore, it is not a regulatory requirement to investigate the residue levels in these commodities. The publication also provides analytical results for 10 honey samples. Only one of these samples was found to contain residues of parent glyphosate above the LOQ of 0.010 mg/kg (at 0.011 mg/kg). None of the honey samples showed detectable residues of AMPA (i.e. these residues were < 0.001 mg/kg). Since according to SANTE/11956/2016 rev. 9 it is possible to derive MRLs in honey based on monitoring data, these results are deemed relevant.

The publication concludes that, based on the observed residue levels, the intake of pollen, beeswax and honey by consumers does not cause any health issue. While this conclusion is certainly correct some of the details of the risk assessment are questionable. For instance, the considered ADI of 0.3 mg/kg bw/day for parent glyphosate is obsolete (and was already obsolete at the time when the publication was issued). Furthermore, the long-term residue intakes were calculated based on maximum residue levels and high percentile consumption figures, which does not correspond to the standard approach.

The publication also includes extensive considerations on bee safety, which, however, are not relevant to this section of the dossier and, therefore, are not discussed here.

1. Information on the study

Data point	CA 6.10.1/004
Report author	Thompson, T.S. *et al.*
Report year	2019
Report title	Determination of glyphosate, AMPA, and glufosinate in honey by online solid-phase extraction-liquid chromatography-tandem mass spectrometry
Document No.	DOI 10.1080/19440049.2019.1577993 E-ISSN 1944-0057
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

A simple method was developed for the simultaneous determination of glyphosate, its main degradation product (aminomethylphosphonic acid), and glufosinate in honey. Aqueous honey solutions were derivatised offline prior to direct analysis of the target analytes using online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry. Using the developed procedure, accuracies ranging from 95.2 % to 105.3 % were observed for all analytes at fortification levels of 5, 50, and 150 µg/kg with intra-day precisions ranging from 1.6 % to 7.2 %. The limit of quantitation (LOQ) was 1 µg/kg for each analyte. Two hundred honey samples were analysed for the three analytes with AMPA and glyphosate being most frequently detected (99.0 % and 98.5 % of samples tested, respectively). The

concentrations of glyphosate were found to range from < 1 to 49.8 µg/kg while those of its degradation product ranged from < 1 to 50.1 µg/kg. The ratio of glyphosate to AMPA was found to vary significantly amongst the samples where both analytes were present above the LOQ. Glufosinate was detected in 125 of 200 samples up to a maximum concentration of 33.0 µg/kg.

Materials and Methods

Reagents and standards

Reagent water (>18 MΩ resistivity) was produced using a Barnstead NANOPure reverse osmosis system. Acetonitrile (ACN; HPLC grade) was purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Ammonium carbonate (ACS reagent grade), sodium carbonate (ACS reagent grade), 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl), and neat reference materials of glyphosate, AMPA, and glufosinate ammonium were obtained from Sigma Aldrich Canada (Oakville, ON, Canada). Isotopically labelled forms of the analytes, specifically $^{13}C_2$,^{15}N-glyphosate, ^{13}C,^{15}N-AMPA, and D_3-glufosinate hydrochloride, were purchased from Toronto Research Chemicals (North York, ON, Canada).

Individual stock standard solutions of glyphosate, AMPA, and glufosinate were prepared by dissolving 10 mg of each analyte in 10 mL of reagent water). A mixed working spike solution containing 1 µg/mL of each analyte in water was prepared from the stock standard solutions. A second working spike solution containing 0.1 µg/mL of each analyte was prepared by diluting the 1 µg/mL solution ten-fold with water. Stock standards of the isotopically labelled internal standards were likewise prepared in water but at a concentration of 100 µg/mL. A working solution containing 1 µg/mL of each internal standard compound was prepared by mixing 0.1 mL of each stock standard solution and diluting to a final volume of 10 mL.

A 0.1 M solution of sodium carbonate, used to adjust the pH of the honey solutions prior to derivatisation, was prepared in reagent water. A 0.05 % (w/v) solution of FMOC-Cl in ACN was prepared fresh for use in derivatising the analytes and their corresponding internal standards.

Sample preparation

Two gram portions of individual honey samples were weighed into 15-mL polypropylene centrifuge tubes (VWR Canada, Edmonton, AB, Canada). The samples were fortified with 50 µL of the working internal standard solution and allowed to sit for 10 min prior to the addition of 5 mL of reagent water. The centrifuge tubes were capped and mixed on a mechanical shaker until the honey was completely dissolved.

Due to difficulties encountered in obtaining a honey sample which did not contain traces of glyphosate, calibration standards were prepared in reagent water. To compensate for the final volume of the honey solution obtained by dissolving 2 g of honey in 5 mL of water, the volume of reagent water added to each 15-mL centrifuge tube was 6.5 mL. A series of 9 calibration standards were prepared by spiking the reagent water aliquots with equivalent analyte concentrations of 0, 1, 5, 10, 20, 50, 75, 100, and 200 µg/kg. Each calibration standard was also spiked with 50 µL of the working internal standard solution. Replicate spiked honey samples for method validation were prepared by fortifying portions of a honey sample which was found to be free of all three analytes at the LOQ values (1 µg/kg) of the proposed method. The levels of fortification for the spiked replicates were chosen at equivalents of 5, 50, and 150 µg/kg).

Prior to analysis by LC-MS/MS, 0.5 mL aliquots of all honey solutions and calibration standards were pipetted into a 2-mL polypropylene microvial to which 0.5 mL of 0.1 M sodium carbonate solution was added. The tubes were capped and mixed by inverting several times. A 0.2 mL portion of the FMOC-Cl in ACN solution was added to each microvial which was then recapped and mixed using a high-speed orbital shaker (Bead Ruptor 12, Omni International Inc., Kennesaw, GA, USA) for two 90 s cycles at maximum speed. Next, the micro-vials were mixed for an additional 60 min using a rocking bed mixer. After derivatisation, the honey mixtures were filtered using 25 mm nylon filters (0.25 µm pore size) directly into polypropylene LC vials (Chromatographic Specialties, Brockville, ON, Canada).

Instrumental analysis

The configuration of the online SPE-LC-MS/MS setup is illustrated in **Figure 1**. The Shimadzu liquid chromatograph system included a SIL30AC autosampler, two LC30AD solvent delivery pumps, and a CBM20A module controller. A six-port, two-position, electronically actuated switching valve (Rheodyne MXT715, Scientific Products and Equipment, Oshawa, ON, Canada) was used to incorporate the online SPE cartridge within the LC-MS/MS system via contact closure through the LC module controller. An Oasis HLB extraction cartridge, 20 × 3.9 mm with 5 µm particles (Waters Ltd., Mississauga, ON, Canada) was employed for the online SPE step. The extraction cartridge was protected by a 4 × 2 mm i.d. RP-1 polymeric guard cartridge (Phenomenex, Torrance, CA, USA). The analytical column was an Agilent Zorbax Extend-C18 column (50 × 2.1 mm, 1.8 µm) preceded by a guard column with similar stationary phase material (5 × 3.0 mm). A binary gradient elution programme employing 10 mM ammonium carbonate in water and ACN as the two mobile phases was used for the online SPE step and the final chromatographic separation. The parameters for the gradient elution programme including the switch positioning of the six-port valve are listed in **Table 1**. The LC was re-equilibrated at initial conditions for 4 min prior to the next injection. The injection volume for all analyses was 50 µL.

A Sciex 4500 quadrupole tandem mass spectrometer was interfaced to the LC using an electrospray ionisation (ESI) probe. The MS/MS was operated in the negative ESI mode with the following general parameters: probe temperature = 700°C; ion spray voltage = −3.5 kV; curtain gas = 20 units; source gases 1 and 2 at 70 units each; collisionally activated dissociation (CAD) gas value = 8 units. All analyses were performed using multiple reaction monitoring (MRM) with the analyte-specific parameters provided in **Table 2**. The dwell time for each MRM transition was 50 ms. A programmable six-port switching valve incorporated into the MS/MS was used to divert flow from the analytical column to the MS/MS only from 6.5 to 9 min during the LC gradient elution programme in order to minimise contamination of the MS ion source.

Figure 1: Schematic diagram of online solid-phase extraction coupled to LC-MS/MS showing solvent flow with switching valve in (a) position #1 for flushing bulk matrix to waste and (b) position #2 for elution and chromatographic separation of analytes prior to MS/MS detection.

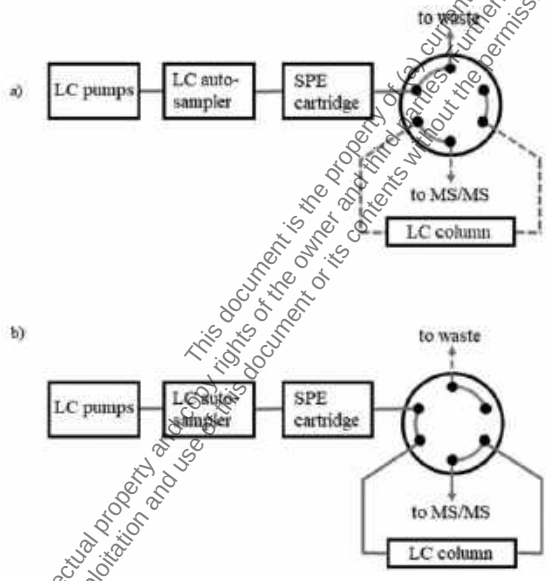


Table 1: LC gradient elution program and six-port switching valve position.

Time (min)	%A (10 mM $(NH_4)_2CO_3$)	%B (ACN)	Flow rate (mL min^{-1})	Valve position
0	100	0	1.00	to waste
2.9	100	0	1.00	to waste
3.0	100	0	0.35	to waste
5.5	*73.6**	*26.4**	0.35	to analytical column
12.0	5	95	0.35	to analytical column
12.5	5	95	0.35	to analytical column
13.0	5	95	0.60	to analytical column
15.5	5	95	0.60	to analytical column
16.00	5	95	0.35	to analytical column
17.00	98	2	0.35	to analytical column
19.00	98	2	0.35	to waste

*Estimated composition based on programmed gradient from 100% A at 3.0 min to 5% A at 12.0 min.

Table 2: MRM parameters for analytes and corresponding internal standards.

Compound	Precursor > product ions*	DP (V)	CE (eV)
glyphosate	***390 > 168***	−40	−16
	390 > 150	−40	−34
$^{13}C_2$,^{15}N-glyphosate	***393 > 170***	−40	−16
AMPA	***332 > 110***	−40	−16
	332 > 136	−40	−20
^{13}C,^{15}N-AMPA	***334 > 112***	−40	−16
glufosinate	***402 > 180***	−45	−14
	402 > 206	−45	−20
D_3-glufosinate	***405 > 183***	−45	−14

*Transition in bolded italics used for quantitation.

Results and Discussion

Considerations for proposed analytical method

There were two main considerations which dictated the direction taken for the development of the method to determine trace residues of glyphosate, AMPA, and glufosinate in honey. The first consideration was the desired LOQ which was established based on the maximum residue limit (MRL) for glyphosate and glufosinate in honey. While Health Canada has not established an MRL for either glyphosate or glufosinate in honey, the EU has set the maximum acceptable concentration at 50 µg/kg for each compound (European Union: Pesticides database 2016). It was decided that the targeted LOQ value should not exceed one-tenth of this MRL value (in other words be 5 µg/kg or lower). The main reason for this targeted LOQ was to have a method which would permit its application to a general survey to establish baseline residue levels rather than determine compliance with existing MRL values.

The second consideration was the necessity to isolate the analytes from the honey matrix which is comprised mainly of the monosaccharides fructose and glucose as well as lower amounts of disaccharides and various other carbohydrates (Bell 2007). On the basis of weight, water typically accounts for less than 20 % of the honey matrix with the majority of the remaining components consisting of simple sugars. The challenge of separating the highly polar analytes of interest from the relatively large quantities of highly polar carbohydrates prior to MS/MS analysis was a significant factor in the development of the proposed testing method.

One of the major advantages of LC over GC is the amenability of the former for the determination of analytes with polar functional groups without the necessity of performing derivatisation. There are, however, still two inherent benefits to performing derivatisation of highly polar analytes such as glyphosate, AMPA, and glufosinate prior to analysis by LC-MS based techniques. Derivatisation of highly polar analytes can result in increased retention using reversed phase stationary phases and increased sensitivity in electrospray ionisation MS (Toss *et al.* 2017). While direct determination of non-derivatised analytes is desirable in that it simplifies the analytical method, there has been mixed success in the development of such procedures. Ibanez *et al.* (2005) attempted to determine glyphosate, AMPA,

and glufosinate without derivatisation but encountered difficulties including reduced sensitivity and lack of robustness of their proposed hydrophilic interaction liquid chromatography (HILIC) method. This ultimately resulted in their decision to employ derivatisation with FMOC-Cl. Similarly, Ehling and Reddy (2015) explored the direct analysis of glyphosate and AMPA using a variety of chromatographic stationary phases but also reported problems with lack of ruggedness, poor chromatographic peak shapes, and inadequate ESI-MS/MS sensitivity. Liao *et al.* (2018) stated that direct determination of glyphosate did not provide adequate sensitivity and selectivity to permit its analysis in baby food samples at concentrations as low as 10 µg/kg. For these reasons, derivatisation with FMOC-Cl has remained a popular procedure in numerous LC-MS-based methods (Arkan and Molnar-Perl 2015).

Based on initial investigations in our lab, it was observed that the sensitivity obtained for FMOC-Cl derivatives of the target analytes was significantly greater than for the non-derivatised compounds under negative electrospray ionisation conditions. A further complication of the direct determination of non-derivatised glyphosate, AMPA, and glufosinate in honey is the fact that the highly polar analytes of interest are difficult to separate from the polar carbohydrates which comprise the bulk of the honey matrix (approximately 80 % by weight simple sugars). While ion exchange solid-phase extraction (SPE) remains an option for isolating glyphosate, AMPA, and glufosinate from the sugars, the inclusion of an offline SPE clean-up step was undesirable due to the additional associated increases in labour, cost, and time. Derivatisation with FMOC-Cl increases the retention of glyphosate, AMPA, and glufosinate on reversed phase stationary phases making it possible to separate the derivatised analytes from highly polar carbohydrates which constitute the bulk of the honey matrix.

Numerous groups have employed online solid-phase extraction methods for the determination of one or more of glyphosate, AMPA, and glufosinate in water samples after offline derivatisation using FMOC-Cl (Vreeken *et al.* 1998; Meyer *et al.* 2009; Sanchis *et al.* 2012; Poiger *et al.* 2017). The advantages of online SPE versus offline SPE are three-fold: firstly to automate the clean-up procedure thereby reducing labour and preparation time; secondly to permit the direct transfer of the analytes of interest from the extraction column/cartridge to the analytical column; and thirdly to facilitate the refinement of the conditions under which the analytes are trapped and subsequently eluted for direct determination. The capability to monitor the chromatographic behaviour of the analytes during online SPE coupled to LC-MS/MS simplifies method development. It was therefore decided to investigate an analytical procedure employing offline derivatisation of glyphosate, AMPA, and glufosinate followed by online SPE separation of the derivatives from the bulk honey matrix with subsequent direct determination by LC-MS/MS.

Derivatisation using FMOC-Cl

Two challenges were encountered in establishing the derivatisation procedure. Firstly, derivatisation of the analytes using FMOC-Cl was discovered to not work efficiently when sodium tetraborate was used in the presence of the honey matrix. Honey is quite acidic in a relatively concentrated solution (2 g of honey plus 5 mL of water) and the borate solution did not have enough buffering capacity to permit the pH of the resulting mixture to be approximately 9 as commonly established in the derivatisation reaction employing FMOC-Cl (Arkan and Molnar-Perl 2015). Sodium carbonate has been used in the derivatisation reaction with FMOC-Cl with aminophosphonic acids (Huber and Calabrese 1985) while a carbonate buffer was used in conjunction with FMOC-Cl and tertiary amphetamines (Herraez-Hernandez and Campins-Falco 2000). Descombes *et al.* (1991) reported that borate and carbonate buffers both worked well in providing alkaline conditions (pH = 9.5) under which the derivatisation of catecholamines and amphetamines could be achieved with FMOC-Cl. Upon switching to 0.1 M sodium carbonate for pH adjustment, it was observed that the derivatisation step proceeded smoothly.

The second challenge was realised when it became obvious that relatively dilute solutions of FMOC-Cl in ACN (e.g. 1 to 10 mg/mL) were not adequate to fully derivatise the analytes in the presence of the honey matrix. Nedelkoska and Low (2004) noted that excessive amounts of FMOC-Cl relative to the quantities of glyphosate present in the sample are required for complete derivatisation of the target analyte due to the reactivity of FMOC-Cl with matrix compounds containing primary and secondary amine functional groups. According to Ehling and Reddy (2015), concentrations of FMOC-Cl solutions used to derivatise glyphosate and AMPA have been previously reported to range from 1 to 28 mg/mL. Toss *et al.* (2017)

used 0.14 mL of a 30 mg/mL solution of FMOC-Cl in acetonitrile to derivatise glyphosate and AMPA in surface water samples containing high levels of organic matter.

Honey is a complex matrix which may contain up to 1 % (w/w) of free amino acids and 0.2–1.6 % protein (Santos-Buelga and Gonzalez-Paramas 2017) which will potentially react with the FMOC-Cl. It was determined that increasing the FMOC-Cl concentration to 50 mg/mL in CAN and utilising 0.2 mL of this solution was necessary to provide the successful derivatisation of the analytes and their corresponding internal standards in the presence of the honey matrix.

Development of online SPE-LC-MS/MS method

The major sugars present in honey were poorly retained by the HLB extraction cartridge and could be flushed to waste without ever reaching the analytical LC column. The derivatised analytes were retained by the extraction cartridge and switching the position of the six-port valve allowed them to be subsequently eluted onto the analytical LC column for further chromatographic separation.

Figures 2 and **3** show the reconstructed MRM ion chromatograms obtained for the determination of a nominally blank honey (*i.e.* all analytes below the LOQ of 1 µg/kg) and the same honey fortified with 5 µg/kg each of glyphosate, AMPA, and glufosinate. During initial method development work it was discovered that it was virtually impossible to find a honey sample which was completely free of all three analytes. Each pair of ion chromatograms for the unspiked and spiked honey samples have been plotted on the same scale for each analyte. While there are additional peaks present in the chromatograms for both glyphosate and glufosinate in the blank honey, these peaks elute after the target analytes and therefore do not interfere in their analysis .

Figure 2: Reconstructed ion chromatograms for blank honey fortified with 25 µg/kg of each isotopically labelled internal standard. The quantitation and confirmatory MRM transitions (respectively) are: (a)+(b) glyphosate; (d)+(e) AMPA; and (g)+(h) glufosinate. The quantitation MRMs for the internal standards are: (c) 13C2,15N-glyphosate; (f) 13C,15N-AMPA; and (i) D3-glufosinate.

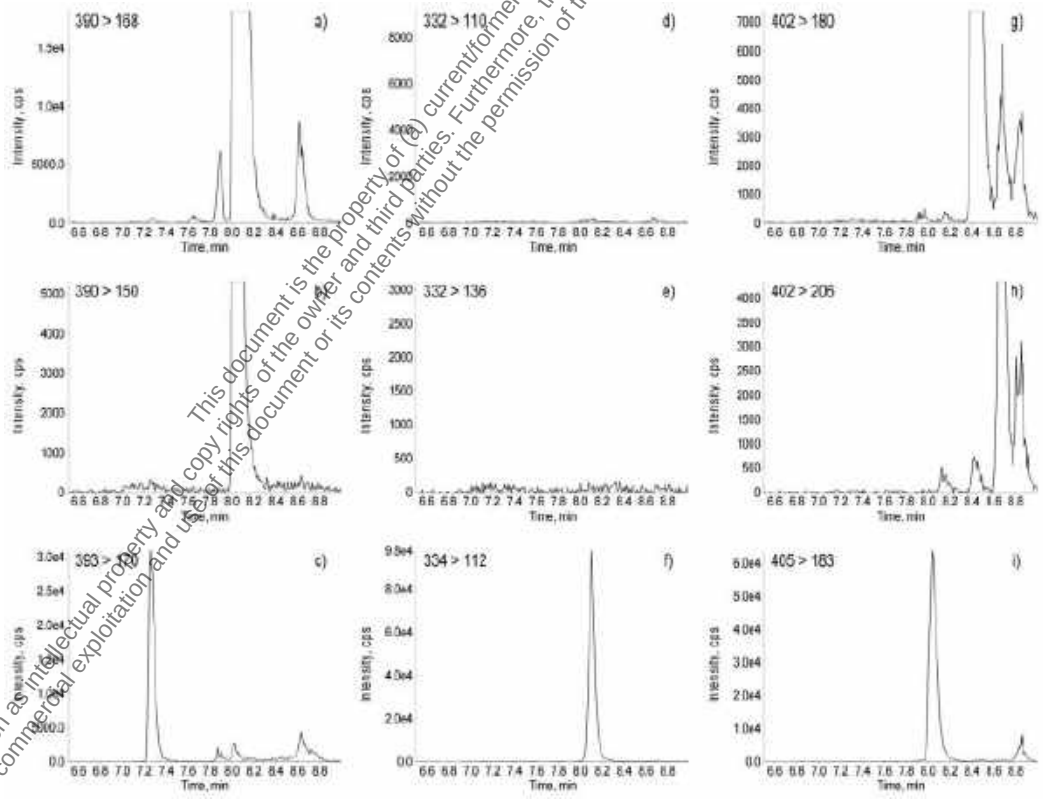
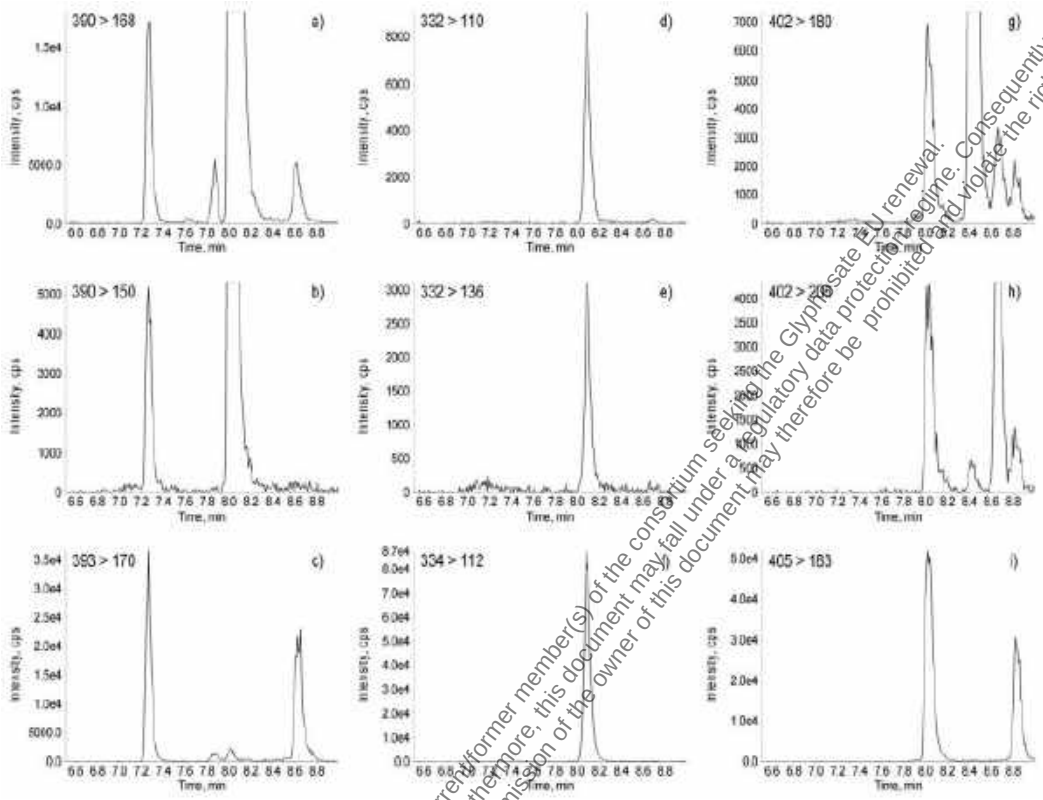


Figure 3: Reconstructed ion chromatograms for blank honey fortified with 5 µg/kg each of glyphosate, AMPA, and glufosinate as well as 25 µg/kg of each isotopically labelled internal standard. The quantitation and confirmatory MRM transitions (respectively) are: (a)+(b) glyphosate; (d)+(e) AMPA; and (g)+(h) glufosinate. The quantitation MRMs for the internal standards are: (c) 13C2,15N-glyphosate; (f) 13C,15N-AMPA; and (i) D3-glufosinate.



Criteria for confirmation of analyte identity

Two MRM transitions were monitored for each incurred analyte in order to permit confirmation of compound identity. A chromatographic peak must be present in both reconstructed ion traces within ± 0.05 min of the retention time of the associated isotopically labelled internal standard. The ratio of the peak areas for the quantitation and confirmation reconstructed MRM traces must be within ± 30 % relative to that obtained for authentic reference material analysed under the same set of operational parameters within the same analytical batch.

Evaluation of matrix effects

Matrix effects were evaluated by comparing calibration curves obtained for standards prepared in reagent water and honey solutions. Unfortunately it was extremely difficult to find a truly blank honey and it was decided that a set of calibration standards would be prepared using a nominally blank honey which did not contain any of the analytes above the LOQ of 1 µg/kg. The results of the calibration curves obtained for standards prepared in either water or honey are given in **Table 3**. The calibration curves were determined using two techniques: firstly by external standardisation and secondly by internal standardisation using each analyte's respective isotopically labelled analogue. The matrix effect (ME) was calculated based on the ratio of the slopes obtained for the calibration curves in matrix versus reagent water:

ME = 100 x (slope of calibration curve in honey) / (slope of calibration curve in reagent water)

where ME = 100 would indicate no matrix effect while ME < 100 or ME > 100 would indicate ionisation

suppression or enhancement, respectively. When the calibration is performed using external standardisation, there is minor ionisation enhancement (ME > 100) observed for glyphosate where ME = 109 %. The opposite ionisation effect (suppression) is observed for both AMPA and glufosinate which have ME values of 51 % and 54 %, respectively. However, when the calibration curves are established using internal standardisation by isotope dilution, the ME values are all within 100 ± 10 %. Based on these results it was concluded that the use of isotopically labelled internal standards for quantitation would adequately overcome the ionisation effects observed because of the honey matrix. Reagent-based calibration standards were subsequently used for all method validation experiments.

Table 3: Comparison of calibration standards prepared in reagent water and honey.

Compound	Standardisation	Equation of curve prepared in reagent water (r^2)	Equation of curve prepared in honey solution (r^2)	Matrix effect (ME*)
glyphosate	external	$y = 6540.7x + 780.0$ ($r^2 = 0.99944$)	$y = 7134.5x + 13125.5$ ($r^2 = 0.99988$)	109%
AMPA	external	$y = 105014.4x - 121.9$ ($r^2 = 0.99972$)	$y = 5317.0x + 1117.5$ ($r^2 = 0.99992$)	51%
glufosinate	external	$y = 9029.6x + 386.2$ ($r^2 = 0.99976$)	$y = 4901.0x + 1918.4$ ($r^2 = 0.99953$)	54%
glyphosate	internal	$y = 0.07890x + 0.02175$ ($r^2 = 0.99826$)	$y = 0.08481x + 0.01717$ ($r^2 = 0.99923$)	107%
AMPA	internal	$y = 0.02129x + 0.00153$ ($r^2 = 0.99866$)	$y = 0.02059x + 0.00457$ ($r^2 = 0.99930$)	97%
glufosinate	internal	$y = 0.02574x + 0.00171$ ($r^2 = 0.99856$)	$y = 0.02480x + 0.03888$ ($r^2 = 0.99818$)	96%

*ME = 100 × (slope of curve in honey)/(slope of curve in water).

Method validation

The analytical method was validated by analysing a series of spiked replicate honey samples. A honey sample which had no analytes at a concentration above the LOQ of 1 µg/kg was found after a large number of honeys were screened using the proposed methodology. A set of spiked replicates fortified at three different concentrations were analysed in order to determine the accuracy and precision of the proposed method. The results of these analyses are summarised in **Table 4.** The inter-day reproducibility was also evaluated by carrying out the analysis of replicate samples over three separate days. The calculated accuracies obtained for the daily analysis of six spiked replicates at each of three concentration levels (5, 50, and 150 µg/kg) ranged from 95.2 % to 105.3 % for all three compounds. The daily precision (standard deviation) for all three analytes at all fortification levels ranged from 1.6 % to 7.2 %. The inter-day accuracy and precision for all three compounds at the three different levels studied over three separate days (a total of 18 replicates at each concentration level) were calculated to be between 97.7 % to 103.1 % and 2.1 % to 5.4 %, respectively. Based on these results, the method was deemed to be fit for purpose.

Table 4: Method validation data.

Compound	Fortification level (µg kg^{-1})	Accuracy ± SD			
		Day 1 (n = 6)	Day 2 (n = 6)	Day 3 (n = 6)	Inter-day (n = 18)
glyphosate	5	105.3 ± 5.4	102.9 ± 4.5	101.2 ± 6.3	103.1 ± 5.4
	50	100.4 ± 2.1	96.2 ± 4.4	104.5 ± 2.8	100.4 ± 4.6
	150	100.2 ± 4.3	96.1 ± 2.2	101.8 ± 3.1	99.3 ± 4.0
AMPA	5	98.9 ± 6.4	101.5 ± 4.4	96.0 ± 3.1	98.8 ± 5.1
	50	103.6 ± 4.4	103.0 ± 3.1	96.8 ± 3.5	101.1 ± 4.7
	150	100.1 ± 1.7	99.1 ± 3.5	96.6 ± 2.5	98.6 ± 2.9
glufosinate	5	101.8 ± 3.8	99.3 ± 3.4	97.3 ± 7.2	99.5 ± 5.1
	50	99.8 ± 3.4	99.2 ± 1.6	95.2 ± 2.5	98.0 ± 3.2
	150	97.7 ± 1.7	98.8 ± 2.4	96.6 ± 1.8	97.7 ± 2.1

The measurement uncertainty for each analyte was estimated using in-house method validation data according to the procedure described in the Codex guidelines on estimation of uncertainty of results (Codex Alimentarius Commission 2011). Method validation data obtained for the analysis of spiked replicates at the three different concentration levels covering a range from 5 to 150 µg/kg was used to calculate an expanded uncertainty (U′) with a coverage factor of 2 (95 % confidence interval) for each

analyte. The expanded uncertainties were estimated as U′ = 14 % for glyphosate, 13 % for AMPA, and 11 % for glufosinate.

Application to honey samples

Two hundred randomly chosen honey samples, which were submitted to our laboratory for other testing, were analysed using the online SPE-LC-MS/MS method to obtain information regarding baseline levels of glyphosate, its main degradation product AMPA, and the other acidic herbicide, glufosinate. The results of these analyses are summarised in **Table 5**. Glyphosate was detected in almost all honey samples analysed with 197 out of 200 samples (98.5 %) having residues equal to or above the LOQ of 1 µg/kg. The maximum concentration of glyphosate residue in the honey samples analysed was 49.8 µg/kg. AMPA was also frequently detected (198 or 99.0 % of 200 samples tested) up to a maximum concentration of 50.1 µg/kg. There were no samples where both glyphosate and AMPA were below the LOQ value.

Table 5: Concentrations of glyphosate, AMPA, and glufosinate in incurred honey samples.*

Compound	# of Detections	Median (µg kg^{-1})	90th Percentile (µg kg^{-1})	95th Percentile (µg kg^{-1})	Maximum (µg kg^{-1})
glyphosate	197	4.9	14.2	19.2	49.8
AMPA	198	10.3	20.8	28.7	50.1
glufosinate	125**	1.4	6.1	9.9	33.0

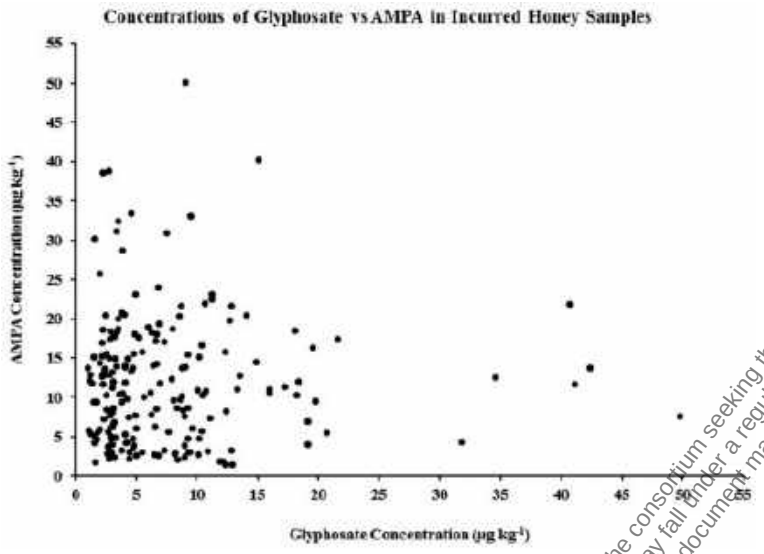
*n = 200 samples analysed.
**One sample did not meet the required ion ratio criterion for confirmation of compound identity and was not included in this value.

The third analyte, glufosinate, was detected much less frequently than either glyphosate or AMPA and also at lower levels in general. Glufosinate was found to be present in 125 of 200 samples analysed with the maximum concentration detected being 33.0 µg/kg. It must be noted that there was a single honey sample where the ratio of the two precursor > product ion MRM transitions for glufosinate was not within the acceptable relative ratio of ±30 % (average ion ratio for calibration standards = 59.3 % while the ion ratio for the sample was 7.0 %). Assuming that there was an interference in the quantitative MRM transition (thereby giving the unacceptably low relative ion ratio), if the confirmatory MRM transition was used for quantitation, the glufosinate concentration was estimated to be just above 1 µg/kg. All samples of honey containing either glyphosate or AMPA at concentrations above the LOQ of 1 µg/kg were successfully confirmed based on the criteria established for compound identification.

Interestingly, the ratio of glyphosate to AMPA was found to vary considerably in samples that contained both analytes. In some cases the two analytes were roughly equal in concentration while in others one of the pair was significantly higher than the other compound. This is illustrated by the scatter plot shown in **Figure 4** where the concentration of glyphosate is plotted versus the concentration of AMPA in the 200 honey samples which were analysed (note that only samples containing both glyphosate and AMPA at or above the LOQ were included in this plot). There are multiple factors which may influence the relative amounts of glyphosate and its degradation product AMPA. Differences in the chemical composition of the honeys tested as well as their age and handling/storage conditions prior to receipt by the laboratory may be important factors. The long-term stability of glyphosate and AMPA in honey has not been established. Other factors which may influence the relative ratios of the two compounds may include agricultural practices such as the timing of herbicide application relative to honey bee foraging, environmental decomposition of the targeted analytes, and differences in crops treated and subsequently pollinated by the bees. The contribution of glyphosate and AMPA residues present in the ambient environment to contamination of plant nectar and subsequently honey itself is further complicated by the variations in the levels of these compounds in environmental matrices such as soil and surface water. No conclusions can be drawn regarding any trend in the relative amounts of these glyphosate and AMPA in honey. The ratio of the concentration of glyphosate to that of AMPA present in samples containing both analytes at ≥1 µg/kg (195 samples) ranged from 0.05 to 9.16. It should also be noted that there were two samples containing glyphosate ≥1 µg/kg (7.7 and 8.8 µg/kg where the concentration of AMPA was below

the LOQ. Conversely, there were three samples with AMPA concentrations ≥1 µg/kg (2.7, 9.0, and 10.6 µg/kg) where the glyphosate concentration was below 1 µg/kg. The concentration of glyphosate exceeded that of AMPA in 63 out of 200 honey samples tested.

Figure 4: Scatter plot of glyphosate versus AMPA concentrations in samples containing both analytes at or above the limit of quantitation (LOQ = 1 µg/kg).



Comparison of residue levels in honey to other reported studies

Table 6 provides a comparison between the residues of glyphosate present in honey samples analysed in this study and those previously reported by other research groups. Bo *et al.* (2007) developed an analytical method for the determination of glyphosate and AMPA residues in a variety of foods including honey. Their reported LOQ was 50 µg/kg and while the method was employed for the analysis of several different food types it does not appear that it was actually applied to honey samples. In several subsequently reported studies, LOQ values were in the range of 10–50 µg/kg (Rubio *et al.* 2014; Chamkasem and Vargo 2017; Karise *et al.* 2017; Berg *et al.* 2018) which permitted frequent detection of glyphosate residues in honey. Zoller *et al.* (2018) and our work both achieved LOQ values of 1 µg/kg and also each had greater than 90 % of tested honey samples containing quantifiable residues of glyphosate. None of the honey samples in either our baseline study or in the survey of honey sold on the Swiss market (Zoller *et al.* 2018) had glyphosate residues above the EU MRL of 50 µg/kg. In a study of honey from numerous countries around the world (Rubio *et al.* 2014), 22 out of 69 samples tested contained glyphosate residues above the MRL of 50 µg/kg up to a maximum of 163 µg/kg. Glyphosate levels in honey samples mainly from the USA and a small number from other countries exceeded the MRL of 50 µg/kg in only 4 of 28 samples tested but with one sample containing 653 µg/kg (Chamkasem and Vargo 2017). Only 2 out of 33 honey samples from Estonia had glyphosate residues above the MRL of 50 µg/kg with a maximum of 62 µg/kg being detected (Karise *et al.* 2017). Berg *et al.* (2018) obtained 59 honey samples from Hawaiian beehives as well as 26 samples from commercially available products. A total of 8 of the 26 merchant samples had detectable residues, three of which were above the MRL of 50 µg/kg. A total of 16 of the 59 samples collected directly from beehives were determined to contain glyphosate residues above the LOQ of 15 µg/kg with 12 samples above the MRL of 50 µg/kg. The maximum concentrations of glyphosate detected in the merchant and hive samples were 87 and 342 µg/kg, respectively. John and Liu (2018) measured glyphosate residues in water, various food matrices, and human urine using an ELISA method. Only one honey was tested amongst the samples and was found to contain 22 µg/kg of glyphosate. In the 2016 EU report on pesticide residues in food (EFSA (European Food Safety Authority) 2018a), 18 of 220 honey samples were found to have detectable residues of glyphosate. The report does not include specific details regarding either the analytical methods used by the reporting laboratories or their LOQs for glyphosate in honey. Six honey samples contained glyphosate

residues above the EU MRL of 50 µg/kg with levels ranging from 90 to 610 µg/kg.

Table 6: Glyphosate residues in honey from various studies.

	Country of study					
	USA (Rubio et al. 2014)	USA (Chamkasem and Vargo 2017)	Estonia (Karise et al. 2017)	Switzerland (Zoller et al. 2018)	USA (Berg et al. 2018)	Canada (this study)
Testing method	ELISA	LC-MS/MS	LC-MS/MS	LC-MS/MS	ELISA	LC-MS/MS
Source of honeys	Various countries of origin	Mainly from USA	Estonia	Not specified	Mainly from USA (Hawaii)	Mainly western Canada
# Samples tested	69	28	33	16	85	200
# Positives (%)	41 (59.4%)	17 (60.7%)	3 (9.1%)	15 (93.8%)	24 (28.2%)	197 (98.5%)
LOQ (µg kg^{-1})	15	10 to 16	50 (LOD = 10)	1	15	1
Maximum (µg kg^{-1})	163	653	62	15.9	342	49.8

Neither AMPA nor glufosinate were detected, with LOQs of 16 and 18 µg/kg respectively, in 19 honey samples analysed by direct determination of the underivatised analytes using LC-MS/MS (Chamkasem and Vargo 2017). None of the 16 honey samples analysed by Zoller *et al.* (2018) contained AMPA residues above the LOQ of 2.5 µg/kg. Considering the low levels of glyphosate found in these samples (median concentration of 3.0 µg/kg), it is entirely plausible that AMPA could be undetected since its LOQ was 2.5 times higher than for glyphosate.

It should be noted that the LC-MS/MS methods employed by Chamkasem and Vargo (2017) as well as by Karise *et al.* (2017) both involved the determination of glyphosate residues without derivatisation or subsequent extract clean-up. The combination of FMOC-Cl derivatisation and online SPE coupled directly to LC-MS/MS as performed in our method made it possible to achieve LOQ values which were at least one order of magnitude lower by comparison. The analytical method used by Zoller *et al.* (2018) did not employ a derivatisation step but did carry out an offline SPE clean-up step followed by extract dilution prior to LC-MS/MS analysis. Their LOQ values for glyphosate and AMPA were equal to and just slightly higher, respectively, than those obtained with our procedure.

Considerations for future studies

It should be noted that the current EU MRL for glyphosate in honey only includes the parent compound as the marker residue (EU 2016). A recent review by the European Food Safety Authority (EFSA) indicates that there is a proposal to include other related analytes in the residue definition for glyphosate in different foods (EFSA 2018b). While there is no specific mention of honey, it has been proposed that the residue definition for numerous other commodities be expanded to include the sum of glyphosate, AMPA, and the metabolite N-acetyl-glyphosate for enforcement purposes. It has also been recommended that residue analysis for risk assessment include glyphosate, AMPA, N-acetyl-glyphosate, and N-acetyl-AMPA. While several studies to date, including the work described herein, have reported residues of glyphosate and AMPA in honey, there is a need for the N-acetylated metabolites of these compounds to be considered for addition in future studies. The current EU MRL for glufosinate in honey includes the sum of the parent compound plus its metabolites 3-[hydroxyl(methyl)phosphinoyl]propionic acid (MPP) and N-acetyl-glufosinate (NAG) (European Union: Pesticides database 2016). While glufosinate was not detected in honey according to a single previously reported study (Chamkasem and Vargo 2017), its presence in honey samples analysed in our survey suggests the need to investigate MPP and NAG residues in future work.

Conclusion

A relatively simple method was developed for the determination of glyphosate, AMPA, and glufosinate residues in honey with an LOQ of 1 µg/kg for each analyte. A key component of the method was the utilisation of isotopically labelled internal standards to overcome matrix effects associated with the samples. Following a simple derivatisation step, it was possible to use online solid-phase extraction for the isolation of the derivatised analytes from the bulk of the honey matrix with subsequent direct determination of the residues by LC-MS/MS. A survey of honey samples from western Canada indicated the widespread contamination of these samples by glyphosate, AMPA, and glufosinate, albeit at low

concentrations. While Health Canada has not currently established an MRL for either glyphosate or glufosinate in honey, in consideration of the EU MRLs of 50 µg/kg for each compound the risk to consumer health appears to be quite low based on the residues detected.

Chromatographic conditions

Chromatograph:	Shimadzu 30 LC System (SIL30AC autosampler, two LC30AD solvent delivery pumps, CBM20A module controller)
Column:	Agilent Zorbax Extend-C18 (50 mm x 2.1 mm, 1.8 µm)
Column oven temperature:	Not provided
Injection volume:	50 µL
Mobile phases:	(A) 10 mM ammonium carbonate in water (B) Acetonitrile

Gradient (linear transitions):

Time (Min)	Eluent A (%)	Eluent B (%)	Flow rate (mL/min)	Valve position
0.0	100	0	1.00	to waste
2.9	100	0	1.00	to waste
3.0	100	0	0.35	to waste
5.5	73.6	26.4	0.35	to column
12.0	5	95	0.35	to column
12.5	5	95	0.35	to column
13.0	5	95	0.60	to column
15.5	5	95	0.60	to column
16.0	5	95	0.35	to column
17.0	98	2	0.35	to column
19.0	98	2	0.35	to waste

Retention time:	Glyphosate: ~ 8.1 min 13C2,15N-glyphosate (IS): ~ 8.1 min AMPA: ~ 8.1 min 13C,15N-AMPA (IS): ~ 8.1 min Glufosinate: ~ 8.1 min D3-glufosinate (IS): ~ 8.1 min
Detector:	Sciex 4500 quadrupole tandem mass spectrometer
Scan type:	MRM
Ion source:	ESI negative

Source gas:	70 units	Source temperature:	700°C
CAD gas:	8 units	Source voltage:	−3500 V
Curtain gas:	20 units		

Analyte	Precursor ion Q1 (amu)	Product ion Q3 (amu)	Declustering potential (V)	Collision energy (eV)	Scan time (ms)
Primary transition (quantification)					
Glyphosate	390	168	−40	−16	50
Glyphosate (IS)	393	170	−40	−16	50
AMPA	332	110	−40	−16	50

AMPA (IS)	334	112	−40	−16	50
Glufosinate	402	180	−45	−14	50
Glufosinate (IS)	405	183	−45	−14	50
Secondary transition (confirmation)					
Glyphosate	390	150	−40	−34	50
AMPA	332	136	−40	−20	50
Glufosinate	402	206	−45	−20	50

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes the development and validation of a method for the analysis of glyphosate, AMPA, and glufosinate in honey. Aqueous honey solutions were derivatised offline prior to direct analysis of the target analytes using online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Method validation fulfil EU requirements. The method showed good performance for all analytes with a LOQ of 1 µg/kg for each analyte.

The method can be considered valid for monitoring purposes and has been applied for the analysis of two hundred randomly chosen honey samples from Canada. Virtually all the samples were found to contain measurable residues of glyphosate and/or AMPA, which is at least in part due to the extremely LOQ (1 µg/kg). The ratio between parent glyphosate and AMPA was very variable, which is also in contrast to the findings of the EU monitoring (where no measurable residues of AMPA were found) but may also be accounted for by the very low LOQ. In spite of the large number of samples analysed, none showed residues of parent glyphosate exceeding the current EU MRL of 0.05 mg/kg.

According to SANTE/11956/2016 rev. 9 it is possible to derive MRLs in honey based on monitoring data. As honey available to European consumers may originate from outside the EU, it is appropriate to consider honey residue data from outside the EU to derive the EU MRL. Therefore, the publication is considered relevant and reliable. It also includes a useful discussion of the residue levels of glyphosate in honey reported by other authors.

1. Information on the study

Data point	CA 6.10.1/005
Report author	Chiesa, L.M. *et al.*
Report year	2019
Report title	Detection of glyphosate and its metabolites in food of animal origin based on ion-chromatography-high resolution mass spectrometry (IC-HRMS)
Document No.	DOI 10.1080/19440049.2019.1583380 E-ISSN 1944-0057
Guidelines followed in study	SANTE/11813/2017
Deviations from current test guideline	None
GLP/Officially recognised testing	Not applicable

facilities	
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

Glyphosate and glufosinate are broad spectrum herbicides, widely used in agriculture and in inhabited or industrialised areas, and aminomethylphosphonic acid is a degradation product of glyphosate. In 2015, the International Agency for Research on Cancer reported that glyphosate is a probable carcinogenic. In 2017, however, a scientific opinion of the European Chemicals Agency concluded that glyphosate is not proven to be carcinogenic, mutagenic or to have negative effects on reproduction. Nevertheless, aminomethylphosphonic acid was not considered. Due to their chemical-physical characteristics, these molecules present difficulties that have not yet allowed routine monitoring to be carried out. For these reasons, we developed and validated a simple and versatile liquid extraction, before IC-HRMS analysis, of three different complex matrices: honey, bass fish and bovine muscle. Among the satisfactory validation parameters, the LOQs in the range of 4.30 – 9.26 ng/g demonstrated high method sensitivity, compared to the few works present in literature. Finally, the method was applied to real commercial samples, which showed no traces of the selected pesticides.

Materials and Methods

Chemicals and reagents

Glyphosate, glufosinate ammonium, aminomethyl-phosphonic acid (AMPA) and the internal standard Glyphosate-2-^{13}C,^{15}N were purchased from Merck (Sigma–Aldrich, Merck KGaA, Darmstadt, Germany). All solvents used were of LC-MS or analytical grade. Formic acid (98–100 %) was obtained from Riedel-de Haën (Sigma–Aldrich). Water was purified by a Milli-Q system (Millipore, Merck KGaA, Darmstadt, Germany).

Standard solutions

Stock standard solutions (1 mg/mL) for each standard were prepared in water and kept at −20°C. Working solutions containing each of the studied analytes at a concentration of 100 ng/mL were prepared daily in methanol containing 1 % of formic acid, as suggested by EU Reference Laboratory for pesticides (Anastassiades *et al.* 2016). Each working solution was maintained at 4°C during the method validation procedures. Plastic flasks and stoppers were used due to the fact that these pesticides tend to interact with glass surfaces.

Sample collection

Three different food matrices of animal origin were selected for the method validation: honey, fish (bass) and bovine muscle. Five commercial samples of each matrix were homogenised to create a pool to be used for the validation. After homogenisation, the samples were stored at −20° C until analyses.

Ten real Italian commercial samples each of organic honey, bass and bovine muscle were also collected from different supermarkets of Milan for the application of the method.

Sample extraction

The extraction procedure was very simple and identical for the three different matrices. Homogenised samples (1 ± 0.05 g) of honey, or minced fish or bovine muscle were weighed into 15 mL polypropylene centrifuge tubes. Samples were spiked with the internal standard: 0.2 µg/g for honey and 0.4 µg/g for fish and bovine muscle samples. Three mL of methanol was added followed by 7 mL of acidified deionised water (1 % formic acid). The samples were mixed for 1 min using a vortex and then sonicated for 15 min. After centrifugation (2500 × g, 4°C for 10 min), 1 mL of the supernatant was filtered through a mixed cellulose syringe filter (0.45 µm) directly into a plastic 2 mL vial, ready for determination by IC-MS/MS.

IC-HRMS orbitrap analyses

The analyses were performed by an Ionic Chromatography (IC) Dionex ICS-5000+ system (Sunnyvale, CA, USA) made up of Dual Pump (DP), a Conductivity Detector (EG), a Detector/Chromatography Module (DC) and an Autosampler (AS-AP). The ion chromatography separation column was a Thermo Scientific Dionex IonPac AS19-4 µm (2 × 250 mm, 4 µm particle size) with a guard column Dionex IonPac AG19-4 µm (2 × 50 mm, 4 µm particle size) maintained at 30°C. The eluent flow rate was 0.30 mL/min with a gradient from 15 mM KOH (aq), held for 8 min, increased to 55 mM KOH (aq) at 20 min, held in these conditions for 4 min and back to 15 mM KOH (aq) at 24.1 min, with a cycle time of 30 min. The KOH eluent was neutralised using a Dionex AERS 500, 2 mm electrolytically regenerated suppressor (Thermo Scientific). The injection volume was 50 µL.

The detector was a Thermo Q-Exactive Orbitrap™ (Thermo Scientific, San Jose, CA, USA), equipped with heated electrospray ionisation (HESI) source. Capillary temperature and vaporizer temperature were set at 330°C and 280°C, while the electrospray voltage was set at 3.50 kV operating in negative mode. Sheath and auxiliary gas were set at 35 and 15 arbitrary units, with S lens RF level of 60.

Instrument calibration was done every analytical session with a direct infusion of an LTQ Velos ESI Negative Ion Calibration Solution (Pierce Biotechnology Inc., Rockford, IL, USA). The Full Scan acquisition (FS) was combined with an Independent Data Acquisition mode (DIA), providing the MS2 spectra for confirmatory response, based on an inclusion list. The resolving power of FS was set at 70,000 Full Width at Half Maximum (FWHM). On the basis of our compound list, a scan range of m/z 50–250 was chosen; the automatic gain control (AGC) was set at 1×10^{-6} and the maximum injection time was 100 ms. The DIA segment operated in negative mode at 35,000 FWHM. The AGC target was set to 5×10^{-4}, with an auto maximum injection time. The precursor ions are filtered by the quadrupole which operates at an isolation window of 1 m/z. Fragmentation of precursors was optimised as three- stepped normalised collision energy (NCE) (10, 25 and 50 eV). Detection of analytes was based on the retention time of target compounds, on calculated exact mass of the deprotonated molecular ions, and at least one specific and typical fragment. The formula of the compounds, with the exact theoretical mass of the parents and the diagnostic transition used to confirm glyphosate and its metabolites are reported in **Table 1**. Chromeleon™ software (Thermo Fisher Scientific, Waltham, MA) was used to control the IC system while Xcalibur™ 3.0 software (Thermo Fisher Scientific, San Jose, CA, USA) was used to control the HRMS system, determine the exact mass of the compounds, record and elaborate data.

Table 1: Main information (formula, retention time (tr), precursors, main products and polarity) about AMPA, glyphosate, glufosinate and the relative internal standard (IS) analysed by IC-HRMS.

Compound IC-HRMS	Formula	t_r (min)	Precursor (m/z)	Main products (m/z)			Polarity
AMPA	CH_6NO_3P	14.25	110.00125	62.96417	78.95904	80.97468	(-)
Glyphosate	$C_3H_8NO_5P$	23.87	168.00673	62.96417	124.01687	149.99612	(-)
Glufosinate	$C_5H_{12}NO_4P$	14.14	180.04312	85.02955	94.99042	136.05329	(-)
IS: Glyphosate-2-13C, 15N	$[^{13}C_2C^{[15]}NH_8O_5P$	23.85	171.01048	62.96415	80.97471	152.99988	(-)

Validation parameters

Validation was carried out following the European Commission (2017) SANTE/2017 Guidance document on method validation & quality control procedures for pesticide residues analysis in food & feed. The selectivity of the method was evaluated by injecting extracted blank honey, fish and bovine muscle samples. The absence of signal above a signal-to-noise ratio of 3 at the expected retention times of the target compounds was the parameter used to show the absence of interferences.

The matrix-matched calibration curves were obtained by spiking 1 g of the three different matrices with an appropriate volume of the standard working solution to cover the concentration range from 5 to 100 ng/g (five calibration points: 5, 10, 20, 50 and 100 ng/g). The limit of quantification (LOQ) of the methods was the lowest validated spiked level meeting the requirements of recovery within the range of 70–120 % and an RSD ≤ 20 % (European Commission. 2017. SANTE/11813/2017). The repeatability, evaluated as a coefficient of variation, CV %, was calculated by analysing six replicates at two

fortification level (10 and 50 ng/g). Recoveries were calculated by comparing the concentrations of the extracted compounds, spiked before extraction, with those spiked at the end of the extraction procedure at two fortification level (10 and 50 ng/g) for all compounds. The matrix effect was also evaluated using the Matuszewski *et al.* (2003) approach by comparing the corresponding peak areas for standards, spiked after extraction into the extracts, to the peak areas obtained in neat solution standards, expressed as percentage.

Results and Discussion

Extraction procedure

During the preliminary phase, the QuPPe extraction method proposed by EU Reference Laboratories for Residues of pesticides (Anastassiades *et al.*, 2016) was followed, with good results for glyphosate and AMPA but found not suitable for glufosinate in our matrices after the IC-HRMS analysis. In particular, we observed a different and opposite extraction and chromatographic behaviour of the molecules (in particular for AMPA and glufosinate) on the basis of the different solvents used and injected during the ion chromatography separation. Moreover, the final dilution 1/10 suggested by the Anastassiades *et al.* (2016) or by others (Adams *et al.*, 2017) did not improve chromatographic problems when AMPA or glufosinate was hardly detectable.

So we decided to modify the method starting from a smaller amount of matrix (1 g instead of 5 g) to decrease interferences, investigating also the influences of the different tested extraction solvents compatible with our instrumentation: water, methanol and the related acidified solutions with 1 % of formic acid. In particular, using only water (**Figure 1a**) or only methanol (**Figure 1b**) as extraction solvent we had poor results for AMPA, but very satisfactory chromatographic peaks for glyphosate and glufosinate. By using 1 % of formic acid in water (**Figure 1c**) we had an improvement of AMPA signal, but it was not yet satisfactory, while with 1 % of formic acid in methanol (**Figure 1d**) we observed the reverse situation, good chromatography for AMPA but not for glufosinate, which eluted with a too-jagged and wide peak. So after different trials, changing the percentage of formic acid and the composition of the methanol and acidified water mixture we reached the best compromise with 30 % of methanol and 70 % of acidified (1 % formic acid) water as extraction solution. **Figure 2** reports the extracted parent ion chromatograms from full-scan IC-HRMS analysis and from data-independent acquisition mode with the relative fragmentation mass spectra of the three selected analytes after method optimisation.

Figure 1: Extracted parent ion chromatograms from full-scan IC-HRMS analysis of AMPA, glyphosate and glufosinate and influences of the different tested extraction solvents: water (a), methanol (b), 1 % formic acid in water (c) and 1 % formic acid in methanol (d).

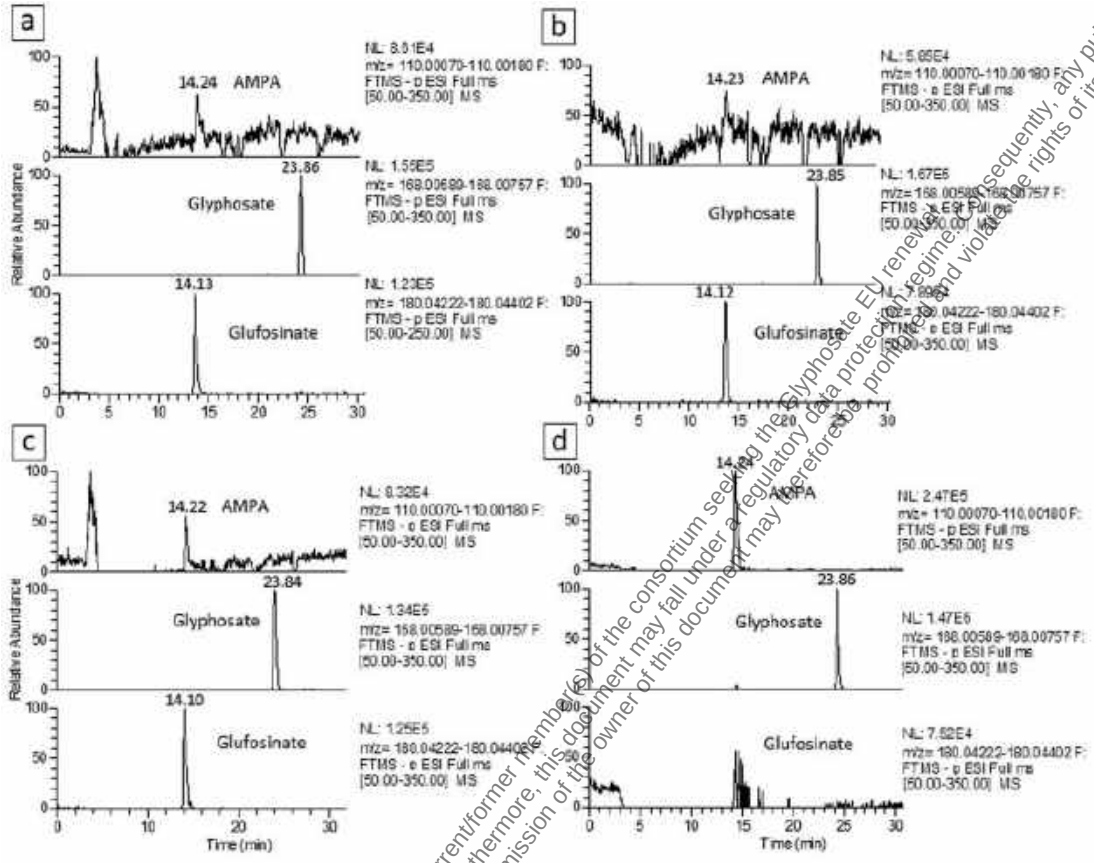
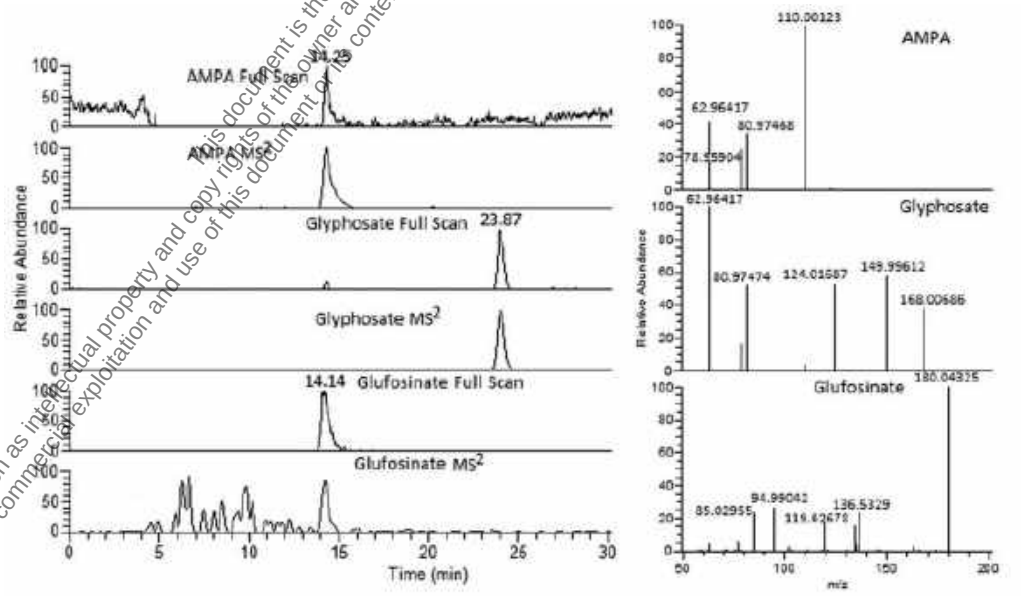


Figure 2: Extracted parent ion chromatograms from full-scan IC-HRMS analysis and from data-independent acquisition mode with the relative fragmentation mass spectra of the three selected analytes (concentration of 10 ng/g) after method optimisation.



IC-HRMS validation parameters

All instrument validation parameters are reported in **Table 2**. The method applied to the three different matrices (honey, bass fish, bovine muscle) showed high specificity, without any interference close to the retention time where the investigated compounds were expected to elute. The good selectivity of the method was demonstrated with a S/N ratio higher than 3 in presence of analytes at the lowest detectable concentrations. All identification criteria passed including retention time stability compared to the standard solution. The mean recoveries ranged between 75 and 112 %, indicating the efficiency of the extraction protocol. Matrix validation curves demonstrated a good linearity over the working range with a good fit ($R^2 > 0.99$) for all compounds. Repeatability was calculated using one-way analysis of variance (ANOVA); the CVs were substantially lower than 20 %, satisfying the criteria required by the European Commission (2017).

Table 2: Validation parameters about glyphosate, glufosinate and AMPA in the three different matrices analysed by IC-HRMS.

	LOQ (ng g^{-1})	Matrix effects %	CV % (at 2 Levels*)	Recovery % (at 2 Levels*)	Matrix calibration curve	Linearity R^2
HONEY						
AMPA	9.26	84	11, 4	91, 95	y = 0.15747x-0.514838	0.9996
Glyphosate	4.30	94	7, 7	99, 100	y = 0.295172x+0.858303	0.9975
Glufosinate	5.05	84	13, 12	100, 101	y = 0.442863x+0.00858678	0.9957
FISH MUSCLE						
AMPA	5.38	95	4, 2	94, 95	y = 0.0897754x-0.231747	0.9951
Glyphosate	5.08	93	8, 8	80, 93	y = 0.072573x+0.112081	0.9985
Glufosinate	4.36	96	7, 6	112, 96	y = 0.127407x-0.176602	0.9976
BOVINE MUSCLE						
AMPA	6.44	99	12, 9	75, 79	y = 0.091067x-0.113588	0.9925
Glyphosate	6.47	107	13, 10	75, 80	y = 0.063115x+0.0920263	0.9922
Glufosinate	6.25	106	8, 2	76, 80	y = 0.120407x-0.107902	0.9962

*The two concentration levels were 10 and 50 ng g^{-1}.

Regarding the LOQs in the range from 4.30 to 9.26 ng/g, our satisfactory results showed high method sensitivity for glyphosate and its metabolites, when compared to the few reports present in the literature. In fact, Picò *et al.* (2007) reported LOQ of 0.05 mg/kg for glyphosate and AMPA in plant products, such as rice, wheat, vegetables, fruits and tea, pig and chicken muscles, aquatic products, chestnut, honey, etc. using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry; Krüger *et al.* (2014) reported validation parameters spiking at 100 µg/g of glyphosate in animal and human residues through ELISA followed by GC-MS/MS analysis. The overview of approximate LOQs reported by Anastassiades *et al.* (2016) in the range of 0.01–0.02 mg/kg obtained by the QuPPE extraction followed by LC-MS/MS analysis or those of Chamkasem *et al.* (2016) in the range of 4–26 ng/g using LC-MS/MS system are a little higher than our results. In **Table 3** we report all the LOQs and other information on the different studies presented in the literature about glyphosate, AMPA and glufosinate in food of animal origin. Matrix effects were modest in the three different matrices with a percentage variation lower than the 20 % (from 84 % to 107 %) recommended by the European Commission (2017).

Based on our results the use of high-resolution mass spectrometry and hyphenation with ion chromatography has been demonstrated to be very effective for the analysis of these challenging analytes in very complex matrices of animal origin. Particularly, as stated by Rajski *et al.* (2018) in their analogous study on anionic pesticides in fruits and vegetables, the high ion-exchange capacity, the efficiency, the diameter reductions and the characteristic chemistry of bonded functional groups of IC columns are a major factor for the separation and identification of the highly polar pesticides, scarcely retained in reversed-phase LC, avoiding moreover any derivatisation step. The high-resolution mass spectrometry allowed obtaining low background matrix signals, improving the sensitivity in terms of LODs and efficient trapping and stability of low m/z ions, improving selectivity. The high MS resolving power and mass accuracy down to 1 ppm, combined with the rapid scan speed, also provide high specificity (Chiesa *et al.* 2018). The possibility to do retrospective analyses is an added value.

Table 3: Literature about glyphosate in products of animal origin.

Authors	Matrix	Analyte	LOQ	Analytical Instrumentation
Wang, Jaw, and Chen	Fish	Glyphosate	/	Beckman LS 1000C liquid scintillation counter.
Alferness and Iwata (1994)	Muscle, kidney, liver and fat beef, eggs and milk	Glyphosate, AMPA	0.01 μg g^{-1}	Capillary Gas Chromatography with Mass-Selective Detection
Picó et al. (2007)	Plant products, vegetables, fruits and tea, pig and chicken muscles, aquatic products, chestnut, honey	Glyphosate, AMPA	0.05 μg g^{-1}	Liquid chromatography/ tandem mass spectrometry
Bo et al. (2007)	Vegetables, fruits, cereals, pig and chicken muscles, aquatic products, honey	Glyphosate, AMPA	0.05 μg g^{-1}	Liquid chromatography/ tandem mass spectrometry
Krüger et al. (2014)	Liver, kidney, lung, spleen, muscle and intestine of cow	Glyphosate	100 μg g^{-1}	ELISA- Gas Chromatography-Mass Spectroscopy
Chamkasem et al. (2016)	Milk	Glyphosate, Glufosinate, and AMPA	100 μg g^{-1}	Liquid chromatography/ tandem mass spectrometry
Anastassiades et al. (2016)	Foods of plant origin, cereals and honey	Glyphosate, Glufosinate, AMPA and others	4–26 ng g^{-1}	Liquid chromatography/ tandem mass spectrometry
Liao et al. (2018)	Milk-based baby foods and other milk products, infant formulae, yogurt	Glyphosate, Glufosinate	/	Liquid chromatography/ tandem mass spectrometry
Chamkasem et al. (2018)	Honey	Glyphosate, Glufosinate, and AMPA	0.25 μg g^{-1}	Liquid chromatography/ tandem mass spectrometer

Application to real commercial samples

Finally, we applied the proposed method for the analysis of 30 real samples: 10 organic honeys, 10 beef muscle pools and 10 sea bass muscle pools, each thoroughly homogenised. All the samples were of Italian origin, taken from different supermarkets of Milan. None of the selected samples showed any traces of glyphosate or metabolites, ensuring the good quality of the samples, especially when it comes to organic products such as honey, demonstrating the absence of pesticide contamination both of the sample and of the production area.

Conclusions

In this study, we developed and validated a new and versatile IC-HRMS method for the detection of glyphosate, AMPA and glufosinate in three complex different matrices, honey, bass fish and bovine muscle. These results are of great importance and topical in the field of food safety because of the scarce data regarding this topic, the extractive and analytical difficulties related to these analytes in relation to complex matrices, and the legislative situation not yet outlined on the use of glyphosate and residues in consumer products. The application of the method to real commercial samples did not show any traces of the pesticides. Further studies of the method's application and statistical evaluation are necessary to form a more complete view on this matter.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The purpose of the publication is to describe and discuss the performance of a residue analytical method for glyphosate, AMPA and glufosinate in, food of animal origin. As such, the publication is not relevant to risk assessment. However, since it also reports residue levels for the investigated compounds in 10 honey samples and since according to SANTE/11956/2016 rev. 9 it is possible to derive EU MRLs in honey based on monitoring data, the publication may be considered relevant to risk assessment and MRL setting. Based on the provided validation results, the method is considered reliable. The LOQ (defined as the lowest fortification level yielding acceptable recoveries) was 0.010 mg/kg for both glyphosate and AMPA (although different values, presumably estimated from the signal to noise ratio, are stated in Table 2). None of the 10 analysed honey samples showed residues of glyphosate or AMPA above the LOQ. However, it is important to note that all the samples were from organic production and this may need to be taken into

account in the evaluation.

1. Information on the study

Data point	CA 6.10.1/006
Report author	Berg, C.J. *et al.*
Report year	2018
Report title	Glyphosate residue concentrations in honey attributed through geospatial analysis to proximity of large-scale agriculture and transfer off-site by bees
Document No.	DOI 10.1371/journal.pone.0198876 E-ISSN 1932-6203
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Executive Summary

Honey taken directly from 59 bee hives on the Hawaiian island of Kaua'i was analyzed for glyphosate residue using ELISA techniques. Glyphosate residue was detected (> LOQ) in 27 % of honey samples, at concentrations up to 342 ppb, with a mean = 118 ppb, S.E.M. 24 ppb. Of 15 honey samples store-purchased on Kaua'i, glyphosate was detected in 33 %, with a mean concentration of 41 ppb, S.E.M. 14. Glyphosate residue was not detected in two samples from the island of Molokai but was in one of four samples from the island of Hawai'i. Presence and concentration of glyphosate residues were geospatially mapped with respect to Hawaiian land divisions. Mapping showed higher occurrence of glyphosate that was over LOQ (48 %) and concentrations of glyphosate (mean = 125 ppb, S.E.M. 25 ppb; N = 15) in honey from the western, predominantly agricultural, half of Kaua'i versus the eastern half (4 %, mean = 15 ppb; N = 1). Geographic Information System analysis of land use percentage was performed within a circular zone of 1 km radius around each hive. Various land use types within each circular zone were transcribed into polygons and percent land use calculated. Only agriculture land use showed a strong positive correlation with glyphosate concentration. High glyphosate concentrations were also detected when extensive golf courses and/or highways were nearby. This suggests herbicide migration from the site of use into other areas by bees. Best management practices in use for curtailing pesticide migration are not effective and must be carefully re-assessed.

Materials and Methods

Sample collection

Honey samples were collected directly from hives by beekeepers on the island of Kaua'i in three batches from 2013 through 2016 (**Table 1**). Samples were opportunistically obtained from all accessible parts of the island. Collections were constrained by lack of bee hives in the area or beekeepers' unwillingness to provide samples. A strict confidentiality agreement was needed to get participation in the study. For some sites, sample batches were collected over time, to increase sample size. The timing was irrespective of seasonality of honey production by the bees. Each sample came from a single unique hive and its location was precisely recorded. Two other batches of honey were obtained from merchants and comprised honey from many hives under control of the manufacturing company.

In the fall of 2013 (Batch 1) two honey samples were collected by beekeepers, by scraping the honey

comb with the open mouth of a clean glass mason jars and sealing the jars. These samples were stored at room temperature in a closed box, in a cabinet, until shipment to Microbe Inotech Laboratories, Inc., St. Louis, MO, for analysis of glyphosate concentration.

During the spring of 2015 (Batch 2) 36 samples of honey were collected directly from their unique hives by beekeepers of Kaua'i, using only the certified pre-cleaned 40 ml amber borosilicate glass vials provided to collect and store the honey. Vials were immediately sealed under a signed and dated custody seal by the collector and delivered directly to one of the authors (CJB, RK), along with a signed confidentiality statement containing contact information, date of collection, and hive location. Samples were stored at room temperature in a closed box, in a cabinet until shipment for analysis.

In fall of 2016 (Batch #3) 21 samples were collected by beekeepers and delivered to one of the authors (CJB), under the same procedures and stored for shipment as Batch #2.

In the winter of 2016 (Batch #4) 23 samples of honey were purchased from local famers' markets, produce stands, and stores. Honey was decanted into glass vials, sealed, and stored as above. Commercially produced honey is a composite from many hives. Source location was broadly determined from the label or from discussion with merchants. Date of honey collection is unknown. Samples were sent to Abraxis Inc, laboratory for analysis.

Batch #5 comprises three honey samples. Two samples were from the island of Molokai. One was purchased at a store on Molokai and the other was obtained from the beekeeper's bottled supplies. Both samples were a composite from hives at each beekeepers' farm. The farms' hives, which were located on Google Earth Pro™, were widely separated and thus represented different bee foraging sites. The third sample was purchased at a Kauai store and the source locality identified as from the island of Hawaii by its label.

Table 1: Honey collection data and laboratory where glyphosate was analyzed by ELISA.

Batch Number	Sample ID	Date Collected	ELISA analysis location
Hive Samples			
Batch #1	37, 38	Fall 2013	Micro Inotech Lab.
Batch #2	1 to 36	Summer 2015	Surfrider Lab.
Batch #3	39 to 59	Fall 2016	Surfrider Lab.
Merchant Samples			
Batch #4	91 to 23	Winter 2016	Abraxis Lab.
Batch #5	60, 61, 62	Winter 2016	Surfrider Lab.

Sample analysis

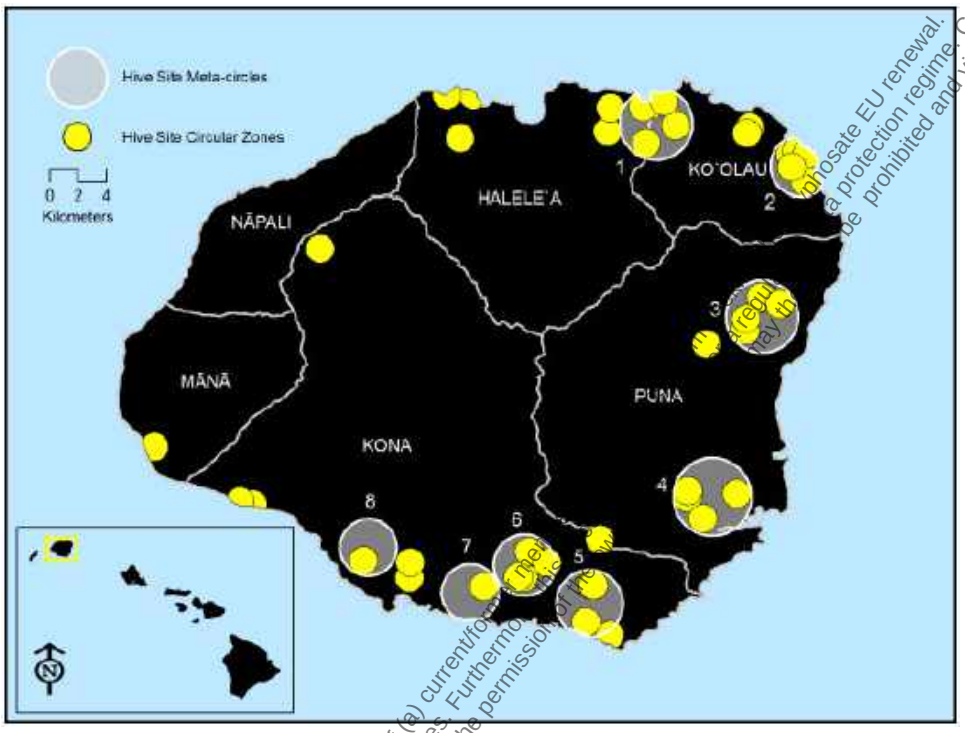
ELISA analysis was performed at each laboratory using the Abraxis method [1]. Abraxis test kit (cat. #500086) and microtiter equipment were used. The sample preparation method for honey followed published procedures [1, 17] (S1 Appendix). Samples were processed and read with a microplate reader Model 4303 [18] from Abraxis Inc. and analyzed using Molecular Devices Soft max pro evaluation program (4-Parameter). Results from Surfrider laboratory analysis were certified correct by Abraxis staff. Limit of quantitation (LOQ) was 15 ng/mL (15 ppb). Samples are stated as having detectable levels of glyphosate only if they are > LOQ.

Abraxis' ELISA methods for analysis of glyphosate have been compared to standard liquid chromatography and tandem mass spectrometry methods but not for honey. Therefore, 14 samples from Batch #2 were analyzed by both methods for validation. The results closely correlated with $R^2 = 0.99$ (S2 Appendix). Only ELISA derived data were used in this study.

Geospatial analysis

Presence and concentration of glyphosate residues were geospatially mapped with respect to general geography of the island and land use. Ancient Hawaiian biogeographical and management land divisions (Moku) (**Figure 1**) [19] were identified using the Google Earth Pro™ (GEP).

Figure 1: Distribution of 1 km radius circular zones (yellow) around hives on island of Kaua'i. Meta-circles of grouped circular zones are shaded in grey and numbered (N = 8). Moku divisions are indicated by white lines and each Moku is named.



Circular zones

Bees have been reported to forage as far as 9.5 Km from the hive [20,21] with a mean distance closer to 1 km at times subject to patchiness of flowering resources [21]. Depending upon resource availability, the probability of plant visitation decreased non-linearly from the hive and > 85 % probability of visitation was at less than 1 km [22]. Beekeepers note that bees forage as close to the hive as possible [23], especially on Kaua'i where naturally occurring plants and crops bloom year-round. Foraging on Kauai may also be constrained by discrete watersheds, bounded by mountainous ridges.

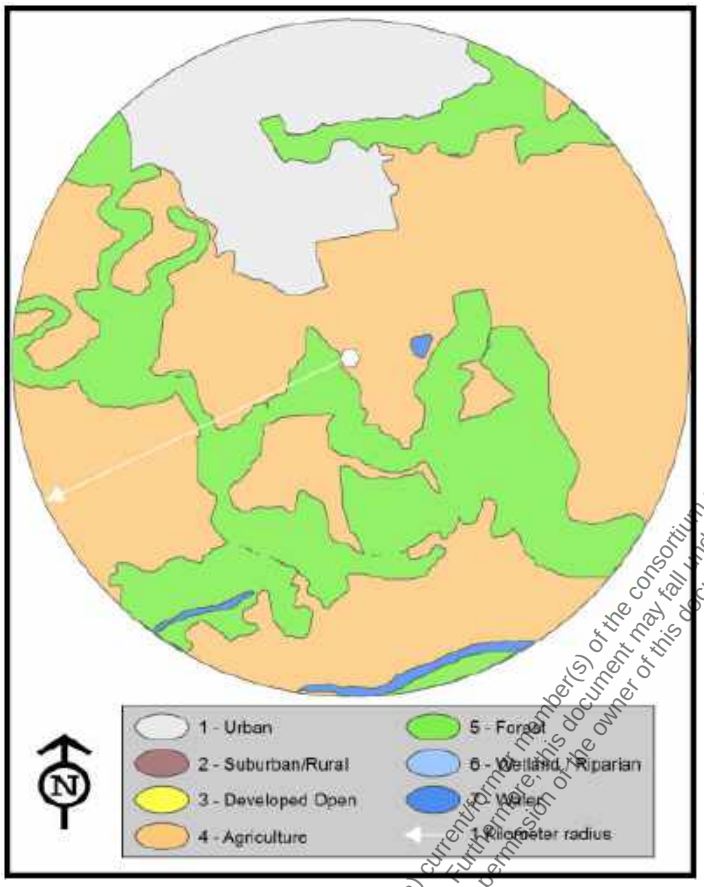
Based on this information, and to avoid overlapping of foraging sites, a 1 km radius was used to define the bees' foraging zone around each hive. Geospatial information analysis was applied using the GEP program with Digital Globe™ (DG) images to delineate circular zones 1 km in radius around each hive (**Figure 1**).

The land area within each circular zone was further sub-divided into discrete polygons, based upon land cover designations derived from NOAA C-CAP twenty-one classifications [24] (**Table 2**). Habitat codes were reclassified to seven land use categories.

Individual polygons were delineated in GEP using an optical mouse and area covered was calculated. The land area of each habitat type was then summed to provide a measure of the total land area (m^2) in each land use polygon (**Figure 2**). Each circular zone comprised 314.16 hectares, unless ocean area was excluded. A total of 18,872 hectares of land area were processed using the latest GEP images (years 2013–2014) and knowledge of current land use. Visual ground truthing was performed on sites known to

differ from GEP images.

Figure 2: Circular zone around a central hive, drawn with 1 Km radius. Polygons represent different land uses categories. Site #16 provided as an illustration.



The percent of the current land use was calculated for each habitat type represented by the polygons within the hive sites' circular zones. These percentages were then correlated with the concentration of glyphosate residue from the hive in the circular zone. One hive (#48, Mānā Moku) was excluded from polygon land use calculations, as it had been moved among sites within the Moku.

A second independent geospatial analytical method for land use categorisation used the NOAA Coastal–Change Analysis Program (C-CAP) [24] and ArcGIS Version 10.5 [25] (S3 Appendix). It derived area (m^2) within the 1 km radius circular zones using a program that automatically identified different types of ground cover (**Table 2**). A comparison of the two methods for accuracy in determining current land use patterns showed GEP preferable, so it was used in this study (S3 Appendix).

Table 2: Land use NOAA C-CAP classification descriptions.

This Study	Land use category	C-CAP	Land use classifications	Description of ground cover
		1	Unclassified	
1	Urban	2	High Developed	heavily built-up urban centers as well as large constructed surfaces in suburban and rural areas. Large buildings
		3	Medium Developed	constructed surface mixed with substantial amounts of vegetated surface. Small buildings.
2	Suburban/Rural	4	Low Developed	class 3, with the addition of streets and roads with associated trees and grasses
3	Developed Open	5	Developed Open	parks, lawns, athletic fields, golf courses, and natural grasses occurring around airports and industrial sites
4	Agriculture	6	Orchard	herbaceous (cropland) and woody (e.g., orchards, nurseries, and vineyards) cultivated lands
		7	Pasture land	grasses, legumes or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops
		8	Grassland	Grassland: grasses and non-grasses (forbs) that are not fertilized, cut, tilled, or planted regularly
		20	Bare land	bare soil, rock, sand, silt, gravel, or other earthen material with little or no vegetation
5	Forest	9	Deciduous forest	Deciduous Forest areas dominated by single stemmed, woody vegetation
		10	Evergreen forest	67 percent of the trees remain green throughout the year. Both coniferous and broad-leaved
		11	Mixed Forest	areas in which both evergreen and deciduous species are growing and neither predominate
		12	Scrub/shrubs	woody vegetation: true shrubs,young trees and trees or shrubs that are small
6	Wetland/ Riparian	13	Palustrine Forested Wetland	non-tidal wetlands dominated by woody vegetation >5m
		14	Palustrine Scrub/Shrub Wetland	non-tidal wetlands dominated by woody vegetation less than or equal to 5 meters
		15	Palustrine Emergent Wetland	non-tidal wetlands dominated by persistent emergents, emergent mosses, or lichens
		16	Estuarine Forest Wetland	tidal wetlands dominated by woody vegetation >5m, salinity >0.5ppt
		17	Estuarine Scrub/Shrub Wetland	tidal wetlands dominated by woody vegetation <5m, salinity >0.5ppt
		18	Estuarine Emergent Wetland	erect, rooted, herbaceous hydrophytes. Perennial plants usually dominate these wetlands
7	Water	19	Unconsolidated Shore	substrates lacking vegetation: beaches, bars, and flats
		21	Open water	open water with less than 25 percent cover of vegetation or soil.

Meta-circles

Analysis was done to determine if non-glyphosate using areas (e.g. containing forest, water, organic farms and residential) could be differentiated from areas of higher glyphosate use, as determined by conversations with the beekeepers. Eight meta-circles were made, comprising multiple 1-km circular zones that were grouped as having the same general land use description (**Table 2**, **Figure 1**) and situated in grouped watersheds. These meta-circles were encircled within a computer-generated circumference (mean 1707 hectares) that fully contained 3 to 9 circular zones of the same land-use practices (ranging from 1256 to 2365 hectares). In total, 41 samples were included within these eight meta-circles.

Large-scale divisions (East-West side of island, Moku)

The island of Kauai is divided by mountainous ranges and orographic rainfall in to two different biogeographical zones [16]. The drier leeward west-side of Kaua'i comprises the Moku of Kona, Nāpali, and Mānā for approximately 73,710 hectares, 51.3 % of the island's area, while the wetter windward east-side comprises the Moku of Puna, Ko'olau, and Halele'a for approximately 70,049 hectares, 48.7 % of the island's area. Moku are identified by geological and biogeographic features [19] (**Figure 1**).

Statistical analysis

Data was analyzed with Microsoft Excel and Access (means, medians, S.D., S.E.M, t-tests, linear and exponential line fits). Analyse-it, a plug-in for Excel, was used for correlations and AICc line fits. TIBCO Spotfire Analyst® was used to produce the Trellis plots and non-parametric Kruskal-Wallis analysis.

Results

Island-wide

ELISA measured glyphosate concentrations in honey taken directly from the hive ranged from < LOQ to 342 ppb (**Table 3**). Sixteen (27.1 %) of 59 samples had glyphosate concentrations detected over the ELISA limit of quantitation (LOQ = 15 ppb).

Calculations of mean concentrations were done in two manners: using all sample ELISA data (N = 59, mean = 33.5 ppb, standard error of the mean, S.E.M. = 9.3) or for only those samples with ELISA values greater than the LOQ (N = 16, mean = 118.3, S.E.M. = 24.0).

Spatial and temporal variations at hive sites

Six separate sites had samples taken from multiple unique hives on those sites. At two of these six sites (Samples # 52, 53; 54, 56, 58), all hives had no glyphosate detected. At three of these six sites (Samples # 18, 59; 8, 14, 20, 21; 34, 35, 36), all hives had glyphosate > LOQ. At one site (Samples # 55, 57), only one hive had detectable glyphosate (Sample # 57) (27 ppb), while the other hive had none detected.

An extremely large feral beehive sampled in 2013 had 92 ppb glyphosate (**Table 3**, Sample # 37). In 2015, this site had four samples taken from widely spaced parts of the hive (Samples 8, 14, 20 & 21). Analysis yielded values ranging from 33 ppb to 342 ppb (mean = 147.7 ppb, S.E.M. = 69.7 ppb).

Two different sites were sampled in 2015 and again in 2016. Each of these two sites had multiple hives. Both sites showed an increase in concentration levels over time (0 ppb to 27 ppb for samples 55 & 57; 25 ppb to 95 ppb for Samples 18 & 59).

Of the store-bought samples (**Table 4** and Table A in S4 Appendix), 33.3 % of those from Kaua‘i had glyphosate residue > LOQ (mean = 41 ppb, S.E.M. = 14.2)

East-West side of island

Presence and concentration of glyphosate residues were mapped with respect to ancient Hawaiian biogeographical and management land divisions (Moku) [19]. When all 59 samples were analyzed, there was a higher glyphosate concentration (mean = 61.6 ppb, N = 31, S.E.M. = 16.2) (**Table 5** and Tables B and C in S4 Appendix) in honey from the leeward western half of Kaua‘I versus the windward eastern half (mean = 2.4 ppb, N = 28, S.E.M. = 0.9). Mean values between the western and eastern sides are different (t-test, p = 0.001, df = 57) (Table D in S4 Appendix).

If only glyphosate values > LOQ are used (N = 16), the western Moku had 15 samples, 48.4 % of which had glyphosate > LOQ (mean = 125.1 ppb). The eastern Moku had only 1 sample over the LOQ (3.6 %). This sample value (15.2 ppb) is just greater than the LOQ.

A Trellis plot was made showing the glyphosate concentration across samples, grouped by side of island and by Moku. When all 59 samples are plotted, there is a clear pattern of the higher glyphosate concentrations in the western Moku vs the eastern Moku (Figure 3). No samples were collected from the remote western Moku of Napali.

Moku

Moku differed greatly in the and mean concentration of glyphosate in honey (Table B in S4 Appendix). Puna and Ko’olau Moku had no samples >LOQ and Halele’a had only one > LOQ. No samples were collected from remote Napali and only one sample from Mana. Concentrations from the west side Kona Moku were different from the three east-side Moku ($p < 0.003$) (Table E in S4 Appendix).

Since it is not known if these samples are from a normally distributed population, a non-parametric Kruskal-Wallis test was performed. This test confirmed the above parametric tests that glyphosate distributions were different depending upon the side of the island and the Moku ($p = 0.0008$ and 0.004, respectively) (Table F in S4 Appendix).

Source location of honey purchased from merchants on Kaua‘i was obtained from the label and discussions with vendors. Percentage of samples with glyphosate residue > LOQ and mean concentrations of glyphosate differed among Moku sampled (**Table 6** and Table A in S4 Appendix). Area with the greatest percentage of samples with glyphosate was in the agricultural district of Kona on the west side of the island. This is the same trend seen as with the hive samples (**Figure 3**).

Table 3: Glyphosate concentration and percent of land use (by category) within the circular zones surrounding the hives.

Google Earth Polygon Land Use Classification								[Glyphosate]
Sample #	% Urban	% Suburbs	% Open	% Ag	% Forest	% Wetland	% Water	ppb
1	71.4%	1.1%	6.6%	3.1%	0.0%	17.6%	0.2%	< LOQ
2	0.0%	30.1%	0.0%	0.0%	67.6%	2.4%	0.0%	
3	0.0%	13.5%	0.0%	70.9%	15.5%	0.0%	0.0%	
4	31.1%	0.0%	9.0%	30.0%	29.7%	0.0%	0.3%	
5	22.6%	0.0%	13.3%	21.0%	42.8%	0.0%	0.3%	< LOQ
6	19.8%	0.0%	3.3%	76.9%	0.0%	0.0%	0.0%	49
7	0.0%	10.4%	66.5%	3.2%	19.8%	0.0%	0.0%	
8	5.5%	1.8%	0.2%	90.5%	0.0%	0.0%	1.9%	61
9	0.0%	46.6%	23.1%	1.1%	29.2%	0.1%	0.0%	
10	0.0%	6.5%	87.5%	4.4%	0.0%	0.0%	1.6%	< LOQ
11	0.0%	0.0%	4.2%	69.7%	26.1%	0.0%	0.0%	
12	0.0%	0.0%	48.2%	19.8%	32.0%	0.0%	0.0%	15
13	0.0%	30.9%	26.6%	8.6%	34.0%	0.0%	0.0%	
14	5.5%	1.8%	0.2%	90.5%	0.0%	0.0%	1.9%	342
15	15.58%	13.8%	1.4%	43.6%	23.1%	0.0%	2.6%	
16	15.3%	0.0%	0.0%	53.8%	30.1%	0.0%	0.9%	
17	0.0%	4.0%	1.8%	0.0%	94.2%	0.0%	0.0%	
18	25.4%	0.0%	74.1%	0.1%	0.0%	0.0%	0.3%	25
19	52.9%	0.0%	44.6%	2.4%	0.0%	0.0%	0.0%	< LOQ
20	5.5%	1.8%	0.2%	90.5%	0.0%	0.0%	1.9%	155
21	5.5%	1.8%	0.2%	90.5%	0.0%	0.0%	1.9%	33
22	0.0%	45.8%	3.0%	33.9%	13.3%	0.0%	4.1%	
23	0.0%	50.2%	14.8%	0.4%	34.6%	0.0%	0.0%	
24	0.0%	1.5%	11.2%	64.3%	22.9%	0.0%	0.0%	
25	6.8%	10.1%	57.1%	2.3%	23.4%	0.0%	0.3%	
26	0.0%	1.0%	6.7%	68.6%	23.7%	0.0%	0.0%	
27	18.9%	0.0%	50.5%	0.0%	29.4%	0.0%	1.2%	
28	0.0%	0.0%	47.5%	11.5%	41.0%	0.0%	0.0%	< LOQ
29	0.0%	14.4%	0.5%	75.0%	10.1%	0.0%	0.0%	
30	0.0%	7.0%	30.2%	7.7%	54.1%	0.0%	0.0%	
31	0.0%	30.1%	0.0%	0.0%	67.6%	2.4%	0.0%	
32	0.0%	30.2%	8.3%	1.8%	57.7%	0.0%	2.0%	
33	22.2%	5.4%	61.4%	3.5%	7.8%	3.2%	0.0%	
34	0.0%	11.9%	1.5%	71.9%	7.4%	7.1%	0.2%	187
35	0.0%	11.9%	1.5%	71.9%	7.4%	7.1%	0.2%	178
36	0.0%	11.9%	1.5%	71.9%	7.4%	7.1%	0.2%	172
37	5.5%	1.8%	0.2%	90.5%	0.0%	0.0%	1.9%	92
38	36.2%	0.0%	0.0%	61.0%	0.0%	0.0%	0.0%	78
39	20.7%	4.2%	44.3%	3.5%	27.4%	0.0%	0.0%	
40	0.0%	49.0%	0.0%	12.9%	38.1%	0.0%	0.0%	< LOQ
41	17.1%	0.3%	0.0%	58.9%	23.8%	0.0%	0.0%	60
42	0.0%	0.0%	1.4%	67.9%	30.7%	0.0%	0.0%	
43	0.9%	1.0%	81.8%	4.7%	11.4%	0.0%	0.0%	
44	0.0%	27.2%	0.0%	25.3%	44.2%	0.0%	2.3%	< LOQ
45	0.0%	0.0%	0.0%	0.0%	95.5%	0.0%	1.3%	
46	6.8%	56.2%	0.0%	0.0%	37.0%	0.0%	0.0%	< LOQ
47	0.0%	0.0%	0.0%	19.5%	80.5%	0.0%	0.0%	
48	19.7%	0.0%	10.2%	16.3%	50.2%	0.0%	3.6%	292
49	20.0%	49.6%	0.0%	0.0%	29.0%	0.0%	1.3%	
50	0.0%	1.5%	0.0%	50.4%	48.1%	0.0%	0.0%	
51	0.0%	16.4%	0.0%	0.0%	83.6%	0.0%	0.0%	
52	19.0%	4.2%	2.2%	51.6%	21.4%	0.0%	1.6%	
53	19.0%	4.2%	2.2%	51.6%	21.4%	0.0%	1.6%	
54	19.0%	4.0%	2.2%	47.3%	24.1%	0.0%	3.4%	
55	18.0%	4.2%	2.2%	42.6%	31.2%	0.0%	1.8%	
56	19.0%	4.0%	2.2%	47.3%	24.1%	0.0%	3.4%	
57	15.6%	13.8%	1.4%	43.6%	23.1%	0.0%	2.6%	27
58	19.0%	4.0%	2.2%	47.3%	24.1%	0.0%	3.4%	
59	25.4%	0.0%	74.1%	0.2%	0.0%	0.0%	0.3%	95

Table 4: Concentration and percentage of glyphosate detected in store-bought honey. Samples originated from three Hawaiian islands and international blends. Samples categorised as Organic or Non-Organic.

Category			Samples N	> LOQ %	> LOQ Mean ppb
Location					
	Hawaii	Island:			
		Kaua'i	15	33.3	41.0
		Hawai'i	4	25.0	16.4
		Molokai	2	0	NA
	International		5	40.0	51.5
Type					
	Organic		5	20.0	30.0
	Non-Organic		21	33.3	41.0

Table 5: Glyphosate concentration by side of island and the six Moku. All 59 sample values used. Napali Moku had no samples ("ns").

Moku	Glyphosate Mean ppb	Median	S.D.	S.E.M.	Count
Windward:					
Koolau	0	0	0	0	10
Puna	2.5	0	5.1	1.7	9
Haleiea	4.1	0	6.3	2.1	9
Totals	2.41	0	4.8	0.92	28
Leeward:					
Kona	53.9	11.7	80.9	14.8	30
Mana	292.2	292.2	na	na	1
Napali	ns	na	na	na	ns
Totals	61.61	13	90.3	16.2	31

Figure 3: Glyphosate concentrations across samples by side of island and within each Moku. Mean glyphosate (ppb) is shown by the horizontal line for each Moku. Side of the island and Moku names are listed at the top of the plot. Samples from the western Moku are shown as orange triangles and eastern Moku as blue circles.

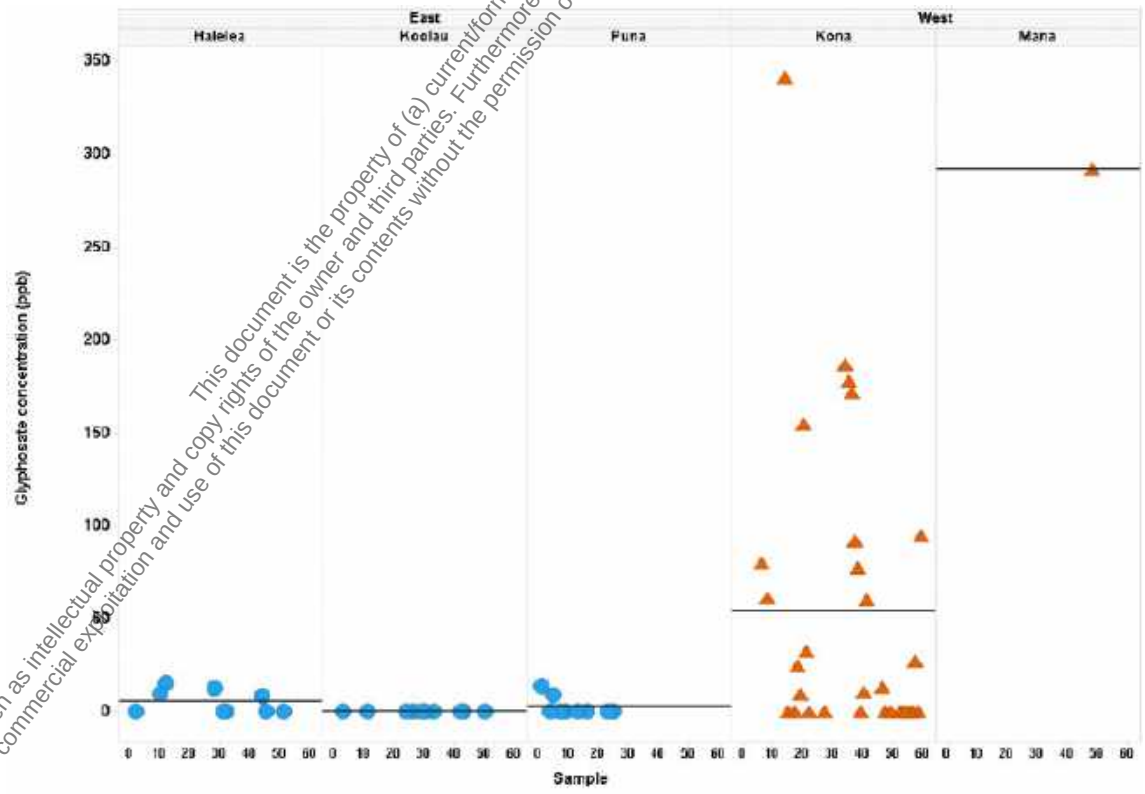


Table 6: Prevalence and concentration of glyphosate in Kauai honey from store-bought samples.

Moku	All samples N	> LOQ N	> LOQ % total	> LOQ mean	> LOQ SEM
Puna	6	1	16.7	15.0	na
Koolau	5	1	20.0	61.8	na
Kona	4	3	75.0	43.1	22.2

Circular zones and land use polygons

Land use within an area of 1 Km radius around each of the hives was determined using Google Earth Pro™ (GEP) (N = 59 hives from Kaua'i). These circular zones were divided into single land use polygons and the total meter^2 coverage for each of the seven land types was calculated. The percent of the total allocated to each of the seven land use types of each site was summarised with the glyphosate concentration found in the samples from that site (**Table 3**).

AICc analysis was performed to determine correlations between presence of glyphosate in honey and various land uses. Non-zero glyphosate data (N = 23) was used for these analyses. The exponential model for land use and glyphosate was chosen, as it has the highest correlation and strongest AICc values, compared with other line fits (Table G in S4 Appendix). Agriculture land use in the immediate 1 km radius vicinity of the hive showed the highest positive correlation with glyphosate concentration (**Table 7**, $R^2 = 0.594$) and the strongest AICc compared with the other land use categories. Open, Suburbs, Urban, and Forest land use all showed weak negative correlations (negative Parameter Estimates) between land use and glyphosate concentration. Wetland and Water land use showed very weak positive correlations. The negative correlations (e.g. Forest) is due to these land use types not being independent variables; rather, they are multicollinear (Figure A in S4 Appendix).

Concentration of glyphosate in honey was plotted versus the percent land use in agriculture. Samples with non-zero glyphosate were used (N = 23). **Figure 4** shows that the higher glyphosate concentrations are correlated with sites that have high percent agriculture land use (> 60 % agriculture).

The hives in the western Moku (orange triangles) have a strong correlation with higher glyphosate when there is higher percent land use as agriculture. Hives in the eastern Moku (blue circles) had very low glyphosate, even with 60 % to 80 % of the area in agriculture (**Table 3**).

Table 7: Correlation of glyphosate concentration (ppb) in honey samples and the percent land use.

Land Use	R^2	AICc	SE of fit (RMSE)	Parameter estimate	95% CI	95% CI	SE	p-value	Exponential Equation
Agriculture	0.594	-8.664	0.782	2.552	1.594	3.511	0.461	0.000	$Y = 12.58 * 12.84^X$
Forest	0.326	2.967	2.010	-3.977	-6.572	-1.383	1.247	0.004	$Y = 65.24 * 0.01874^X$
Open	0.123	9.030	1.922	-1.465	-3.242	0.311	0.854	0.101	$Y = 50.03 * 0.231^X$
Suburbs	0.086	9.973	2.123	-2.276	-5.638	1.087	1.617	0.174	$Y = 46.98 * 0.1027^X$
Urban	0.049	10.897	2.200	-1.422	-4.274	1.430	1.371	0.311	$Y = 47.01 * 0.2412^X$
Wetlands	0.017	11.668	1.220	3.659	-9.110	16.427	6.140	0.558	$Y = 36.23 * 38.8^X$
Water	0.011	11.854	1.224	13.180	-44.017	70.377	27.504	0.637	$Y = 34.83 * 5.296e+05^X$

Meta-circles

In order to expand land use to watersheds or larger areas, meta-circle analysis was done on eight clusters of circular zones situated all around the island (**Figure 1**). They comprise similar environments. Discussions with beekeepers were used to develop a general description of each meta-circle (**Table 8**) as to predominant land use and glyphosate use.

The percent of each of the seven types of land use was calculated for each circular zone in each meta-circle (**Table 3** and Table H in S4 Appendix). Then the mean percent of each type of land use was calculated for each meta-circle. The highest percent land use was used to describe the meta-circle, if that land use type was at least 70 %. If it was less than 70 %, then a composite was used; the second highest type of land use was added to the highest land use type. This process was repeated until the composite land use designation comprised at least 70 % of the meta-circle. This composite description is shown in **Table 8**, in the column "Composite land use type".

The mean concentration of glyphosate in honey was calculated using all samples within each meta-circle (N = 48 samples total). The percentage of samples which had glyphosate > LOQ was also calculated (N = 16 total). Only three meta-circles had significant glyphosate residues and all were in areas on the western side of Kaua'i. The two meta-circles with the most glyphosate, Ag. 1 and Ag. 2, were in areas of large scale agriculture use. The Koloa meta-circle had some agricultural use and contained the circular zones with large amounts of golf courses and or highway present, as discussed below.

A Trellis plot was made to show glyphosate concentration across samples, grouped by meta-circle (**Figure 5**). Within each meta-circle, samples are plotted versus the percentage of agriculture for that sample. There is a clear pattern of the higher glyphosate concentrations for samples in the western meta-circles (orange) vs samples in the eastern meta-circles (blue). The samples with glyphosate > LOQ (triangles) are also all in the western meta-circles, while the eastern meta-circles all have glyphosate < LOQ (circles) (**Figure 5**).

Figure 4: Glyphosate concentration versus the percent land use in agriculture (N = 23). Samples from the western Moku are shown as orange triangles and eastern Moku as blue circles. Exponential fit is $Y = 12.6\ e^{12.8X}$, $R^2 = 0.594$.

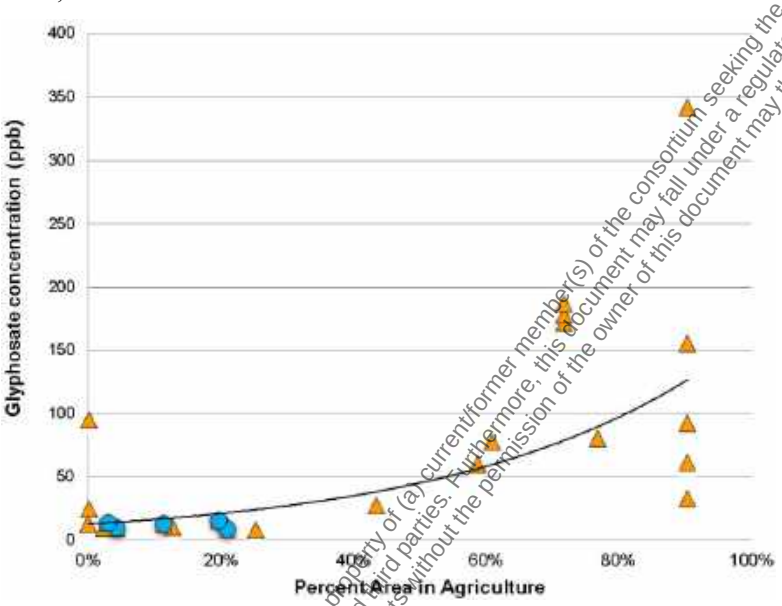
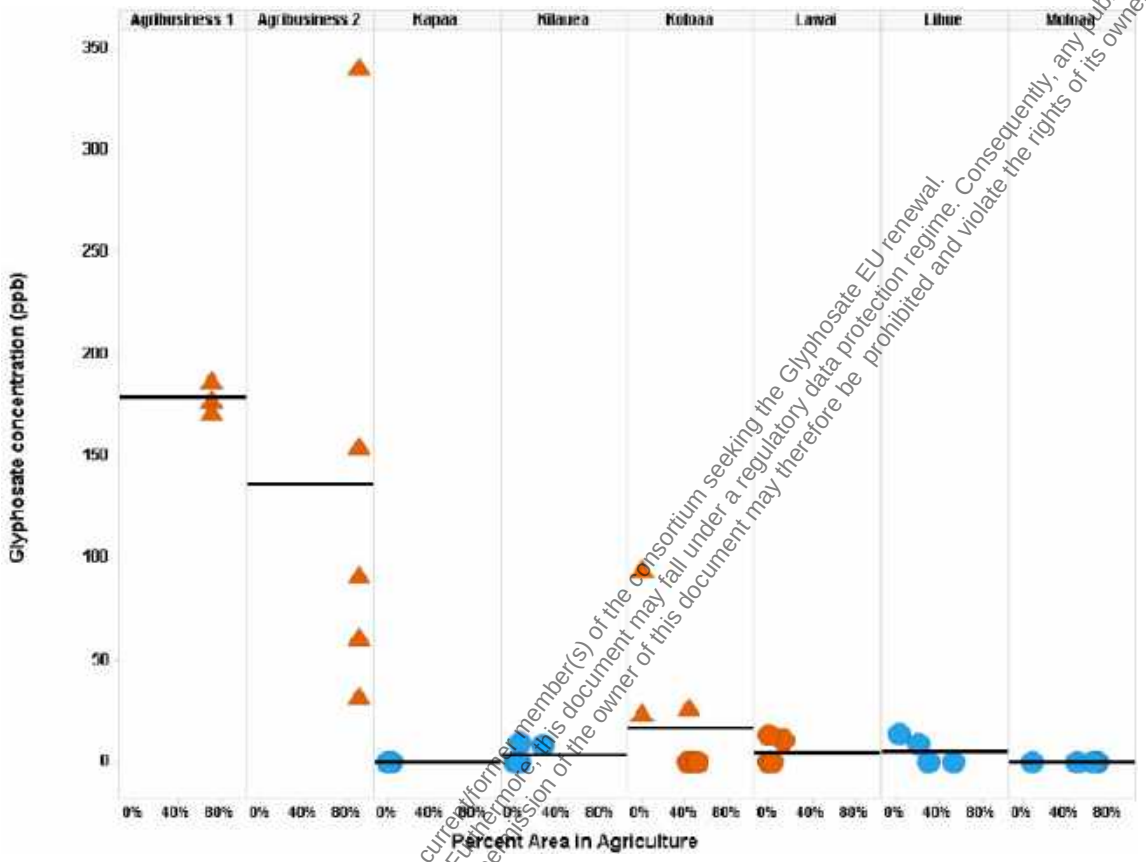


Table 8: Meta-circle composition, mean glyphosate concentration, and percent prevalence. Meta-circle # corresponds to Figure 1.

Meta-circle #	Meta-circle	Number of Circular Zones	General Description	Composite % land use	Composite land use type	Mean ppb	% > LOQ
1	Kilauea	5	Rural, Suburbs	72.0%	Open, Forest	< LOQ	0%
2	Moloa'a	6	Organic farming	89.2%	Agriculture, Forest	< LOQ	0%
3	Kapa'a	4	Suburbs	82.0%	Open, Forest, Suburbs	< LOQ	0%
4	Lihue	4	Urban, open, agriculture	87.7%	Urban, Agriculture, Forest	< LOQ	0%
5	Koloa	9	Suburbs, golf, resort	74.7%	Agriculture, urban, Forest	16.3	33%
6	Lawai	5	Suburbs	82.9%	Forest, Suburbs, Open	< LOQ	0%
7	Ag. 1	3	Large scale agriculture	71.9%	Agriculture	179.0	100%
8	Ag. 2	5	Large scale agriculture	90.5%	Agriculture	136.6	100%

Figure 5: Glyphosate concentrations across samples within each meta-circle. Mean glyphosate (ppb) is shown by the horizontal line for each meta-circle. Meta-circle names are listed at the top of the plot. Samples from the western Moku are shown as orange and eastern Moku as blue. Samples with glyphosate > LOQ are shown as triangles, while those < LOQ are as circles.



Golf courses and highways

A smaller specific land use, golf course, was identified from GEP images, but was subsumed in the "Developed Open" C-CAP category (**Table 2**). There were only eight circular zones which encompassed golf course(s) and all had glyphosate residues in honey (**Table 9A**). Percent area in golf course varied from 1.2 % to 16.2 %. Three of those samples (samples #34, 35, 36) were from different hives on the same farm and were also associated with high percent (> 70 %) agricultural land use. Two hives with the highest percent land use as golf course (samples # 18 and #59) were from the same residence with very low agricultural land use.

Major highways were identified as another small specific land use. These were subsumed under the Urban and Suburban/Rural categories (**Table 2**). Portions of highways were contained within 76 % of the circular zones (Table I in S4 Appendix). Those in the top 10 % of cumulative length of highway (> 4.6 km) had three samples with glyphosate > LOQ (25 to 95 ppb) (**Table 9B**). Frequent spraying of golf courses and highways may explain the presence of glyphosate (> LOQ) in samples # 18, 57, and 59.

Table 9: (A) 8 samples with highest % area Golf; (B) 6 samples with highest km highway present.

A				
Sample #	**Glyphosate ppb**	**% Ag**	**% Golf**	**Km Highway**
34	187	71.9%	1.2%	1.1
35	178	71.9%	1.2%	1.1
36	172	71.9%	1.2%	1.1
19	10	2.4%	1.6%	3.4
1	14	3.1%	4.8%	2.0
28	13	11.5%	13.7%	2.0
18	25	0.1%	16.2%	4.7
59	95	0.2%	16.2%	4.7
B				
55	0	42.6%	0.0%	4.6
57	27	43.6%	0.0%	4.6
59	95	0.2%	16.2%	4.7
18	25	0.1%	16.2%	4.7
52	0	51.6%	0.0%	4.7
53	0	51.6%	0.0%	4.7

Discussion

The presence of glyphosate residue in honey samples taken directly from the hive has been shown to correlate with areas that geospatial analysis has identified as comprised mainly of large-scale mono-crop agriculture. This suggests both a source and a pathway whereby pesticides migrate from site of use into other areas. Glyphosate residue >LOQ was found in 27.1 % of the hives and 33.3 % of store bought honey from Kauai, lower than the 59 % in store bought honey from around the world [1]. With hive-collected honey, geospatial analysis was able to further identify: which side of the island (west), which Moku (Kona and Mana), which areas (agriculture meta-circles), and most specifically which land use (agriculture) had the greatest prevalence and greatest concentration of glyphosate in honey.

Purchased samples from the other Hawaiian islands had lower mean concentrations and a smaller percentage contaminated than those from Kauai. The mean concentration of glyphosate from international samples purchased on Kauai was 51.5 ppb, similar to the 64 ppb in Rubio [1]. Samples from Brazil and a sample from a blend of USA and Argentina approximated values reported earlier, while the blend from Brazil, Mexico and Uruguay did not [1].

One of five Kauai purchased samples (20 %) labeled organic had glyphosate residues > LOQ (mean 30.6 ppb) compared to 45 % (mean 50 ppb) reported elsewhere [1]. This supports supposition of some migration of pesticides from areas of application to organic farms. The twenty-one Kauai samples not labeled as organic had both a higher occurrence (33.3 %) and higher mean concentration (42.0 ppb) of glyphosate than the organic labeled samples, suggesting application of glyphosate near the hives. Honey from traditional agriculture sites around the world had 62 % with glyphosate > LOQ and mean 66 ppb [1], expressing widespread use of glyphosate in agriculture.

The actual process of how Kauai bees obtained, carried and processed glyphosate is not known and was not addressed in this study, but is discussed elsewhere [13,14]. As honey was obtained directly from the hive using clean vials, this eliminated the possibility of contamination occurring during processing. Each sample was unique to a single hive, not blended from various sites. A survey of beekeepers confirmed that their hives did not get sprayed with glyphosate. Uptake could have occurred if the bees themselves got sprayed while foraging, if flowers frequented by the bees contained glyphosate from either direct spraying or aerial drift, or if water that the bees drank on plants or on the ground was contaminated in some way. In all cases, contamination could have occurred at a distance from the hive. Geospatial analysis mallowed the determination that within a 1 km radius of the hive, glyphosate contamination was most closely associated with large scale agriculture. The proximity of golf courses and highways were also associated, but to a lesser degree. General land use changes and landscape composition may have indirect detrimental effect on bee fitness, although the association between pesticide and landscape composition was not investigated.

The presence of both restricted use pesticides and glyphosate in bee pollen and honey, even at very low

levels, identifies an important pathway whereby pesticides migrate from site of application to the hive and into the human food supply [12–14, 26]. Geospatial analysis can help honey producers estimate spatial pesticide exposure risks inherent in intensive agriculture. When bees are used for commercial large-scale crop pollination, hive placement can be optimised so that the bee colonies are not seriously impacted by pesticides that the bees must endure while foraging [26–27]. Linking spatial and temporal dynamics of flowering crops, agri-environmental schemes, and pesticide applications would lead to better understanding of environmental risk assessment, management of pollination services, and protecting biodiversity [26–28].

Supporting information

Supporting information with is available online:

S1 Appendix. Abraxis technical bulletin.
https://doi.org/10.1371/journal.pone.0198876.s001

S2 Appendix. ELISA verification with mass spectrometry.
https://doi.org/10.1371/journal.pone.0198876.s002

S3 Appendix. Geospatial analytical method comparison.
https://doi.org/10.1371/journal.pone.0198876.s003

S4 Appendix. Glyphosate data from Kauai hives and store-bought honey.
https://doi.org/10.1371/journal.pone.0198876.s004

This information is summarised at the end of this document.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The publication provides residue levels for glyphosate in honey produced in Hawaii (majority of samples) but also Argentina, Brazil, Canada, Mexico, Uruguay and USA (mainland). It is considered relevant to the setting of a suitable MRL for glyphosate in honey since according to SANTE/11956/2016 rev. 9 it is possible to derive MRLs in honey based on monitoring data. As honey available to European consumers may originate from outside the EU, it is appropriate to consider honey residue data from outside the EU to derive the EU MRL.

The samples were analysed by means of an ELISA method which was validated indirectly by comparison with an LC-MS/MS method. A total of 14 honey samples were analysed with the two methods and the results were shown to be similar. The publication, however, does not provide validation data for the LC-MS/MS method (recovery rates from fortified samples).

The study showed a higher detection rate of glyphosate than in the EU-monitoring for 2016-2017. Besides the different origin of the samples, this may also be due to the use of different analytical methods with different LOQs. In line with the EU-monitoring the publication shows that glyphosate can occur in honey at levels > 0.05 mg/kg and that it is, therefore, appropriate to increase the existing EU-MRL. The highest measured residue level was 0.342 mg/kg, which is less than the maximum value found during the EU-monitoring for 2016-2017.

S1 Appendix. Abraxis Technical Bulletin

Glyphosate in Honey and Corn Syrup Sample Preparation

1. Intended Use

For the detection of Glyphosate in honey and corn syrup.

2. Sensitivity

0.015 ppm in matrix

3. Materials and Reagents Required

Analytical balance
Microcentrifuge tubes
4 mL glass vials with Teflon-lined caps
Disposable pipettes
Micropipettes with disposable plastic tips
Vortex mixer
Microcentrifuge
Timer
Plate shaker or Micro-well plate holder with insert retainer for vortex mixer
1 N Hydrochloric Acid (HCl)
Glyphosate sample diluent
Abraxis Glyphosate Plate ELISA Kit

4. Notes and Precautions

This procedure is intended for use with honey and corn syrup (light and dark). Other matrices should be thoroughly validated before use with this procedure.

Hydrochloric Acid must be handled with care. Wear appropriate protective clothing (gloves, glasses, etc.). Avoid contact with skin and mucous membranes. If contact occurs, wash with copious amounts of water and seek appropriate medical attention.

Due to the viscous nature of the prepared samples, the microtiter plate should be placed on a plate shaker or vortex mixer fitted with a micro-well plate holder adapter for the incubations with the antibody and conjugate solutions. This will allow for the appropriate mixing of all reagents in the microtiter wells.

5. Sample Preparation Procedure

5.1 Weigh 0.5 g of sample into an appropriately labeled microcentrifuge tube.

5.2 Add 0.5 mL of 1 N HCl. Vortex for 2 minutes.

5.3 Add 3.96 mL of Glyphosate Diluent to a clean, appropriately labeled 4 mL glass vial. Add 40 µL of the acid-treated sample (from step 5.2) to the Glyphosate Diluent in the vial (1:100 sample dilution). Vortex. This will then be analyzed as sample, see Derivatisation of Standards, Control, and Samples in the Reagent Preparation section of the Glyphosate Plate ELISA Kit user's guide.

6. Evaluation of Results

The Glyphosate concentration in the samples is determined by multiplying the ELISA results by a factor of 200.

Samples showing a concentration lower than standard 1 (0.075 ppb) should be reported as containing < 15 ppb of Glyphosate. Samples showing a higher concentration than standard 5 (4.0 ppb) can be reported as containing > 800 ppb of Glyphosate or diluted further and re-analyzed to obtain an accurate quantitative result.

7. Performance Data Recovery

Honey samples were spiked with various amounts of Glyphosate, prepared as described above, and then derivatised and assayed using the Glyphosate Plate Assay. Average recovery was 113 %.

Corn syrup samples (light and dark) were spiked with various amounts of Glyphosate, prepared as described above, and then derivatised and assayed using the Glyphosate Plate Assay. Average recovery was 104 %.

S2 Appendix. ELISA verification with mass spectrometry

To verify ELISA techniques for measuring glyphosate in a honey matrix (LOQ of 15 ng/g, 15 ppb) honey remaining in 14 vials from the Batch 2 samples analysed with ELISA were sent to Quality Services International GmbH (QSI), (Bremen, Germany) for analysis of herbicide residue by gas chromatography/mass spectrometry (GC-MS/MS) and/or liquid chromatography mass spectrometry (LC-MS/MS) methods (QSI method # 88505) with a LOQ of 0.01 mg/kg (10 ppb) (Table A). All concentrations derived from ELISA were used in analysis, however QSI did not report readings for levels < 10 ppb, so a value of zero was assigned for data analysis.

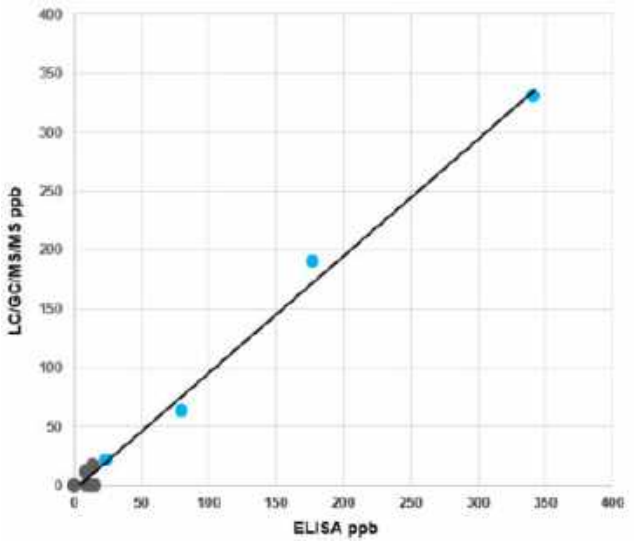
Table A: Glyphosate concentrations in honey matrix using either ELISA techniques or LC-GC-MS/MS techniques. Bold face numbers exceeded both techniques' LOQ and were plotted separately.

	Glyphosate ppb	Glyphosate ppb
Sample #	ELISA	QSI
1	13.6	17
5	8.8	10
6	**80.2**	**63**
7	0	0
10	9.2	0
12	15.2	0
14	**341.6**	**330**
16	0	0
18	**24.6**	**21**
19	9.6	12
25	0	0
27	0	0
28	12.6	0
35	**178.0**	**190**
LOQ=	15	10

Results for all 14 samples analysed by both methods correlated well (Figure A). Standard error of y for each x-value is 8.6 ppb. Only 4 samples had both the ELISA and LC/GC/MS/MS values over their respective LOQ, but the correlation coefficient remained high (Figure A).

Although sample size was small, a correlation coefficient $R^2 = 0.99$ supports the ELISA tests for accuracy, in addition to the use of blank and standards within each test run [1]. Comparison of ELISA techniques for monitoring glyphosate with chromatography-mass spectrometry have consistently found high correlations between the techniques in tests of various matrices, e.g. water [2,3], animal urine and animal tissues [4]. The use of Abraxis methods of ELISA determination of glyphosate in honey is well substantiated.

Figure A: Correlation of glyphosate concentration in honey split-samples using ELISA and LC-GC-MS/MS techniques. Linear fits: Y = 0.99 x – 3.1, R^2 = 0.993 (N = 14; black and blue circles); Y = 0.99 x + 6.1, R^2 = 0.994 (N = 4; blue circles).



S3 Appendix. Geospatial analytical method comparison

Development, application and comparison of two means for quantifying current land use practices within 1 km radius of a bee hive.

Geospatial analysis was performed in ArcGIS 10.5 on two separate Habitat datasets

1. Coastal Change Analysis Program (C-CAP) High Resolution Land Cover (1-4 meter resolution). Derived from high resolution imagery and analyzed according to the Coastal Change Analysis Program (C-CAP) protocol to determine land cover.

2. Vector polygons digitised in Google Earth Pro™ (GEP) using Digital Globe™ (DG) images (2013-2014) of 30-50 cm resolution as a base layer.

Coastal Change Analysis Program (C-CAP)

C-CAP analysis was conducted in January of 2016. Data downloaded was produced at a 1-4 meter resolution and utilised 35 full or partial WorldView2 multispectral scenes and the 2005 high-resolution Kauai C-CAP data set. The imagery and base classification were included in a multi-step semi-automated change detection process to extract land cover features in the 2010 imagery. Habitat within this dataset is classified into one of 21 different habitat classifications using a 2.5 meter cell size.

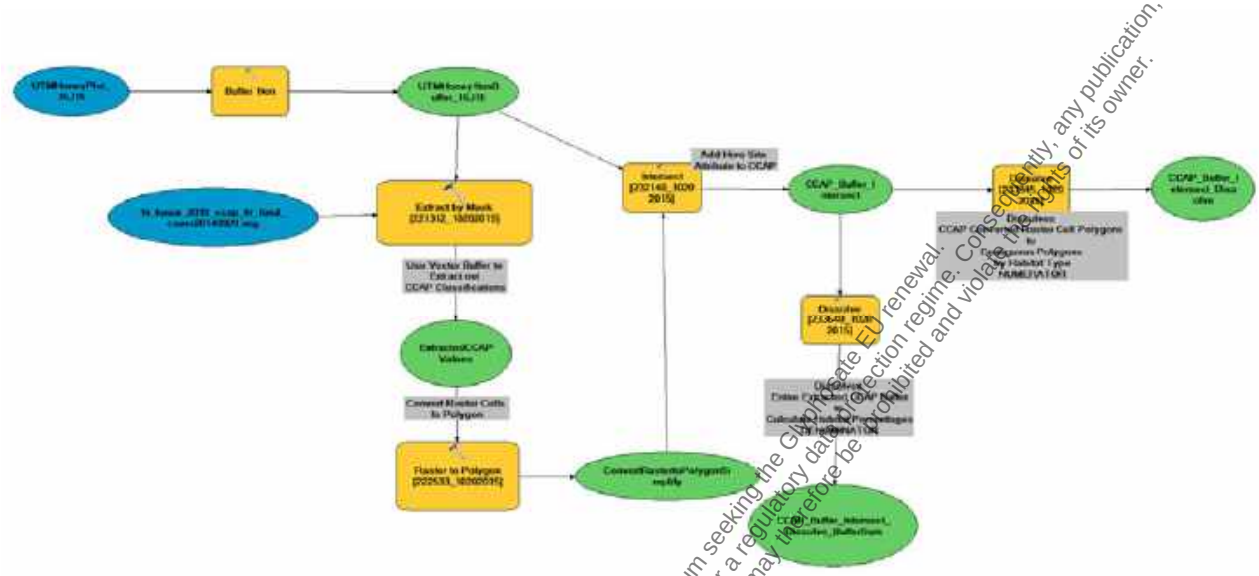
In order to extract out raster cells within the 2 kilometer boundary (1 Km radius) per hive site, the data set was masked using a vector dataset. This dataset was created by plotting each of the 38 hive sites in ArcGIS using their UTM location. Locations were converted into a point shapefile and then buffered by 1 km to create the 2 kilometer boundary polygon. Individual polygons were dissolved into one record to create the Mask to extract out pixels of the CCAP raster. Masking a raster using a vector is similar to the "Clip" geoprocessing routine done between two vector datasets. A vector representing an outline of the island was used to further mask the raster, removing pixels that were beyond the coastline, seemingly representing ocean (Figure A, B).

In order to quantify the percentage of habitat within each hive site boundary area (buffer a k.a. circular zone), the raster pixels were converted into a polygon feature class (vector) for vector geoprocessing. This polygon conversion resulted in 26,176 records/polygons, representing 26,176 cells within the original

Raster dataset residing in the hive site boundary area. The "Intersect" geoprocessing tool was used next to assign to each record the corresponding hive site number it fell within. Habitat codes were reclassified, reducing the number of habitats considered by the analysis to seven land use categories. These were used in identifying the candidate habitats bees are believed to be foraging. Using the "Dissolve" geoprocessing tool, the habitat polygons were dissolved by Hive Site and reclassified Habitat Code, and the results stored in a geodatabase so that the area for each habitat could be reported using the Shape Area field. Totals for the amount of habitat polygon cells residing within each hive site boundary were then summed and the percentage for each habitat within the boundary calculated.

Figure A: ArcGIS 10.5 geoprocessing tools: Clip, Dissolve and Intersect.

Figure B: Schematic of geoprocessing tools used to improve calculations of polygon areas for the C-CAP dataset.



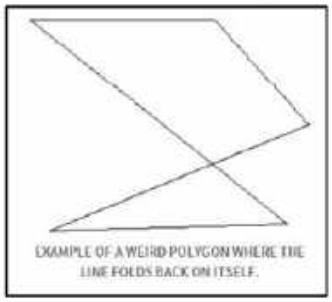
Google Earth Pro™ (GEP)

Digitizing in a Geographic Information System is the process of converting geographic data from a hard-copy or scanned image into a vector dataset by tracing features; features are captured in coordinates and stored as either a point, line or polygon vector dataset. For this analysis, "heads up digitizing" in GEP was used to create discrete habitat polygons based on the reclassified habitats in the C-CAP analysis. Polygons created in GEP were stored as a KML/KMZ file, imported into ArcGIS 10.5 and converted into a feature class residing in a geodatabase so that areas of each habitat polygon could be calculated in square meters.

Upon importing the polygons from Google Earth, numerous topological errors were discovered in the polygons themselves, the most pervasive being knots, loops and slivers. These occur when "…the digitizer has an unsteady hand and moves the cursor or puck in such a way that the line being digitised ends up with extra vertices and/or nodes". Knots and loops result when a line forming a boundary of a polygon folds back on itself, creating small polygon like geometry known as "weird polygons".

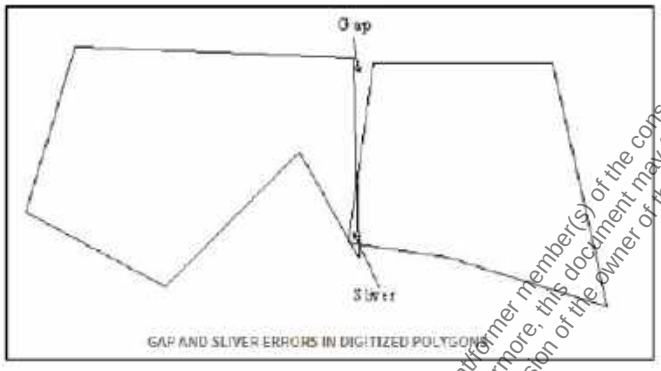
Polygon features are enclosed areas created from a series of vertices that are connected with a continuous line traveling in one direction whereby the starting and ending point are coincident (Fig C). Because the depiction of the polygon begins with a start point and travels in one direction, the resulting geometry of the polygon means the GIS can interpret what area is 'right' as opposed to 'left' of the boundary, as well as what area is enclosed by the boundary of the entire polygon; when a knot or loop occurs, the topology of the polygon actually becomes confounded due to the extra node between them. As a result, right and left sides of the boundary violates the topological relationship of the polygon itself, preventing performance of common geoprocessing tasks (clip, intersect and dissolve).

Figure C from https://www.gislounge.com/digitizing-errors-in-gis/



Another confounding topological error involves slivers. "Slivers are gaps in a digitised polygon layer where the adjoining polygons have gaps between them or where the two adjacent polygons overlap in error". This can inadvertently lead to areas among the polygons to have conflicting attributes as to what habitat the slivers represent (Fig D).

Figure D from https://www.gislounge.com/digitizing-errors-in-gis/



Manual digitizing habitat polygons is time consuming and tedious. For this analysis, and to reduce anticipated issues related to slivers, it was decided early on in the digitizing process that the largest habitat within a circular zone could be left un-transcribed and the void filled utilizing geoprocessing tools in ArcGIS. Unanticipated topological inconsistencies related to knots and loops however prevented these geoprocessing tools to be run, and thus required that topology of all individual polygons to be inspected and corrected.

"Topology in GIS is generally defined as the spatial relationships between adjacent or neighboring features" Planar topology requires that intersections for lines and polygons in a digital data layer is enforced and that no two lines or polygons cross. This process involves removing twisted or self-intersecting polygons (i.e. knots and loops) so as to ensure that the "inside" of the polygon is on the correct side of the boundary. It also includes removing overlaps (i.e. slivers) found by intersecting each polygon with all other polygons.

Tools from ET Geowizards 11.3 were used to correct planar topology, rigorously testing and correcting for topological correctness and verifying the spatial relationships between neighboring polygons. Eight circular zone sites were chosen to validate the hand-drawn polygon designations and to determine if the process would improve calculations of polygon areas. Overestimation of the initial polygons varied by only 2.5% (n=51, t-test no significant difference in paired data p=.875)

Once the topology of the GEP dataset was reconciled, the "Intersect" geoprocessing tool was used to fill voids and assign a habitat code. The dataset was then "clipped" using an "island" polygon to remove those portions of the circular zone that extended past the coastline. Since there were multiple polygons representing a given habitat within a circular zone, the "dissolve" tool was used to consolidate records so that percent habitat calculations could be completed for each circular zone.

Total area of each habitat type for each 1 km hive site circular zone was summed and the percentage calculated (Table 2 in text). Each circular zone comprised approximately 314.16 hectares, unless ocean surface area was removed. A total of 18,872 hectares of land area was classified for the vector polygon dataset. Visual ground truthing was performed to ensure images in the GEP imagery matched images on the ground.

Comparing results between the C-CAP and GEP Datasets

C-CAP high-resolution land cover for 2010, produced at 2.4 m resolution, was applied to the 38 sites from the 2013 and 2015 sampling and compared to the same data grouped and processed using GEP polygons. For Agriculture and Urban land-cover categories, the two methods produced similar mean values, were not significantly different (t-test), and were well correlated (Table A). For Forest, Open and Water land-cover, the mean values were significantly different.

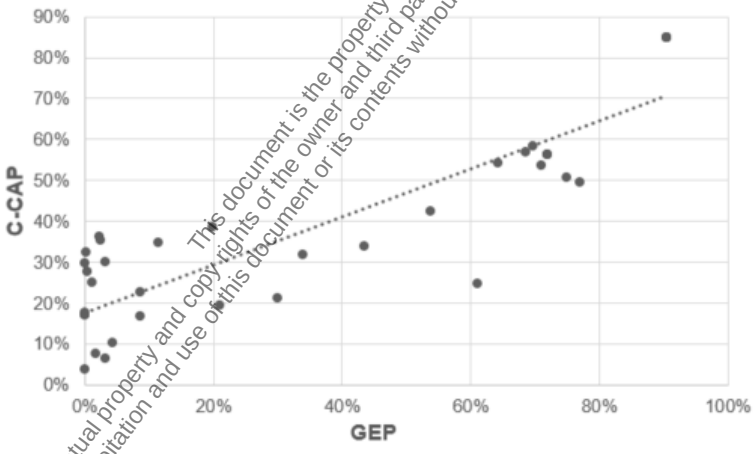
Table A: Glyphosate concentrations in honey matrix using either ELISA techniques or LC-GC-MS/MS techniques. Bold face numbers exceeded both techniques' LOQ and were plotted separately.

	C-CAP Mean	GEP Mean	t-test	correlation
% Ag	39.4%	36.9%	0.423	0.880
% Forest	36.4%	22.4%	0.000	0.836
% Open	12.4%	18.7%	0.078	0.584
% Urban	9.9%	9.6%	0.898	0.832
% Wetland	1.2%	1.2%	0.990	-0.034
% Water	1.0%	0.6%	0.041	0.533

The percentage coverage for Agriculture calculated with the C-CAP method was plotted versus the percentage coverage for Agriculture calculated with the GEP.

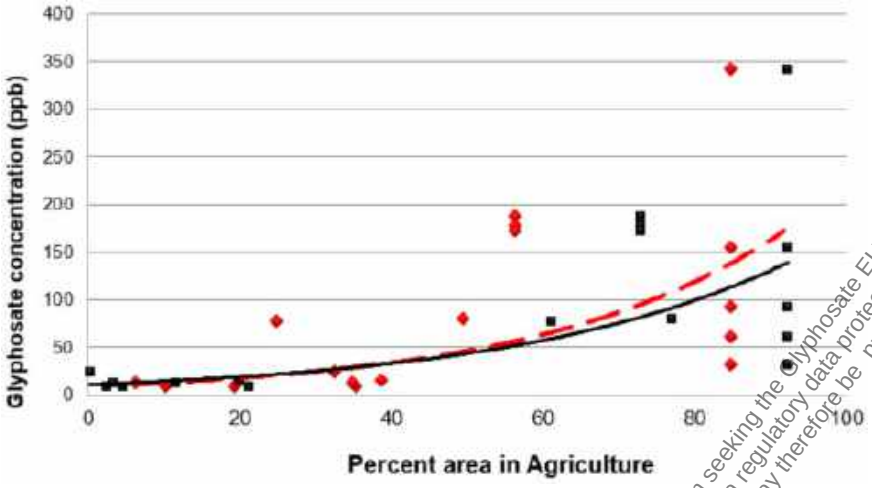
The plot illustrates the difference between GIS analyses of the two datasets and the general under-representation by C-CAP (Figure E).

Figure E: Correlation of % Agriculture in areas surrounding hive sites using C-CAP versus GEP analysis. Linear fit: $Y = 0.586 x + 0.177$, $R^2 = 0.775$.



When glyphosate concentrations are plotted against percent acreage in agriculture using the two methods (Fig F), the general trends as expressed by exponential curves are very similar, but the GEP polygon method produces a stronger correlation ($R^2 = 0.71$, AICc = -9.794).

Figure F: Correlation of % Agriculture and Glyphosate concentration surrounding hive sites using C-CAP and GEP analysis. Excel Analyse It software exponential fits produced $Y = 9.648\ e^{0\ 23121x}$, $R^2 = 0.48$, AICc = 0.173 (C-CAP, red diamonds; dash line) and $Y = 11.02\ e^{0\ 1628x}$, $R^2 = 0.71$, AICc = -9.794 (GEP Polygons, black squares; solid line).



There are many factors that would explain the differences in the land use designation and the choice of GEP polygons as the most accurate method for determining land use contemporary with honey production. These include:

- Cell size is 2.4 m for C-CAP vs Digital Globe has a 30-50 cm range. A smaller cell size allows for finer delineation and identification of objects.
- Date the image was accessed: 2010 for C-CAP but 2013-2014 for GEP with ground-truthing in areas in question.
- C-CAP would designate a ground cover as forest, but GEP showed it to be an orchard.
- C-CAP would identify open fields as "Open", but GEP showed that cattle are on it, so it is “Agriculture”.
- C-CAP does not recognize little streams or ponds but GEP resolution does.
- C-CAP sees "Forest" but Google Earth shows "Riparian"
- C-CAP see “Urban” but finer detail allows designation as “Rural/Suburban”

Conclusion

Although manually digitizing GEP polygon delineations is more tedious and time consuming, for the above stated reasons and the stronger correlation of the GEP derived curve, only the GEP polygon delineation method was used for final analysis of the relationship between land use and glyphosate concentration

S4 Appendix. Glyphosate data from Kauai hives and store-bought honey

Table A: Store-bought honey; sources and glyphosate concentration.

Sample Origin				**Sample #**	**Glyphosate ppb**
Hawaii	**Island:**	**Moku**	**Area**		
	Kauai	Kona	Waimea Valley	5	15.2
		Kona	Koloa	9	0
		Kona	Kalaheo	11	87
		Kona	Poipu	19	27.2
		Koolau	Waipake	3	5
		Koolau	North/Northeast Kauai	4	6.4
		Koolau	North Shore Kauai	6	60.8
		Koolau	Kilauea	8	0
		Koolau	Kilauea	12	11.2
		Puna	Puhi	1	15
		Puna	Hanamaulu	2	6.2
		Puna	Kapa'a	7	0
		Puna	Kapa'a	10	7
		Puna	Puhi	20	10.4
		Puna	Hanamaulu	21	6.4
	Hawaii Island		Hawaii Island	15	12
			Kealakekua, Big Island	16	7.4
			Kealakekua, Hawaii Island	60	16.4
			Big Island and Oahu	18	8
	Molokai		Molokai	61	0
			Molokai	62	0
Country		Product of Brazil and Canada		17	0
		Product of Brazil and Canada		22	30.6
		Product of Brazil and Canada		14	8.2
		Product of Mexico, Brazil and Uruguay		13	0
		Product of USA and Argentina		23	72.4

Table B: Kauai hive samples categorised by side of island and Moku with glyphosate concentration.

Side of island	**Moku**	**Sample #**	**Glyphosate ppb**	**Count**	**Median**	**Mean**	**SD**
WINDWARD							
	Halele'a	2	0				
		10	9.2				
		12	15.2				
		28	12.6				
		31	0				
		32	0				
		44	8.2				
		45	0				
		51	0	9	0	5.0	6.3
	Ko'olau	3	0				
		11	0				
		24	0				
		26	0				
		29	0				
		30	0				
		33	0				
		42	0				
		43	0				
		50	0	10	0	0	0

Side of island	Moku	Sample #	Glyphosate ppb	Count	Median	Mean	SD
	Puna	1	13.6				
		4	0				
		5	8.8				
		7	0				
		9	0				
		13	0				
		16	0				
		23	0				
		25	0	9	0	2.5	5.1
LEEWARD							
	Kona	6	80.2				
		8	61.4				
		14	341.6				
		15	0				
		17	0				
		18	24.6				
		19	9.6				
		20	155.2				
		21	32.6				
		22	0				
		27	0				
		34	187.2				
		35	178				
		36	171.8				
		37	92.2				
		38	77.6				
		39	0				
		40	10.4				
		41	60				
		46	13				
		47	0				
		49	0				
		52	0				
		53	0				
		54	0				
		55	0				
		56	0				
		57	27.4				
		58	0				
		59	95	30	11.7	53.9	80.9
	Mana	48	292.2	1	292.2	292.2	na
	Napali	None	None	None			

Table C: Summary statistics of glyphosate with Kauai hive samples categorised by side of island.

Windward	Count	28
	Median	0
	Mean	2.41
	SD	4.87
Leeward	Count	31
	Median	13
	Mean	61.61
	SD	90.34

Table D: t-test comparing glyphosate from Windward (Eastern) and Leeward (Western) sides of Kauai. Data from Table B1.

Windward-Leeward:	
t-test probability	0.001
degrees of freedom	57

Table E: t-test comparing glyphosate between Moku pairs. Mana Moku had only one sample, thus could not be compared.

Moku differences	**t-test p**
Kona -Koolau	0.001
Kona - Puna	0.002
Kona - Halelea	0.003
Koolau -Halelea	0.043
Puna -Koolau	0.180
Puna - Halelea	0.361

Table F: Kruskal-Wallis analysis of impact of side of island and Moku on glyphosate concentration.

Y (numerical)	**X (categories)**	**H-stat**	**DF**	**N**	**p-value**
Glyphosate	Side	11.3	1	58	0.00077
Glyphosate	Moku	13.3	3	58	0.0041

Table G: AICc analysis of fits for glyphosate concentration vs. % Agriculture.

	Exp.	**Power**	**Linear**	**Log**	**Polynomial**
R2	0.594	0.174	0.417	0.155	0.429
AICc	-8.664	7.662	194.88	195.232	197.055

Table H: Sample #'s included within Meta-circles and their glyphosate concentrations.

Meta-circle #	**Meta-circle Name**	**Sample #**	**Glyphosate ppb**	**Glyphosate ppb Mean**
1	Kilauea	10	9	
		32	0	
		33	0	
		43	0	
		44	8	3.5
2	Moloaa	11	0	
		24	0	
		26	0	
		30	0	
		42	0	
		50	0	0.0
3	Kapaa	7	0	
		9	0	
		23	0	

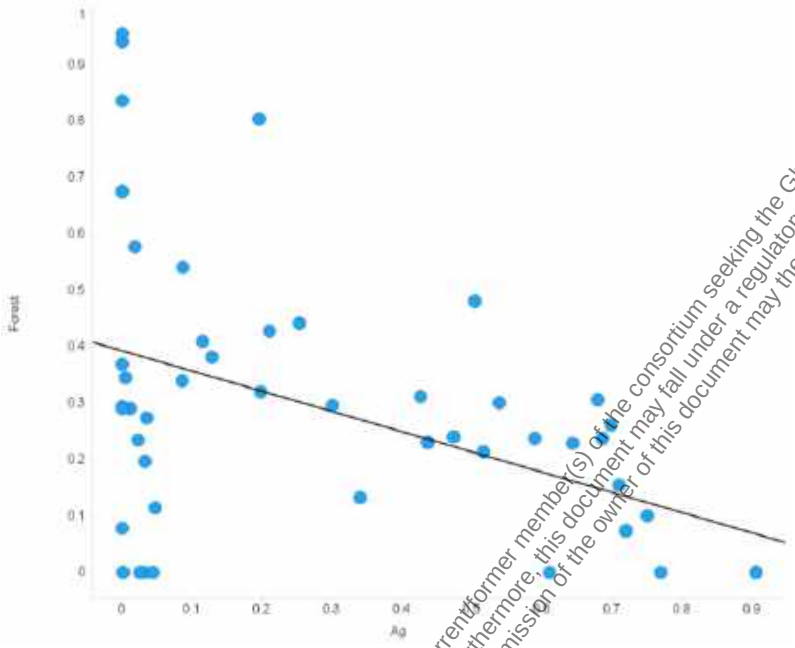
Meta-circle #	Meta-circle Name	Sample #	Glyphosate ppb	Glyphosate ppb Mean
		25	0	0.0
4	Lihue	1	14	
		4	0	
		5	9	
		16	0	5.6
5	Koloa	15	0	
		18	25	
		52	0	
		53	0	
		54	0	
		55	0	
		56	0	
		57	27	
		59	95	16.3
6	Lawai	27	0	
		39	0	
		40	10	
		46	13	
		49	0	4.7
7	Agribusiness 1	34	187	
		35	178	
		36	172	179.0
8	Agribusiness 2	8	61	
		14	342	
		20	155	
		21	33	
		37	92	136.6

Table I: Samples by Side and Moku with % Agriculture, % Golf, Hiway Km, and Glyphosate concentrations.

Sample #	Side	Moku	Glyphosate ppb	% Agriculture	% Golf	Hiway Km
1	East	Puna	13.6	3.1 %	4.8 %	2.00
2	East	Halelea	0	0.0 %	0.0 %	2.39
3	East	Koolau	0	70.9 %	0.0 %	1.59
4	East	Puna	0	30.0 %	0.0 %	2.02
5	East	Puna	8.8	21.0 %	0.0 %	1.74
6	West	Kona	80.2	76.9 %	0.0 %	2.03
7	East	Puna	0	3.2 %	0.0 %	0.00
8	West	Kona	61.4	90.5 %	0.0 %	1.65
9	East	Puna	0	1.1 %	0.0 %	0.00
10	East	Halelea	9.2	4.4 %	0.0 %	2.04
11	East	Koolau	0	69.7 %	0.0 %	0.00
12	East	Halelea	15.2	19.8 %	0.0 %	0.00
13	East	Puna	0	8.6 %	0.0 %	0.00
14	West	Kona	341.6	90.5 %	0.0 %	1.65
15	West	Kona	0	43.6 %	0.0 %	4.53
16	East	Puna	0	53.8 %	0.0 %	0.00
17	West	Kona	0	0.0 %	0.0 %	0.00
18	West	Kona	24.6	0.1 %	16.2 %	4.66
19	West	Kona	9.6	2.4 %	1.6 %	3.36
20	West	Kona	155.2	90.5 %	0.0 %	1.65
21	West	Kona	32.6	90.5 %	0.0 %	1.65
22	West	Kona	0	33.9 %	0.0 %	1.44
23	East	Puna	0	0.4 %	0.0 %	0.00
24	East	Koolau	0	64.3 %	0.0 %	1.66
25	East	Puna	0	2.3 %	0.0 %	0.00
26	East	Koolau	0	68.6 %	0.0 %	1.10
27	West	Kona	0	0.0 %	0.0 %	2.29
28	East	Halelea	12.6	11.5 %	13.7 %	2.04
29	East	Koolau	0	75.0 %	0.0 %	2.03
30	East	Koolau	0	8.7 %	0.0 %	2.06
31	East	Halelea	0	0.0 %	0.0 %	2.36
32	East	Halelea	0	1.8 %	0.0 %	2.63
33	East	Koolau	0	0.0 %	0.0 %	1.98
34	West	Kona	187.2	71.9 %	1.2 %	1.08
35	West	Kona	178	71.9 %	1.2 %	1.08
36	West	Kona	171.8	71.9 %	1.2 %	1.08
37	West	Kona	92.2	90.5 %	0.0 %	1.65
38	West	Kona	77.6	61.0 %	0.0 %	2.18
39	West	Kona	0	3.5 %	0.0 %	0.32
40	West	Kona	10.4	12.9 %	0.0 %	0.52
41	West	Kona	60	58.9 %	0.0 %	0.00
42	East	Koolau	0	67.9 %	0.0 %	0.00
43	East	Koolau	0	4.7 %	0.0 %	1.51
44	East	Halelea	8.2	25.3 %	0.0 %	0.00
45	East	Halelea	0	0.0 %	0.0 %	0.00
46	West	Kona	13	0.0 %	0.0 %	1.20
47	West	Kona	0	19.5 %	0.0 %	0.00
48	West	Mana	292.2	16.3 %	0.0 %	0.00
49	West	Kona	0	0.0 %	0.0 %	2.10
50	East	Koolau	0	50.4 %	0.0 %	2.26
51	East	Halelea	0	0.0 %	0.0 %	2.25
52	West	Kona	0	51.6 %	0.0 %	4.73
53	West	Kona	0	51.6 %	0.0 %	4.73
54	West	Kona	0	47.3 %	0.0 %	4.35
55	West	Kona	0	42.6 %	0.0 %	4.58

Sample #	Side	Moku	Glyphosate ppb	% Agriculture	% Golf	Hiway Km
56	West	Kona	0	47.3 %	0.0 %	4.35
57	West	Kona	27.4	43.6 %	0.0 %	4.60
58	West	Kona	0	47.3 %	0.0 %	4.35
59	West	Kona	95	0.2 %	16.2 %	4.66

Fig A: Multicollinearity amongst land use types. Samples are plotted with their % Forest vs % Agriculture. $Y = 0.39 - 0.36*X$, $R^2 = 0.23$



1. Information on the study

Data point	CA 6.10.1/007
Report author	Karise, R. *et al.*
Report year	2017
Report title	Are pesticide residues in honey related to oilseed rape treatments?
Document No.	DOI 10.1016/j.chemosphere.2017.09.013 E-ISSN 1879-1298
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicble
Acceptability/Reliability:	Yes/Reliable with restrictions

2. Full summary of the study according to OECD format

Executive summary

Pesticide treatments before and during the flowering of honey bee forage crops may lead to residues in honey. In northern regions oilseed rape belongs to the main forage crops that is mostly cultivated by means of intensive agriculture, including several pesticide treatments. However, in addition to the focal forage crops, pesticides from non-forage crops can spread to wild flowers around fields, and thus the residues in honey would reflect the whole range of pesticides used in the agricultural landscape. The aim of our study was to clarify which currently used pesticides are present in honey gathered from heterogeneous agricultural landscapes after the end of flowering of oilseed crops.

Honey samples (N = 33) were collected from beehives of Estonia during 2013 and 2014, and analysed for residues of 47 currently used agricultural pesticides using the multiresidue method with HPLC-MS/MS and GC-MS and a single residue method for glyphosate, aminopyralid and clopyralid. Residues of eight different active ingredients with representatives from all three basic pesticide classes were determined. Although no correlation was detected between the cumulative amount of pesticide residues and percent of oilseed crops in the foraging territory, most of the residues are those allowed for oilseed rape treatments. Among all pesticides, herbicide residues prevailed in 2013 but not in 2014. Despite the relatively small agricultural impact of Estonia, the detected levels of pesticide residues sometimes exceeded maximum residue level; however, these concentrations do not pose a health risk to consumers, also acute toxicity to honey bees would be very unlikely.

Materials and methods

Study location

Honey samples were gathered from Eastern and Southern Estonia (Ida-Viru, Tartu, Polva and Valga Counties) in 2013 (N = 14) and 2014 (N = 19). This area is representative of typical agricultural landscapes in Estonia with mostly intensively managed fields, forested areas and human settlements. Among other field crops, both winter and spring oilseed rape are often grown in Estonia, and both belong to the common forage crops of honey bees. Within a 2 km radius of each hive there is on average 34.6 ± 20.7 % cultivated land (min. 0.81 %, max. 70.2 %), 48.1 ± 20.6 % forest, 5.3 ± 7.6 % waste and vacant land, 7.6 ± 5.0 % grassland and 2.1 ± 3.6 % garden. The average oilseed crop coverage within the foraging territory remained between 0 and 12.9 %.

Pesticide selection

The 47 active ingredients analysed were selected for the survey as being the most commonly used in Estonian fields according to the pesticide ordering lists of the Tartu County Farmers Association for the year 2013-2014. These include the most commonly used contemporary herbicides (21), fungicides (15) and insecticides (10), and plant growth regulator and retardant (1). The active ingredients searched for were: 2,4D, alpha-cypermethrin, amido-sulphuron, aminopyralid, azoxystrobin, clopyralid, cypermethrin, cyproconazole, deltamethrin, dicamba, dimethachlor, dimethoate, ethyl trinexapac, fenoxaprop-p-ethyl, fenpropidin, florasulam, fludioxonil, fluoxastrobin, flutriafol, fuberidazole, glyphosate, imazalil, imidacloprid, indoxacarb, iodosulfuron-methyl-sodium, lambda-cyhalotrin, MCPA, mefenpyr-diethyl, pencycuron, picloram, pinoxaden, prochloraz, propaquizafop, propiconazole, propoxycarbazone-sodium, prothioconazole, pymetrozine, pyroxsulam, quizalofop-p-ethyl, spiroxamine, sulfosulfuron, tau-fluvalinate, tebuconazole, thiacloprid, triadimenol, triasulfuron and tribenuron-methyl.

Sample collection and handling

A total of 33 honey samples were collected from beehives in the eastern and southern part of Estonia (Tartu County and its near vicinity) during 2013 and 2014 for analysis of pesticide residues. Each honey sample originated from a different apiary, each of which consisted of 10-20 honey bee hives. The sampled hive was selected randomly for testing. The distance between sampled apiaries was at least 4 km in 2013 and at least 8 km in 2014 to preclude overlapping of the main forage area. The samples were gathered from honeycombs within beehives during the honey harvest in mid-July after the end of oilseed rape flowering. Due to the funding allocated for this study, it was decided to concentrate only on honey samples, and in order to cover more apiaries from the largest possible territory, we sampled only one hive per apiary. The honey was extracted from the comb wax and thereafter kept at 5°C until analysis.

Chemicals and materials

The reference standards of pesticides were purchased from AccuStandard (New Haven, USA) and Dr. Ehrenstorfer (Germany). HPLC grade acetonitrile and methanol were purchased from Merck-Millipore (Darmstadt, Germany). ACS grade formic acid (≥ 96.0 %), acetic acid (glacial, ≥ 99.85 %), and ammonium formate (99 %) were purchased from SigmaeAldrich (St. Louis, MO, USA). Ultrapure deionised water was generated by a Millipore Milli-Q™ system (Billerica, MA, USA). A buffer-salt mixture (1 g trisodium citrate dihydrate, 1 g sodium chloride, 0.5 g disodium hydrogen citrate sesquihydrate and 4 g of anhydrous magnesium sulphate) and a mixture of dSPE (900 mg anhydrous magnesium sulphate, 150 mg PSA and 150 mg C18E) were obtained from Phenomenex (Torrance, CA, USA).

Stock solutions of approximately 1000 mg/L concentration were prepared by weighing 10 mg of standard in a 10 mL graduated flask and dissolving it in acetonitrile. The purity of the standard was taken into account in the preparation of standard solutions of final concentration. The mix of working standard solution with a concentration of 0.01 mg/L was prepared by diluting the appropriate volume of stock solution in acetonitrile. The stock and working standard solution were stored at – 20°C.

Sample preparation

Different sample extraction and detection procedures were used for analysis of the selected pesticides. Most compounds were analysed using QuEChERS extraction methodology followed by detection using GC-MS and UHPLC-MS/MS. Analysis of glyphosate, aminopyralid and clopyralid was performed as single analyses using extraction with methanol.

For analysis of glyphosate, aminopyralid and clopyralid, 5.0 ± 0.1 g of samples were weighed into a 50 mL polypropylene centrifuge tube, then 10 mL of water and 10 mL of methanol were added for extraction. The samples were shaken for 20 min and centrifuged for 10 min at 4500 rpm. An aliquot of extract was transferred to an autosampler vial for analysis by UHPLC-MS/MS.

UHPLC-MS/MS analysis

An Acquity UHPLC system (Waters, USA) coupled to QTrap 5500 (AB SCIEX, USA) equipped with an electrospray ionisation source was used for the analysis of pesticides in honey. The chromatographic conditions for analysis of glyphosate residues in honey are summarised in Table 1 below.

Table 1: Chromatographic conditions for analysis of glyphosate in honey

UHPLC system and conditions		
Column:	Thermo Scientific, Hypercarb, 100 x 2.1 mm, 5 µm	
Column temperature:	40°C	
Injection volume:	10 µL	
Mobile phase:	1 % acetic acid in water	
Column flow:	0.3 mL/min	
MS system and conditions		
	Quantification	*Confirmation*
Scan type:	MRM	MRM
Ionisation mode:	ESI negative	ESI negative
Ion source temperature:	500°C	500°C
Ion spray voltage [V]:	-4500	-4500
Curtain gas nebulizer [psi]:	45	45
Ion source gas 1 [psi]:	40	40
Ion source gas 2 [psi]:	60	60
Declustering potential [V]:	-50	-50
Collision energy [V]:	-20	-16
Mass transition for evaluation [*m/z*]:	168 → 63	168 → 150

Results

Performance of the method

The performance of the method was evaluated according to the EC guidance document SANCO/12571/2013. The method showed good linearity with the determination coefficients, higher than 0.990 for all compounds included in the study. The mean variation of coefficients for repeatability of the method ranged from 3.0 % to 16 %, and the recovery ranged from 78 % to 115 %.

The limit of quantification (LOQ) for which the S/N ratio exceeds 10 was assumed at a concentration level of 0.01 mg kg for all pesticides with the exception of aminopyralid, clopyralid, glyphosate, dicamba and picloram for which the LOQ was 0.05 mg/kg.

Analysis of the honey samples

The amounts and composition of pesticide residues found in the honey samples differed between years. The residues of glyphosate in honey samples are summarised in Table 2. The agricultural practices generally do not vary so much, but the need for different kinds of pesticides can vary widely from year to year.

Table 2: Concentrations of glyphosate residues found in honey samples in Estonia 2013-2014

Honey sample	**Year**	**% of oilseed rape in foraging range**	**Glyphosate residues [1] [µg/kg]**
1	2013	3.4	n.d.
2	2013	5.7	14
3	2013	6.2	**56**

Table 2: Concentrations of glyphosate residues found in honey samples in Estonia 2013-2014

Honey sample	Year	% of oilseed rape in foraging range	Glyphosate residues [1] [µg/kg]
4	2013	12.1	n.d.
5	2013	10	n.d.
6	2013	9.2	n.d.
7	2013	12.9	**62**
8	2013	9.2	n.d.
9	2013	9.1	n.d.
10	2013	14	n.d.
11	2013	8.6	n.d.
12	2013	5.1	n.d.
13	2013	9.2	n.d.
14	2013	9.3	n.d.
Average	*2013*	*8.86*	***44***
% of samples	*2013*		*21*
15	2014	0	n.d.
16	2014	8.6	n.d.
17	2014	1	n.d.
18	2014	3.8	n.d.
19	2014	0	n.d.
20	2014	2.8	n.d.
21	2014	8.8	n.d.
22	2014	2.7	n.d.
23	2014	11.6	(9)
24	2014	12.3	n.d.
25	2014	8.9	n.d.
26	2014	13.3	n.d.
27	2014	11.6	n.d.
28	2014	1.8	n.d.
29	2014	5.9	n.d.
30	2014	5.4	n.d.
31	2014	8.7	n.d.
32	2014	3.5	n.d.
33	2014	4.9	n.d.
Average	*2014*	*6.08*	*9*
% of samples	*2014*		*16*

[1] The numbers in parenthesis represent values under the limits of detection (LOD). The numbers in bold represent values above the maximum residue limits (MRL).

Honey as a product contains surprisingly few pesticide residues compared to bee bread or pollen (Thompson *et al.*, 2014). Pesticide residues in different matrixes differ in their chemical composition and physical characteristics. Fat or lipid soluble compounds tend to contaminate wax, whereas water-soluble compounds are more readily found in nectar or honey. Besides contaminated nectar, honey contamination may also occur via translocation of the compounds from comb wax to honey (Kochansky *et al.*, 2001; Tremolada *et al.*, 2004).

The relatively large areas with natural vegetation, and the low amounts of pesticides used in Estonian agriculture (Eurostat, 2015) has shaped the notion that the bee forage environment should be unpolluted in Estonia and probably also in other Nordic countries. Our results, however, suggest the situation may be of concern.

Despite the general low input of pesticides compared to the average usage over the European countries (Eurostat, 2015), some compounds found in honey samples exceeded the MRL. On the background of landscape characteristics, this might arise from relatively homogeneous land cover type – in Estonia, as in Ireland and the United Kingdom, the landscape in 2015 is dominated by larger areas composed of the same land cover type, also the number of structural green elements in the landscape is small (Eurostat, 2015). Larger forest areas may serve as barriers for bees, for instance. Forests have been shown to negatively affect bumble bees with larger foraging territories (Diaz-Forero *et al.*, 2011). Such barriers may concentrate bees on other land, thus increasing the risk of forage on polluted plants. Honey bees prefer to forage in larger open areas rich in flowers, and flowering crops make up an important part of the forage. Since it is one of the most profitable crops, oilseed rape crops are common in crop rotations: covering 15 % and 11 % of total cultivated land in 2010 and 2015 accordingly (Statistics Estonia, 2012).

In northern regions, the most common group of pesticides sold are herbicides: these comprise more than 70 % of pesticides sold in Estonia (Eurostat, 2015). The higher amounts of herbicide active ingredients needed for effective treatments compared to insecticides, for instance, may also be one reason why herbicide residues in particular were higher in our samples. The amounts of herbicides used on fields may differ from year to year depending on the weather conditions throughout the spring and summer. The amounts of herbicides sold in Estonia were higher in 2013 compared to 2014 (Eurostat, 2015) and this appears to have been reflected in our honey samples. Although pesticide residues may be retained in soils from the previous year or even from treatments made decades ago (Hilber *et al.*, 2008; Lozowicka *et al.*, 2016), the authors believe this probably did not affect our results because the samples with higher concentrations in 2013 did not show higher residue level in 2014. Most of the locations sampled in 2013 were also sampled in 2014. We suppose that in those cases where we found herbicide residues higher than the MRL, the bees must have foraged on recently treated fields. For instance, glyphosate residues may remain very high in nectar for up to seven days after treatment, as demonstrated by Thompson *et al.* (2014). Glyphosate-based herbicides are the most common herbicides worldwide. Moreover, its usage nowadays has gone beyond pest control purposes – being more of an agricultural instead of a pest management tool (Steinmann *et al.*, 2012). We believe that this is something to consider for reducing the levels of pesticide residue found in food: by excluding the routine spray applications and retaining the weed management purpose of glyphosate, one could facilitate a less polluted environment.

The concentrations of all residues found from honey samples in this study remained below the lethal dose to honey bees. LD50 is measured for 2,4D was 0.0115 mg/bee (Extension Toxicology Network, 1996), clopyralid > 100 mg/bee (Dow AgroSciences, 2007) and glyphosate 100 mg/bee (Thompson *et al.*, 2014), tebuconazole 83 mg/bee, azoxystrobin 200 mg/bee, dimethoate 0.11 mg/bee, thiacloprid 27.89 mg/bee, and tau-fluvalinlate 45 mg/bee (Sanchez-Bayo and Goka, 2014). This means that the concentrations found are definitely below acute lethal dosages, although sub-lethal effects cannot be excluded when considering that at least nurse bees consume the contaminated food until they produce the royal jelly, and also larger larvae are fed with nectar and pollen collected by foragers.

Conclusion

Our results demonstrate that intensively treated oilseed rape fields can be a source for pesticide residue contamination in honey, however no direct correlation was found. We believe that pesticides escape from fields over larger neighbouring areas with wild vegetation and contaminate the nectar of wild plants. Our

study indicates that most of the agrochemical residues in Estonian honey can originate from oilseed treatments, however the same active ingredients are used for different crops, which is why no direct references can be made. The compounds that were represented in the highest amounts belonged to herbicides, the most frequently used pesticide group in Northern European climatic conditions. In the context of honey as human food, the concentrations of pesticide residues do not pose any health risk to consumers, although in some cases the levels detected exceeded the MRLs. Concerning the health of bees, the residues remained below acute lethality, however some sub-lethal effects cannot be excluded.

3. Assessment and conclusion

Assessment and conclusion by applicant:
The publication is considered relevant to the setting of a suitable MRL for glyphosate in honey since according to SANTE/11956/2016 rev. 9 it is possible to derive MRLs in honey based on monitoring data. Although only limited information is given about the validation of the method for the determination of glyphosate residues, the analytical results are most likely reliable. The residue levels found for glyphosate are consistent with the EU-monitoring data published by EFSA for 2016-2017 in that: 1. Most of the samples do not show quantifiable residues of glyphosate. 2. Some samples show residues > 0.05 mg/kg, which indicates that it is appropriate to increase the existing MRL. 3. The measured residue levels are far below the levels found in the tunnel residue study.

1. Information on the study

Data point	CA 6.10.1/008
Report author	Rubio, R. *et al.*
Report year	2014
Report title	Survey of glyphosate residues in honey, corn and soy products
Document No.	DOI 10.4172/2161-0525.1000249
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Yes/Reliable

2. Full summary of the study according to OECD format

Executive Summary

Samples of honey (sixty-nine), pancake and corn syrup (twenty six), soy sauce (twenty eight), soy milk (eleven), and tofu (twenty) purchased in the Philadelphia, US metropolitan area were analyzed for glyphosate residue using ELISA. The limit of quantification (LOQ) and range of the method were determined for honey, pancake syrup, and corn syrup to be 15 to 800 ppb; soy sauce, soy milk, and tofu 75 to 4000 ppb. Glyphosate residues above the limit of quantification were not found in pancake and corn syrup, soy milk, and tofu. Of the sixty-nine honey samples analyzed, forty-one samples, or fifty-nine percent (59 %), had glyphosate concentrations above the method LOQ (15 ppb), with a concentration range between 17 and 163 ppb and a mean of 64 ppb. Eleven of the tested honey samples were organic; five of the organic honey samples, or forty-five percent (45 %), contained glyphosate concentrations above the method LOQ, with a range of 26 to 93 ppb and a mean of 50 ppb. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62 %), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb. In addition to comparison of production method (organic *vs.* conventional), the honey results were evaluated according to pollen source and by country of origin, grouped by GMO usage (prohibited, limited, or permitted). Glyphosate concentrations above the method LOQ (75 ppb) were also found in ten of the twenty-eight soy sauce

samples evaluated (36 %), with a concentration range between 88 and 564 ppb and a mean of 242 ppb; all organic soy sauce samples tested were below the method LOQ.

Materials and Methods

Chemicals and reagents
Chemicals were of reagent grade and were purchased from Sigma Chemical Company, St. Louis MO, USA, except as indicated. Glyphosate (> 98 % purity), Chem Service, West Chester, PA, USA. Glyphosate micro titer plate ELISA, Abraxis PN 500086; Glyphosate sample diluent, PN 500082, Abraxis LLC, Warminster, PA, USA. Glyphosate stock solution was prepared in deionised water to 1.0 mg/mL; spiking solutions were prepared from the working solution using deionised water.

Samples and sample preparation/extraction
In total, 153 representative samples were purchased from markets in the Philadelphia metropolitan area (69 honey, 26 corn and pancake syrup, 28 soy sauce, 11 soy milk, and 20 tofu products).
Honey, corn and pancake syrup samples: A 0.50 g aliquot of sample was weighed into a micro centrifuge tube and 0.50 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/ extraction described above produced a 1:200 sample dilution.
Soy sauce: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, then diluted by adding 40 µL of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.
Soy milk: A 0.10 mL aliquot of sample was transferred into a micro centrifuge tube and 0.90 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then centrifuged at 6000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.
Tofu: A 1.0 g aliquot of sample was weighed into a 20 mL vial and 10.0 mL of 1N HCl was added. The sample was mixed for 2 minutes using a vortex mixer, and then allowed to separate for 2 minutes. Approximately 1 mL of the mixture was transferred into a micro centrifuge tube and centrifuged at 6,000 x g for 5 minutes. The sample was then diluted by adding 40 µL of the middle layer of the acid treated sample into 3.96 mL of glyphosate sample diluent and mixed using a vortex mixer. The sample was then analyzed in the ELISA. The sample preparation/extraction described above produced a 1:1000 sample dilution.

Determination of glyphosate in samples
The instructions provided in the ELISA kit user's guide were followed, in brief, glyphosate calibrators provided in the kit and the samples to be tested are derivatised for ten minutes and then added, along with an antibody specific for glyphosate to micro titer wells coated with goat anti-rabbit antibody and incubated for thirty minutes with shaking. A glyphosate horseradish peroxidase (HRP) enzyme conjugate is then added. At this point a competitive reaction occurs between the glyphosate, in the calibrators or samples, and the enzyme labeled glyphosate for the antibody binding sites on the micro titer well. The reaction is allowed to continue for sixty minutes. After a washing step an enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine) are added. The enzyme-labeled glyphosate bound to the glyphosate antibody catalyzes the conversion of the substrate /chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilised by the addition of diluted acid and read in a Molecular Devices micro titer plate reader (450 nm). Since the labeled glyphosate (conjugate) was in competition with the unlabeled glyphosate (sample) for the antibody sites, the color developed is inversely proportional to the concentration of glyphosate in the sample.

Data analysis
The evaluation of the assay was performed using Molecular Devices Soft max pro evaluation program (4-Parameter). The program calculates the mean absorbance value for each of the standards (Bi) and calculates the %Bi /B0 for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance (B0). The program then constructs a non-linear regression model of a standard curve by plotting the % Bi/B0 for each standard on the vertical linear (y) axis versus the corresponding glyphosate concentration on the horizontal logarithmic (x) axis. The % Bi/B0 for samples is interpolated using the standard curve yielding sample concentration levels of glyphosate from the standard curve. Correlation coefficients of the assays were >0.995 and standard deviation between standard replicate analysis were < 10 %.

Validation, performance and quality control
Specificity had been previously determined (ELISA user's guide), (**Table 1**). Recovery, limit of quantitation, range and limit of quantification were determined to test the validity of the dilution/ extraction procedures of each of the matrices used in combination with the glyphosate ELISA.

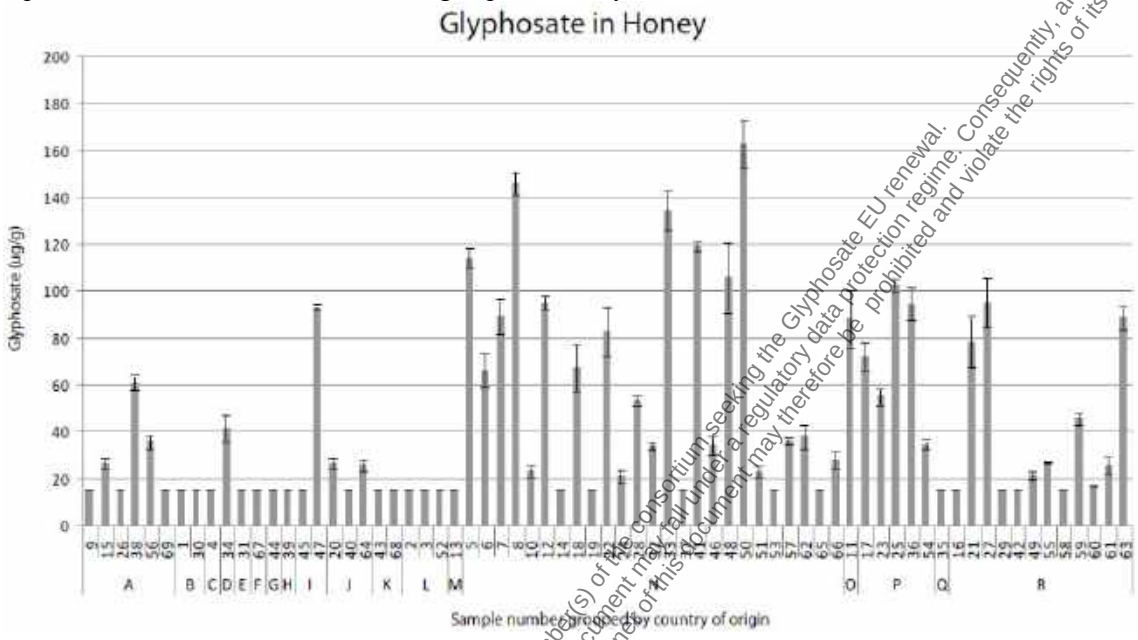
Table 1: Cross-reactivity table. The reactivity of glyphosate to various related compounds expressed as LOD and as the dose required for 50 % absorbance inhibition (50 % B/Bo).

COMPOUND (B/Bo)	LOD(ng/mL)	50% B/Bo(ng/mL)
Glyphosate	0.05	0.5
Glyphosine	50	3,000
Glufosinate	2,000	70,000
AMPA	35,000	>1,000,000
Glycine	>10,000	>1,000,000

Results and Discussion

The method performance for glyphosate analysis was determined by conducting recovery tests on each of the matrices. To determine the accuracy of the glyphosate analysis for the sample matrices analyzed in this study, matrix samples that were glyphosate negative and positive (positive samples were not encountered with tofu, soy milk, pancake and corn syrup) were spiked as follows: 15, 40, 100, 200 and 400 ng/ mL (honey, pancake and corn syrup); 75, 200, 500, 1000 and 4000 ng/mL [soy sauce, soy milk and tofu (ng/g)]. Analysis was performed in duplicate for all unspiked and spiked samples at all levels. Average recovery obtained for glyphosate negative honey samples fortified with glyphosate was 119 %, (SD = 10). Average recovery for glyphosate positive honey (unspiked contained 44 ng/g glyphosate) after fortification was 116 % (SD = 10). Average recovery for negative soy sauce was 94 % (SD = 5), and for positive fortified soy sauce (unspiked contained 417 ng/mL) was 86 % (SD = 5). The limit of quantification and range of the method were determined for honey, pancake and corn syrup to be 15 to 800 ng/g; soy sauce, soy milk, and tofu 75 to 4000 ng/ mL or ng/g, respectively.
In this study, the first sample matrix analyzed for the presence of glyphosate was honey; 69 samples were analyzed and classified into 18 groups depending on the country of origin listed on the bottles: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. The glyphosate concentrations obtained are shown in (**Figure 2**). Forty-one out of the sixty-nine honey samples analyzed, or fifty nine percent (59 %), had glyphosate concentrations above the method LOQ (15 ng/g) with a concentration range between 17 and 163 ng/g and a mean of 64 ng/g.

Figure 2: Concentration of glyphosate (ng/g) in honey samples listed by honey origin: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g). Error bars represent concentrations obtained during duplicate analysis.



The glyphosate concentration in honey grouped by flower (pollen) source is shown in (**Figure 3**). The pollen types listed on the bottles were: clover (12 samples), exotic (11 samples), wildflower (11 samples), unknown (35 samples). (**Figure 4**) depicts the concentration of glyphosate in honey samples grouped by growing method of source pollen: organic (11 samples) and traditional (58 samples); 5 of the 11 organic samples had glyphosate concentrations above the method LOQ with a range of 26 to 93 ng/g and a mean of 50 ng /g. Of the fifty-eight non-organic honey samples, thirty-six samples, or sixty-two percent (62 %), contained glyphosate concentrations above the method LOQ, with a range of 17 to 163 ppb and a mean of 66 ppb.

Figure 3: Concentration of glyphosate (ng/g) in honey samples by flower (pollen) source. Dashed line represents LOQ of method (15 ng/g). Exotic flowers were sophora, manuka, orange, cactus, summer flower, lychee, alfalfa, acacia). Error bars represent concentrations obtained during duplicate analysis.

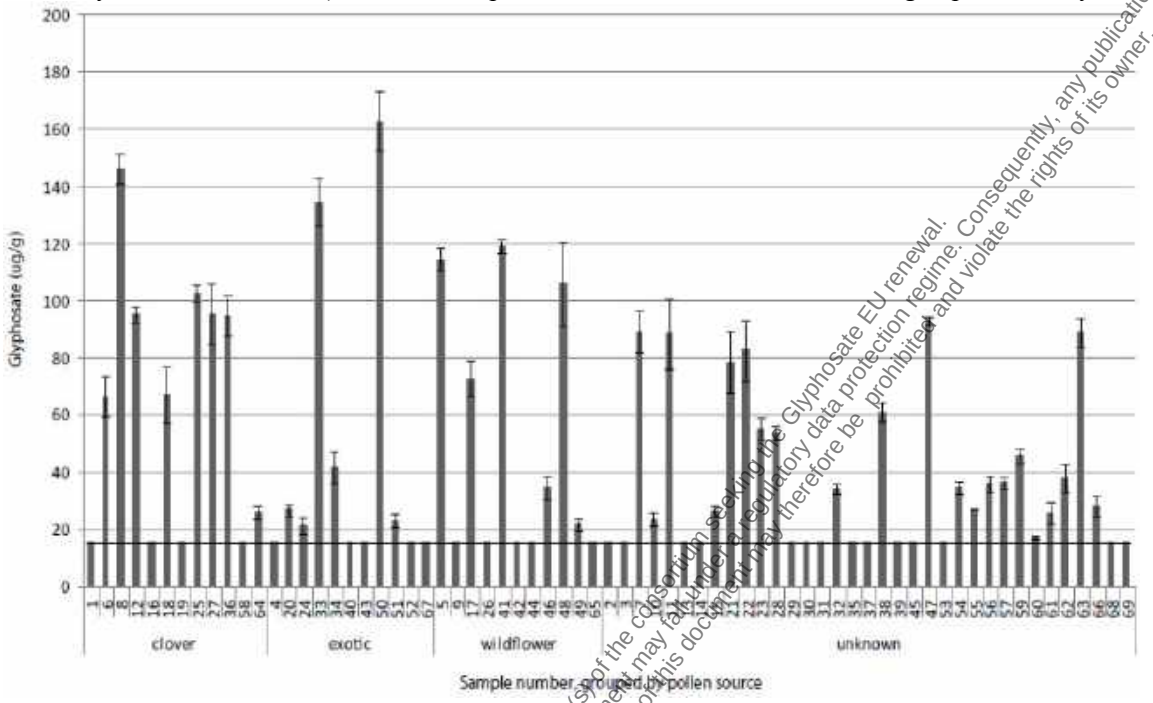


Figure 4: Concentration of glyphosate (ng/g) in honey samples by growing method of source pollen (Organic *vs.* Traditional). Dashed line represents LOQ of method (15 ng/g).) Error bars represent concentrations obtained during duplicate analysis.

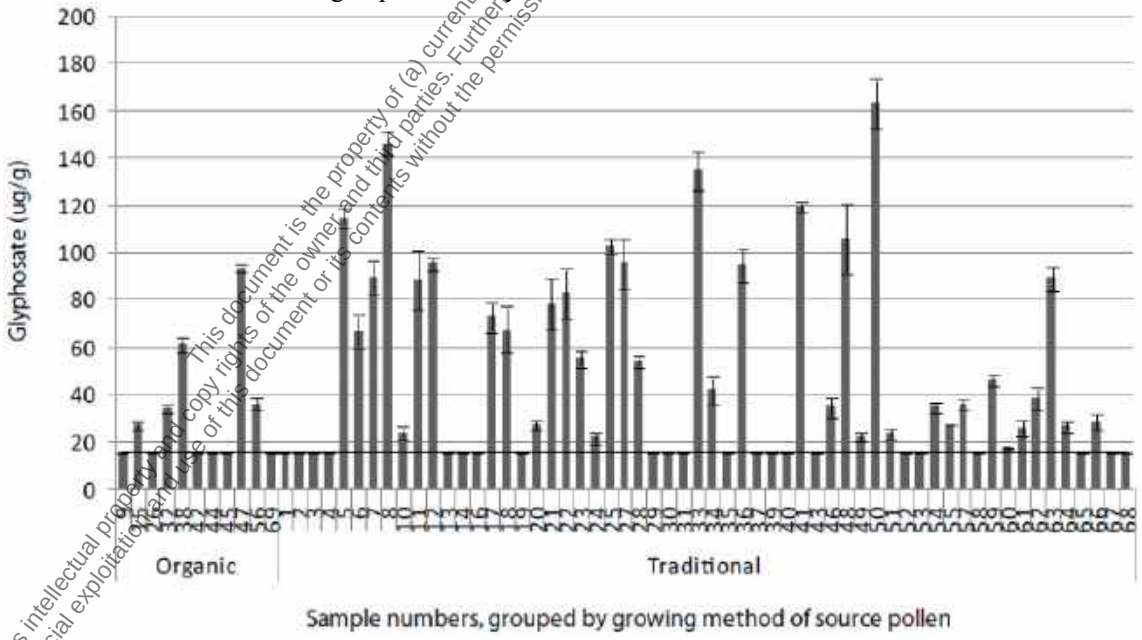
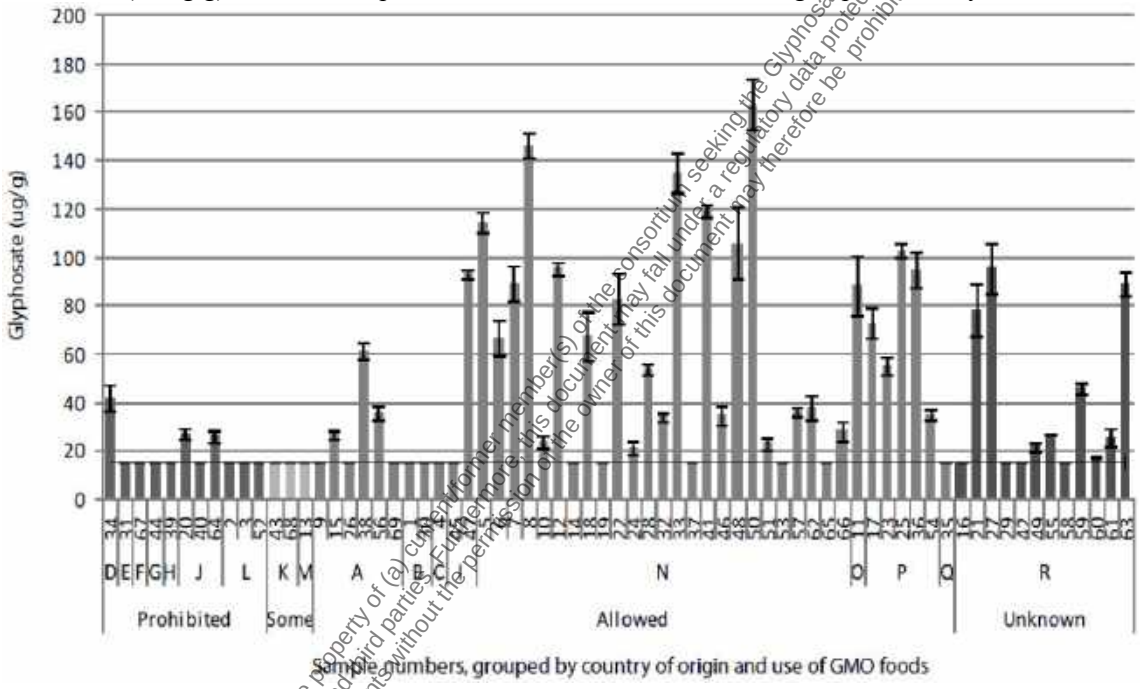


Figure 5 depicts the concentration of glyphosate in honey by country and whether the use of genetically modified organisms (GMO) seeds is prohibited or permitted. The graph also shows where some minimum uses of GMO traits are allowed (Spain, and blend of Vietnam/Ukraine). The glyphosate concentration in honey originating in countries that do not allow or allow limited GMO traits (3 out of 14 samples above the LOQ) ranged from 26 to 41 ng/g with a mean of 31 ng/g. The glyphosate range for those countries that allow GMO (30 out of 43 samples above LOQ) was 21 to 163 ng/g with a mean of 71 ng/g. Samples of unknown origin (8 out of 12 samples above LOQ) ranged from 17 to 95 ng/g with a mean of 50 ng/g.

Figure 5: Concentration of glyphosate (ng/g) in honey samples listed by honey origin and the allowance of GMO use: (A) Brazil, (B) Canada, (C) China, (D) Germany, (E) Greece, (F) Hungary, (G) India, (H) Korea, (I) blend of Mexico, Brazil, and Uruguay, (J) New Zealand, (K) Spain, (L) Taiwan, (M) blend of Ukraine and Vietnam, (N) USA, (O) blend of USA and Argentina, (P) blend of USA, Argentina and Canada, (Q) blend of USA, South America, (R) unknown origin. Dashed line represents LOQ of method (15 ng/g). Error bars represent concentrations obtained during duplicate analysis.



The second matrix group analyzed for glyphosate was soy sauce. The analysis consisted of 28 samples, (**Figure 6**). Ten out of 28 samples (36 %) had glyphosate concentrations above the method LOQ (75 ng/mL) with a concentration range between 88 and 564 ng/mL and a mean of 242 ng/mL. (**Figure 7**) shows the concentration of glyphosate in soy sauce by method of soy bean growing (organic vs. traditional). The recent report from the Chinese Academy of Medical Science and the Beijing Union Hospital [20] reported an average glyphosate concentration in soy sauce of 133 ng/mL in samples that did not specify on the bottle whether or not the raw material was GM soybean. In our study, the small subset of organic labeled samples (three) was all below the limit of quantitation of the test.

Figure 6: Concentration of glyphosate (ng/mL) in soy sauce samples. Dashed line represents LOQ of method (75 ng/mL). Error bars represent concentrations obtained during duplicate analysis.

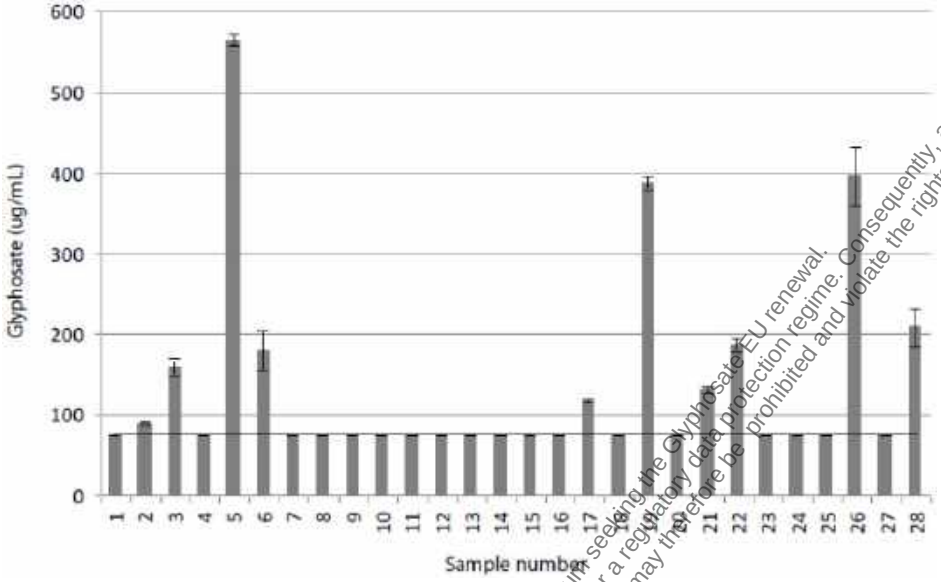
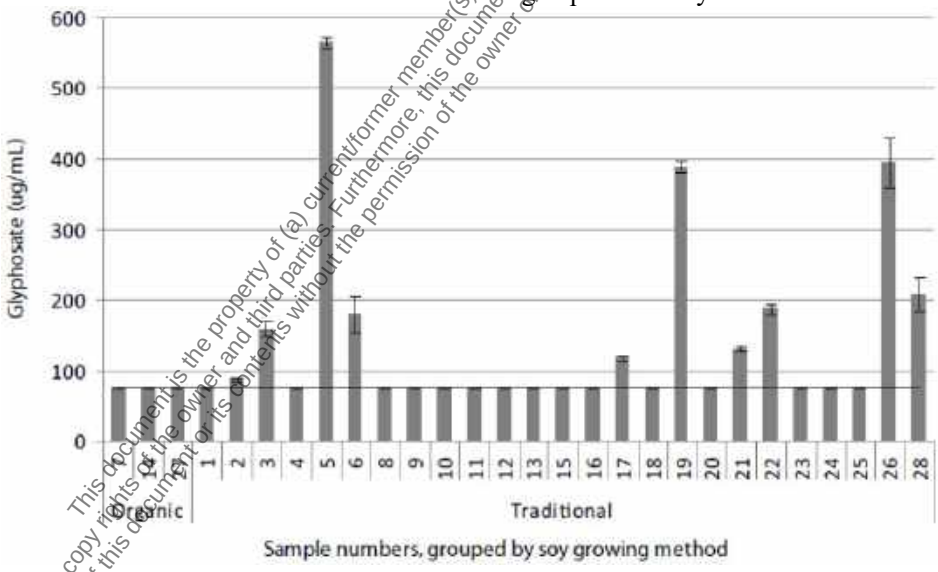


Figure 7: Concentration of glyphosate (ng/mL) in soy sauce samples by growing method of soy beans (Organic *vs.* Traditional). Dashed line represents LOQ of method (75 ng/mL). Error bars represent concentrations obtained during duplicate analysis.



Corn and pancake syrup (26 samples), soy milk (11 samples), and tofu (20 samples) tested were negative for glyphosate at the LOQ of the method (15 ng/g for pancake and corn syrup, and 75 ng/mL or ng/g for soy milk and tofu, respectively).

Studies on glyphosate residues in food are scarce. Among the few studies found was a recent report published on the incidence of glyphosate in soy sauce, conducted by the Chinese government [20]. Searches were conducted by the authors using various scientific databases on the concentration and incidence of glyphosate in honey, but these failed to provide any information. The honey samples analyzed in the present study show that 59 % of all samples contained glyphosate residues (ranging from 17 to 163 ng/g, mean 64 ng/g); the residue concentration does not seem to depend on pollen source or

growing method, even organic honey contained glyphosate residues (5 out 11 samples, or 45 %, mean glyphosate concentration 50 ng/g). Comparing the concentration of glyphosate in honey by countries that use GMO extensively with countries that allow the use of some GMO traits and those that do not allow GMO, shows that, in general, glyphosate levels are lower in samples from countries that do not allow or allow limited use of some GMO traits, such as Spain and Vietnam/ Ukraine blend (mean 31 ng/g), compared to those countries that allow planting of GMO traits (71 ng/g). It should be noted, however, that some residues of glyphosate (although < 50 ng/g) were found in honeys originating from Germany and New Zealand, countries where no GMO planting is allowed.

The European Union has specific guidelines for the labeling of organic honey [25,26]. According to those guidelines, the location of apiaries is strictly controlled and states that "Nectar and pollen sources available over a three-kilometer radius around the apiary sites must consist essentially of organically produced crops or crops treated with low-environmental-impact methods. Apiaries must also be far enough away from any non-agricultural production source that could lead to contamination (*e.g.* urban centers, waste dumps, waste incinerators, etc.). Member States have the option of prohibiting the production of organic honey in certain regions or areas that do not meet these conditions. Organic honey must not contain chemicals residues (synthetic pesticides, etc.)." The United States has no such guidelines for the organic production of honey, but uses organic farming certification for honey labeling purposes; one reason is that it is practically impossible to regulate without testing all honey for residues since bees can fly up to 3 miles in search of nectar and it is difficult to be certain that they do not feed on nectar contaminated by crop spraying or industrial sources. In the EU, glyphosate residues in non-organic honey regulatory limits are 50 ng/g [27], the United States does not have a limit in honey. The limit in drinking water in the United States is 700 ng/mL; the reference dose is 1.75 mg/kg/day; the One-Day Health Advisory level is 20 mg/L [28]. Also, it is widely known that like milk and olive oil, honey is one of the foods that is most commonly mislabeled and adulterated [29] providing yet another source of glyphosate contamination in honeys that, according to the bottle label, originated in non-GMO countries.
Bee colony collapse disorder (CCD) is a growing threat to the efficient production of food around the world. Honey bees pollinate nearly 130 species of plant life [30], such as fruits, vegetables, nuts, and seed crops. Honeybees are therefore indirectly responsible for an estimated one-third of the world food supply [31]. Although several factors are involved in CCD, including numerous pathogens and parasites, the extensive use of pesticides [32,33] such as neonicotinoids have provided evidence that these products are harmful to honey bees and have lead to a recent ban or restriction in the use of three neonicotoids by the European Union [34]. Although glyphosate is not acutely toxic to bees, it is chronically toxic to animals and is reported to disrupt the endocrine system [35,36] and a recent study indicates that honey bees exposed to increasing sub-lethal concentrations of glyphosate exhibit a decrease in acetyl cholinesterase (AChE) activity [37]. The high rate of glyphosate use creates the potential for wide-spread contamination of our food chain. Glyphosate is used throughout the bee foraging period in high amounts and is found in the air, water, and in plant parts frequented by bees, such as flowers and buds, potentially contaminating the nectar collected by bees from contaminated plants [38]. Based on its prevalence in the environment, as well as our findings in honey samples, we propose that future studies should be conducted to determine if glyphosate is in fact a contributing factor in CCD.

Conclusions

This study indicates the presence of glyphosate residues in honey and soy sauce, but not in pancake and corn syrups or soy based products such as soy milk and tofu. Forty one out of sixty nine (59 %) honey samples analyzed contained glyphosate at a concentration above the method LOQ (15 ng/g) with a range between 17-163 ng/g and a mean of 64 ng/g. Ten out of twenty eight (36 %) soy sauce samples contained glyphosate at a concentration above the method LOQ (75 ng/mL) with a range between 88-564 ng /mL and a mean of 242 ng /mL. Future studies should be conducted on many other food products to determine the extent of glyphosate residue contamination.

3. Assessment and conclusion

Assessment and conclusion by applicant:

The article describes a survey of glyphosate residues in honey (n = 69), pancake and corn syrup (n = 26), soy sauce (n = 28), soy milk (n = 11) and tofu (n = 20) purchased in USA, but originating from various countries around the globe. In the context of the dossier for the renewal of the EU approval of glyphosate and with regard to the supported representative uses, the residue data for pancake and corn syrup, soy sauce, soy milk and tofu are not considered relevant. However, the residue data for glyphosate in honey are potentially relevant since according to the guideline SANTE/11956/2016 rev. 9 it is possible to derive MRLs in honey based on monitoring data. Only few of the analysed honey samples originated from Europe but, as honey available to European consumers may originate from outside the EU, it is appropriate to consider honey residue data from outside the EU to derive the EU MRL.

The samples were analysed by means of an ELISA method which was validated by determining the recovery rates from fortified samples. The validation results are not provided in detail, but the average recoveries and relative standard deviations were satisfactory, although the validation was not conducted exactly in accordance with EU or OECD guidelines (i.e. with at least 5 replicates at the LOQ and 5 replicates at a higher level). The limit of quantification was estimated at 0.015 mg/kg. The specificity of the method was investigated by assessing the response of the ELISA test to a series of substances chemically related to glyphosate and it was shown that the response of these substances was at least 1000 times less than that of glyphosate. While this experiment allows to exclude some possible sources of false-positive results, it does not allow to completely rule out that other (not tested compounds) may yield false positive results. Despite these limitations, the obtained analytical results are considered fairly reliable.

59 % percent of the 69 honey samples contained glyphosate residues above the method LOQ (0.015 mg/kg) with a concentration range between 0.017 and 0.163 mg/kg and a mean of 0.064 mg/kg. While the individual results are not provided, it seems that about 31 % of the samples (22 from 69) showed residues of glyphosate above the EU MRL of 0.05 mg/kg. The samples originating from the EU all showed residues < 0.05 mg/kg. Overall, the findings reported in the publication are in line with the results of the EU-monitoring since the publication shows that glyphosate can occur in honey at levels > 0.05 mg/kg and that it is, therefore, appropriate to increase the existing EU-MRL. The highest measured residue level was 0.163 mg/kg, which is less than the maximum value found during the EU-monitoring for 2016-2017.

Glyphosate

Annex M-CA 6-01

ANNEX to the Document M of the technical section[10]:

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

[10] Annex to the Doc ID: 110054 MCA6-Jun_2020

AIR 5 introduction

During the AIR 2 evaluation process of glyphosate, in the Renewal Assessment Report 2015 version[11], the RMS Germany included public literature articles as part of the B.7 section. **All articles** included in that RAR Vol 3 2015 version, have been included in this annex for sake of completeness, with the aim to present to the EU authorities during the AIR 5 EU process, all information available for glyphosate from previous EU evaluations.

All information presented in this Annex 1, is an exact copy of the literature information included in the RAR Vol 3 2015 version. When reading the present annex, please note:

- This annex only presents articles and not regulatory studies.
- The numbering of tables in the present annex has not been changed and remains as original presented in the RAR Vol 3 2015 version.
- If text was highlighted in the RAR Vol 3 2015, then those sentences are also highlighted in the present annex.
- If parts of the text were given in italic style in the RAR Vol 3 2015, then those parts are also given in italic in the present annex.

[11] Renewal Assessment Report, Revised 2015 Vol 3

Renewal Assessment Report, Revised 29 January 2015 Vol 3, Annex B.7

B.7.1.1.1 Fruit crops

Reference: OECD KIIA 6.2.1
Report: [redacted] Degradation of Glyphosate in avocado fruit 10.04.1995, L365, ASB2011-13642
Guidelines: no
Deviations: not applicable
GLP: no
Acceptability: The study is considered to be additional information.

Material and Methods:
In this study conducted in a research project supported by the California Avocado Advisory Board the bahaviour of ^{14}C-radiolabelled glyphosate was investigated in avocado.
The study contained two parts. First a selected leaf was painted with ^{14}C-glyphosate. After up to 10 days samples of leaves near the treated leaf were analysed for radioactive residues.
In the second part of the experiment a mature avocado fruit was picked and a cavity was drilled into the end of the peduncle. The cavity was filled with an aqueous solution containing ^{14}C-glyphosate (453000 cpm) and then kept filled with destilled water for the remainder of 10 days. The fruit was placed in a respiration chamber, monitoring the formation of $^{14}CO_2$.
Analysis of the radioactivity was conducted by LSC and ion exchange HPLC with radiodetection agains glyphosate and AMPA as reference substances.

Findings:
In the first part of the experiment no translocation of radioactivity from the treated leaf into other parts of the plant was observed.
The treated avocado fruit was separated into its different segments and analysed for radioactivity. Results are presented in the following table:

Table B.7.1-16: Recovery of radiolabel from ^{14}C-glyphosate treated avocado fruit

Matrix (weight)	Glyphosate	AMPA	Unasssigned
	cpm (%)	cpm (%)	cpm (%)
Avocado, mesocarp (190.4 g)	290378 (64.1)	1179 (0.26)	4330 (0.9)
Avocado, exocarp (24.9 g)	31029 (6.8)	0 (0)	363 (<0.1)
Avocado, seed (53.9 g)	202 (<0.1)	0 (0)	2107 (0.47)
Avocado, peduncle (2.6 g)	69057 (15.2)	340 (<0.1)	2162 (0.48)
Filter paper	4225 (0.9)	0 (0)	98 (<0.1)
Total recovered	394891 (87.1)	1519 (0.34)	9060 (2)
CO_2	-	-	16239 (3.6)

Conclusions:
The information provided by this study are limited. No translocation of radioactivity from one treated leaf into other parts of the branch were observed. In one avocado fruit directly treated with glyphosate via a drilled cavity in the peduncle, most of radioactivity was present in the mesocarp and peduncle. AMPA was present in very low amounts <1 % of the TRR.

B.7.1.4 Public literature

In several studies the effect of glyphosate on the concentration of secondary plant metabolites was investigated. Bresnahn et al (2003, ASB2012-12365) investigated the levels of shikimic acid, which is involved in the main mode of action for glyphosate in plants, in wheat. It was observed that following

glyphosate application an approximately 2-fold increase in the shikimic acid concentration in the grain was observed compared to control samples or wheat treated with other herbicides.
In studies published by Bohm et al (2008, ASB2012-12366) and Duke et al (2003, ASB2012-12400) the effect of glyphosate to isoflavones in soya beans was investigated. No significant influence was identified.
The activity of gluthathione-S-transferase in maize was investigated by Cataneo et al (2003, ASB2012-12384). The results indicate an increase in the activity of the molecule, probably due to its role in the plant metabolism of the active substance.

In a literature review Duke (2011, ASB2012-12401) comapred the mode of glyphosate resistance in weed, showing that only a minor share of the weeds express a GOX gene. Most of the weeds contained a unchanged glyphosate, suggesting a modification of the EPSPSenzyme. However, the level of resistance in weeds was found to be lower than in genetically modified crops.
Further investigations on secondary plant metabolites in organic, conventional and GM-soya beans and the residues of glyphosate and AMPA were published by Bøhn *et al.* (2014, ASB2014-6353). Organic soybeans showed a nutritional profile with more sugars, such as glucose, fructose, sucrose and maltose, significantly more total protein, zinc and less fibre than both conventional and GM-soya. Organic soybeans also contained less total saturated fat and total omega-6 fatty acids than both conventional and GM-soya. The ration of residues of glyphosate and AMPA (mean 3.3 and 5.7 mg/kg, respectively) confirmed the findings from ^{14}C-radiolabelled plant metabolism studies provided by the applicant.

The metabolism of glyphosate in *Mucuna pruriens var. utilis* (velvet beans) was investigated by Rojano-Delgado et al (2012, ASB2012-12462). The velvet beans are a plant species exhibiting an innate, very high resistance to glyphosate. Using ^{14}C-radiolabelled glyphosate, the uptake and metabolism in velvet beans compared to *Amaranthus retroflexus* was observed. It was shown that velvet beans had a much lower uptake of the radioactivity via the leaves. While amaranthus accumulated up to 94 % of the applied dose within 24h, an uptake of less than 40 % was observed for the velvet beans. After 72 hours 52 % of the applied dose were taken up.
Besides the investigation of the uptake, degradation products found in the velvet bean leaves were analysed to identify the mode of tolerance for this species. In treated plants glyphosate, AMPA, sarcosine and formaldehyde were identified. In comparision to non-tolerant amaranthus, where mainly glyphosate and traces of AMPA were found, a secondary metabolic pathway via sarcosine, which is normally found in bacteria only, was postulated by the author:

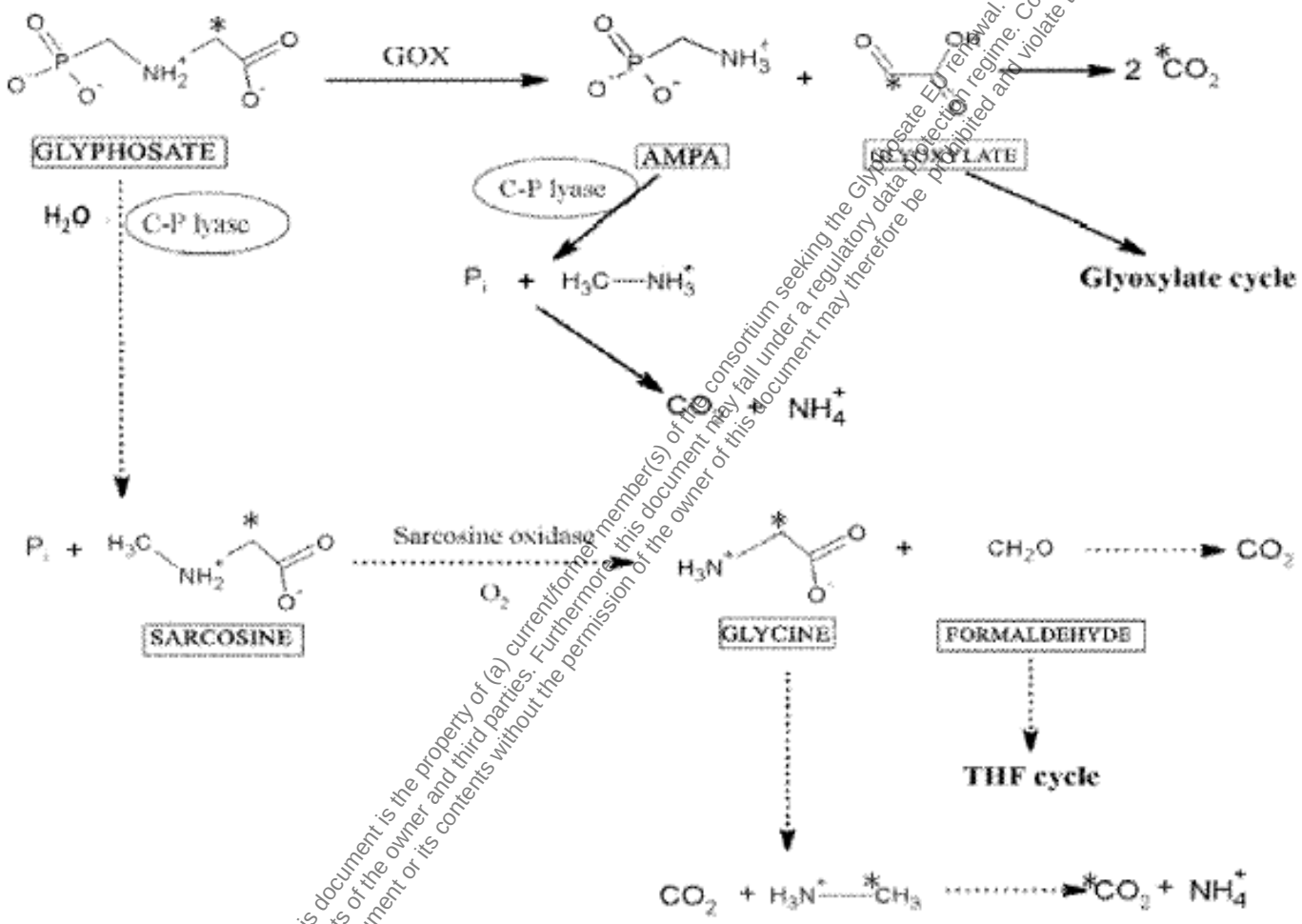


Fig. 6. Potential degradation pathway for glyphosate (⟶ primary pathway in plants and soils, ⋯▸ primary pathway in bacteria).

In summary it was concluded, that the natural tolerance of velvet beans is based on three modes of actions: a natural GOX modification resulting in AMPA, a high-tolerance EPSPS enzyme and the additional degradation into sarcosine, finally resulting in glycine, formaldehyde and their natural products.
In view of the residue situation the observation of an additional metabolic pathway is relevant. However, all degradation products observed in this pathway are identical to already known metabolites (AMPA) or represent natural products commonly available in bichemical cycles (sarcosine, glycine, formaldehyde, glyoxylate). Further investigation in addition to the information already available are not necessary to describe the metabolism of glyphosate in terms of residues.

The sensitivity of different plant species to glyphosate was investigated by Reddy et al (2008, ASB2012-12463). Besides these phytotoxic effects it was investigated, if the metabolism of glyphosate into AMPA is a common factor in the natural resistance of plants against glyphosate. Although non-tolerant crops (especially soya beans) showed increased concentrations of shikimic acid, no correlation of glyphosate or AMPA concentration to the tolerance were found.

The uptake of glyphosate into maize seedlings was investigated by Wagner *et al.* (2003, ASB2012-12484) using ^{14}C-radiolablled active substance. The seedlings were grown in ^{14}Cglyphosate solutions with concentrations of 0-30 mg as/kg). After 26h of exposure the plants were transferred into fresh nutrient solution and grown for 5 additional days. The glyphosate uptake was observed to be 11 % of the theoretical mass flow. If more than 0.6µg/g glyphosate were observed, a decrease in the growth was observed. It could be shown, that radioactivity taken up by the plants was mainly located in the new leaves, suggesting symplastic distribution in the plants.

B.7.6.6.6 Public literature

A study published by Ando C. et al (2003, ASB2012-12350) investigated the residue situation on typical herbs collected by native Americans in the California National Forest. The results are not related to the representative uses and the situation in the EU.

Arregui et al (2004, ASB2012-12351) reported monitoring results of glyphosate residues in transgenic soya beans from Argentina from 1997-1999. An additional study was provided by Lorenzatti et al (2004, ASB2012-12448). The representative uses evaluated within this document do not involve transgenic plants or import tolerances. The respective study was not taken into account.

In Denmark, the residue levels of glyphosate in cereal grain were monitored 1998 and 1999 by Granby et al (2001, ASB2012-12423). In 1998the average concentration was 0.08 mg/kg (n=49) and increased to 0.11 mg/kg in 1999 (n=46). No MRL violations were identified.

B.7.7.3 Open literature

The influence of glyphosate residues during malting after desiccation of barley was investigated by Caierao et al (2007, ASB2012-12382). The residue concentration of glyphosate showed no effect to the malting of barley.

Low et al (2005, ASB2012-12449) investigated the inpact of Saccharomyces cerevisiae on the stability of glyphosate during bread leavening. It was shown that the approximately 20 % of the initial glyphosate concentration was degraded within 1 hour. However, no analysis on the metabolites formed was conducted.

B.7.8.4 Public literature

In paper primarily dealing with determinations of glyphosate in the urine of humans and cattle, Krüger *et al.* (2014, ASB2014-5024) reported data from Danmark also including findings in various tissues. The samples obtained from cows on “conventional husbandry” (compared to “organic husbandry”) were

analysed by means of a not further specified ELISA (Abraxis, USA) but an LOD or LOQ were not mentioned. No numeric values such as mean and standard deviation are given in this very brief paper but only figures. Based on these figures, maximum glyphosate concentrations of up to 0.06 mg/kg in kidney (n = 26), 0.04 mg/kg in liver (n = 41) and 0.02 mg/kg in muscle (n = 6) can be estimated.
The study confirms the findings from livestock animal feeding studies that liver and kidney are the target tissues for glyphosate residues. However, since no linkage to a potentical dietary burden can be made, the study is of limited value for the estimation of maximum residue levels in animal commodities or the dietary intake assessment.

B.7.15.3 Public literature

The chronic dietary intake of numerous pesticides was investigated by Nougadère, A. et al (2011, ASB2012-11982) to introduce a ranking and scoring method for the active substances. The exposure of the Cammeroonian against glyphosate was investigated in a total diet study by Gimou et al (2008, ASB2012-12422). Harris C. et al published a case study to predict the chronic dietary intake of glyphosate based on several intake models in 2004 (2004, ASB2012-12428).
In view of the representative use based evaluation of glyphosate within this document, the provided literature was not applicable to support the dietary intake assessment for glyphosate.