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Glyphosate

Volume 3 – B.7.1 – B.7.4 (AS)

Rapporteur Member State : Assessment Group on Glyphosate (AGG) consisting of FR, HU, NL and SE

Version History

When	What
2021/06	Initial RAR

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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B.7. RESIDUE DATA

B.7.1. STORAGE STABILITY OF RESIDUES

B.7.1.1. Honey

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:	Conclusion applicant : The study is considered to be acceptable (Category 1) Conclusion RMS : The study is considered to be acceptable

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.

Storage stability in honey is part of the study, which reports ILV of method ME-2220-01. The detailed validation of the method is evaluated in the RAR Volume B-5.

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 : 0, 1, 3 and 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 (See Volume 3, B-5)). 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 ionization (HPLC-MS/MS).

The limit of quantification (LOQ) of this method in honey 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%. On day 0 five storage stability samples were determined and no extra concurrent recoveries were analyzed.

II. Results and Discussion

In the control samples the residues were always below 30% of the LOQ. The results are presented in the table B.7.1.1-1 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 B.7.1.1-1: 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)

Table B.7.1.1-1: 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)
		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 is acceptable. Stability of glyphosate and AMPA is demonstrated in honey for 6 months.

Residue data were obtained with analytical methods for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. The method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
However, it does not invalidate the stability studies, since for each study, enough data are available to benchmark trends in residue levels.

B.7.1.2. Plants

B.7.1.2.1. Study 1

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:	Conclusion applicant : The study is considered to be acceptable (Category 2a) Conclusion RMS : The study is considered to be acceptable

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 (2 years).

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 (purchased at a local supermarket)
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 (homogenized whole fruit) stored at ≤ -18°C was investigated. Duplicate samples (homogenized) 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 (See Volume 3, B-5). 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 in investigates matrices.
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 B.7.1.2.1-1: 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)

Table B.7.1.2.1-1: 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)
		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: The study is acceptable. Stability of glyphosate and AMPA is demonstrated in oranges at least for 24 months.
Residue data were obtained with analytical methods for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. The method is considered acceptable to demonstrate storage stability (RAR Volume 3, B-5)

B.7.1.2.2. Study 2

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:	Conclusion applicant : The study is considered to be acceptable (Category 2a) Conclusion RMS : The study is considered to be acceptable

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. 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.
Homogenized 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 (see Volume 3, B-5). 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 B.7.1.2.2-1: 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*

Table B.7.1.2.2-1: 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 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 [2]	**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*

Table B.7.1.2.2-1: 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] (%)*
		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 B.7.1.2.2-1: 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*

Table B.7.1.2.2-1: 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] (%)*
		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:
Storage stability study of glyphosate and AMPA in barley (grain and straw), maize (grain) and sugar beet (root and leaves) was investigated. It is noted that the results of the study are fluctuating in several matrices. It is assumed that this is caused by performance of the method (for example extractions problems). For this reason for some matrices no conclusion could be drawn on stability.

For glyphosate in barley grain and straw and maize grain single recoveries were often below 70%. Recoveries at day 0 and further freshly recoveries were also rather low. Since no decline was observed in time, it can be concluded that glyphosate is stable in barley grain and straw and maize grain for 18 months.
Glyphosate in sugar beet root is stable at least for 18 months and in sugar beet leaves decline was observed in residues after 12 months. It is noted however, that this decline is of no more than 30%.

In barley grain for metabolite AMPA an exact 30% decline in stability is observed compare to day 0. Therefore, it is concluded that AMPA is stable in barley grain up to 12 months and not 18 months as stated by the applicant. It is noted that for metabolite AMPA extraction problems in stored samples were reported by the applicant in barley straw. Taking into account that for procedural recoveries results for AMPA in straw are acceptable, the applicant's explanation is accepted. However, due to extractions problems it cannot be concluded on overall stability in barley straw, since reported stability was only 50-37%.

In maize grain AMPA was demonstrated to be stable for 18 months. In sugar beet roots decline of stability is observed, comparable to barley grain (also high starch content matrix) and stability is demonstrated up to 12 months and not 18 months as stated by the applicant. In sugar beet leaves on the other hand, a single decline is measure at 12 months of storage, with acceptable fresh recoveries. No explanation has been given by the applicant. Since after 18 months interval no decline is observed, it is concluded that AMPA is stable in this period of time.

Residue data were obtained with analytical methods which is considered acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.3. Study 3

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:	Conclusion applicant : The study is acceptable (category 2a) Conclusion RMS : The study is considered to be acceptable

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, stover voor N-acetyl AMPA study), 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.

Homogenized 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 validated during the study DuPont-15444 (see Volume 3, B-5). 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 B.7.1.2.3-1: 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,	90, 87	*103*	90, 87 (89)	*102, 99*

Table B.7.1.2.3-1: 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)*
			0.435 (0.442)	(89)			*(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,**	**64, 64**	*75*	80, 74 (77)	*83, 83 (83)*

Table B.7.1.2.3-1: 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)*
			0.322 (0.321)	**(64)**			
		12	0.369, 0.349 (0.359)	74, 70 (72)	*84*	85, 95 (90)	*82, 77 (80)*
	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)*

Table B.7.1.2.3-1: 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	0.580, 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)*
		12	0.379, 0.374 (0.377)	76, 75 (76)	*83*	90, 103 (97)	*78, 78 (78)*

Table B.7.1.2.3-1: 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	0.476, 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	93, 84 (89)	*100*	-	

Table B.7.1.2.3-1: 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)*
			(0.442)				
		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)*
		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	85, 84 (85)	*93*	78, 85 (82)	*104, 103 (104)*

Table B.7.1.2.3-1: 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)*
			(0.424)				
		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,	106, 113	*117*	92, 99 (96)	*111, 119*

Table B.7.1.2.3-1: 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)*
			0.564 (0.546)	(110)			*(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)*
	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-	0	0.444,	89, 85	*100*	-	

Table B.7.1.2.3-1: 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		0.426 (0.435)	(87)			
		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%; Normalized 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%; Normalized 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: The study is considered acceptable. It is noted that glyphosate, N-acetyl glyphosate and AMPA were applied as one solution to investigated matrices. N-acetyl-AMPA was applied separately.
Storage stability of glyphosate, N-acetyl glyphosate and AMPA was demonstrated in maize green, forage and grain for at least 12 months and for 23 months in maize stover, except for N-acetyl glyphosate in maize stover, where stability up to 12 months only was demonstrated. It is also noted that recovery for AMPA in maize stored at the 23 month interval was 68%. However, fresh recovery at that time point was also lower compared to previous timepoint and therefore stability for 23 months is acceptable. For N-acetyl-AMPA stability was demonstrated for at least 23 months in investigated matrices.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.4. Study 4

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:	Conclusion applicant : The study is acceptable (category 2a) Conclusion RMS : The study is acceptable

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.
Homogenized 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 (see Volume 3, B-5). 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 B.7.1.2.4-1: 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*	-	-

Table B.7.1.2.4-1: 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] (%)*
		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 (0.502)	95, 106 (101)	*94*	98, 99 (99)	*96, 108 (102)*
		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)*

Table B.7.1.2.4-1: 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] (%)*
	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*	-	-

Table B.7.1.2.4-1: 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] (%)*
		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)*
		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	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)*

Table B.7.1.2.4-1: 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] (%)*
		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	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 (0.543)	116, 102 (109)	*105*	80, 98 (89)	*130, 115 (123)*

Table B.7.1.2.4-1: 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	0.416, 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	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*

Table B.7.1.2.4-1: 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] (%)*
		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: The study is considered acceptable.
It is noted that a mix spiking solution with all analytes was used to fortify plant matrices.

Residues of glyphosate, N-acetyl glyphosate and AMPA are stable for 12 months in soybean forage, seeds. It is noted that for glyphoste in hay recoveries are slightly below 70%. It is observed that in all the sampling points recoveries, including fresh recoveries, are rather low, but stable. It is considered acceptable. Residues of glyphosate and N-acetyl glyphosate are stable for 12 months in soybean hay. Residues of AMPA are stable in soybean hay for 9 months, since at the 12 months interval decline to 66% has been observed.
Residues on N-acetyl AMPA are stable for 18 months in the investigated matrices.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.5. Study 5

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:	Conclusion applicant : The study is acceptable (Category 2a) Conclusion RMS: The study is acceptable

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.
Homogenized 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 (see Volume 3, B-5). 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 B.7.1.2.5-1: 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)*

Table B.7.1.2.5-1: 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)*
		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)*
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*	-	-

Table B.7.1.2.5-1: 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)*
		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 B.7.1.2.5-1: 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: The study is considered acceptable.
It is noted that a mixed spiking solution of all analytes was used to fortify investigated matrices.
Residues of glyphosate and N-acetylglyphosate were stable for at least 12 months in corn forage, grain and stover. Residues of AMPA were stable for at least 12 months in corn forage, grain. In stover stability of AMPA was demonstrated up to 6 months.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.6. Study 6

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:	Conclusion applicant : The study is acceptable (Category 2a) Conclusion RMS : The study is acceptable

2. Full summary of the study according to OECD format

Executive Summary
The storage stability of glyphosate in dry 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 for beans, 0.6 mg/kg for rape seed and 5.6 mg/kg for linseed. 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 homogenized, 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 (see Volume 3, B-5) 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 B.7.1.2.6-1: 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 (2.6 mg/kg fortification)	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 (0.6 mg/kg fortification)	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 (5.7 mg/kg fortification)	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: The study is acceptable. Glyphosate is demonstrated stable in dry beans (high protein matrix), rape seeds and linseeds (oil matrix) for at least 18 months when stored frozen.

Residue data were obtained with analytical methods for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted that extraction efficiency was not sufficiently demonstrated in the methods used. However, it does not invalidate the stability studies, since for each study, enough data are available to benchmark trends in residue levels.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.7. Study 7

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:	Conclusion applicant : Supportive (Category 2a) Conclusion RMS : Supportive information

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. Homogenized 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 (homogenized) 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 (see Volume 3, B-5) 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 B.7.1.2.7-1: 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*
		15	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 B.7.1.2.7-1: 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: The samples were stored at -10ºC, which is a higher temperature than requested following the OECD Guideline. Samples of soybean seeds were not analysed at day 0, but the first analysis was done at day 5. Moreover, recoveries from freshly fortified samples are low, often below 70% and rather inconsistent. It can be concluded that method performance in the study is questionable. Therefore, the study is considered as supportive information.

Residue data were obtained with analytical methods for which acceptable procedural recovery data were generated concurrently with the specimens to-be-analysed. It is noted that extraction efficiency was not sufficiently demonstrated in the methods used. However, it does not invalidate the stability studies, since for each study, enough data are available to benchmark trends in residue levels.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.8. Study 8

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 as supportive information (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant : Supportive (Category 2a) Conclusion RMS : Supportive information

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 of the method) 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	45-9B
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 (see Volume 3, B-5) 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 B.7.1.2.8-1: 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: The samples were stored at -10ºC, which is a higher temperature than requested following the OECD Guideline. Samples of grass were not analysed at day 0, but the first analysis was done at day 6. For glyphosate freshly fortified samples had rather high recoveries (122-244%) and therefore the stability data is not reliable. For metabolite AMPA freshly fortified samples had recoveries within acceptable ranges, however the results of stored samples were inconsistent. It can be concluded that method performance in the study is questionable. Therefore, the study is considered as supportive information.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.9. Study 9

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) or not evaluated
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant : The study is acceptable (Category 3a) Conclusion RMS : The study is acceptable

2. Full summary of the study according to OECD format

Executive Summary

The storage stability of glyphosate (*N*-(phosphonomethyl) glycine anion) 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) fortified samples.

2. Description of analytical procedures

Glyphosate
Analysis was done according to procedures described in Residue Analytical Method 245/02 (see Volume 3, B-5). 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 %.

TMS^+
Samples were analysed according to procedures described in RR 93-105B with alternative gas chromatography conditions. TMS^+ was extracted by maceration in phosphate buffer. An aliquot was treated with basic anion exchange resin, phenylisocyanate and barium hydroxide. After filtration the extract was cleaned up by cation exchange resin. Subsequent addition of strong alkali and further heating converted TMS^+ to dimethylsulphide (DMS), which was collected into toluene. Final quantitative determination of DMS was by gas chromatography. The LOQ of the method was 0.05 mg/kg.

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 B.7.1.2.9-1: 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

Table B.7.1.2.9-2: Storage stability of trimethylsulphonium cation (TMS^+) 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)	TMS^+	0	0.48 ; 0.42 ; 0.53 (0.48)	96 ; 84 ; 106 (95)	-	87 ; 89
		6	0.46 ; 0.52 ; 0.54 (0.51)	92 ; 104 ; 108 (101)	-	90 ; 97
		12	0.47 ; 0.5, 0.5 (0.49)	94 ; 100 ; 100 (98)	-	107 ; 111

[1] Residues have been rounded to two significant figures

[2] Fortification level of 0.50 mg/kg

III. Conclusion

In this study, glyphosate (N-(phosphonomethyl)glycine and trimethylsulphonium cation were 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. 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: The study is accepteble. The applicant in the original submission did not include data of trimethylsulphonium cation (TMS^+). For the completenes all the results were reported by RMS (Table B.7.1.2-9b). Investigated samples of glyphosate are stable in banana for 12 months.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.10. Study 10

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:	Conclusion applicant : The study is acceptable (category 2a) Conclusion RMS : The study is acceptable

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) (grain is 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 (see Volume 3, B-5).
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 LOQ of the method for glyphosate was 0.1 mg/kg and for AMPA 0.05 mg/kg.

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 B.7.1.2.10-1: 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*

Table B.7.1.2.10-1: 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)	**0.328**	**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*
		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: The study is consider acceptable.
RMS is of the opinion that in this study stability of glyphosate in wheat grain and rye grain is demonstrated for 45 months.
It is noted, that in wheat grain at 21 months recovery is 65% however at that timepoint also fresh recovery is low (57%), which explains the low recovery of stored sample. Additionally, in rye grain sample, stability at 21 months is demonstrated with acceptable data. At 45 months recovered stability in both wheat and rye grain is

68-69%, with the acceptable fresh recoveries. However, taking into account all the investigated timepoint, no decline in grain is observed.
Residues of glyphosate are demonstrated to be stable up to 45 months in grain and 45 months in straw.
Residues of AMPA are demonstrated to be stable for maximum 10 months in cereal grain and 6 months in cereal straw, since low recoveries especially is straw were reported after 10 months of storage.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.11. Study 11

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	Yes
Acceptability/Reliability:	Conclusion applicant : The study is acceptable (category 2a) Conclusion RMS : The study is acceptable

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.

Besides the investigation of exogeneous residues, samples from various supervised field trials were frozen and analysed for the endogeneous residues present after several months. Because endogenous AMPA residues were very low in all five matrices, two additional crops alfalfa seed and potatoes were added to the study in order to generate additional endogenous residue data.

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 forage, sorghum stover (straw), clover, tomatoes Incurred residues: Maize, soybean forage, sorghum stover (straw), clover, tomatoes, alfalfa, potatoes	
	Type:	Maize, sorghum: Cereals Tomatoes: Fruiting vegetables Soybean forage 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 triplicate).

2. Description of analytical procedures

All samples were analysed using the analytical method based on the well-established method DFG 405 (see Volume 3, B-5). 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.

In addition to the mean values of the stored exogenously spiked samples (as presented in the table B.7.1.2-11b in original dossier submission) the study report allows the recalculation based on single values, given in Appendix D of the study report. As the results differ from the mean values in the report an additional table is given (Table B.7.1.2-11c).
The mean values for exogenously spiked samples as residues in stored samples in mg/kg and as % recovery of the nominal spiking level were newly calculated from the single results of the measurements reported in Appendix D (italic) of the report. Consequently, % recovery of initial value at day 0 and % recovery corrected for procedural recoveries are updated (italic).

Table B.7.1.2.11-1: 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

Table B.7.1.2.11-1: 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 (%)
		61 (5)	2.99	~~85~~	80.7
		75 (6)	2.74	~~78~~	73.2
	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

** RMS disregarded this data, since it has not been accepted to define residues measured at the first timepoint as 100%.

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 B.7.1.2.11-2: 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	0.377	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*

Table B.7.1.2.11-2: 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	0.340	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*
	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*

Table B.7.1.2.11-2: 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)*
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*

Table B.7.1.2.11-2: 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	0.391	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*

[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.

Table B.7.1.2.11-3: 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 (*0.5*)	0	0.39924, 0.37851, 0.40587, 0.42023, 0.42430, 0.43106 *(0.410)*	*79.85, 75.70, 81.17, 84.05, 84.86, 86.21 (82.0)*	*100*	79.85, 75.70, 81.17, 84.05, 84.86, 86.21 *(82.0)*	*100*
		1 (34)	0.39303, 0.37933, 0.41490 (*0.396*)	*78.61, 75.87, 82.98 (79.2)*	*97*	75.53, 81.15, 78.38 *(78.4)*	*101*
		3 (95)	0.39950, 0.39495 *(0.397)*	*79.90, 78.99 (79.4)*	*97*	81.68, 74.38 *(78.0)*	*102*
		6 (181)	0.33011, 0.32138 (*0.326)*	***66.02, 64.28 (65.1)***	*79*	**59.94, 61.53** ***(60.7)***	*107*
		9 (271)	0.27744, 0.30647 (*0.292*)	***55.49, 61.29 (58.4)***	*71*	**60.56, 66.02** ***(63.3)***	*92*
		12 (376)	0.34160, 0.33209 *(0.337)*	***68.32, 66.42 (67.4)***	*82*	**69.86, 67.33** ***(68.6)***	*98*
		18 (528)	0.35388, 0.26388 *(0.309)*	*70.78,* ***52.78 (61.8)***	*75*	**69.37** ***(69.4[2])***	*89*
		24 (742)	0.37624, 0.33226 (*0.354)*	*75.25, 66.45 (70.9)*	*86*	73.48, 70.54 *(72.0)*	*98*
		31 (944)	0.24964, 0.32429 *(0.287)*	***49.93, 64.86 (57.4)***	*70*	81.18, 69.49 *(75.3)*	*76*
	AMPA	0	0.36167, 0.40537, 0.35252, 0.41806, 0.40006, 0.43042 *(0.395)*	*74.65, 83.67, 72.76, 86.29, 82.67, 88.84 (81.5)*	*100*	74.65, 83.67, 72.76, 86.29, 82.67, 88.84 *(81.5)*	*100*
		1 (34)	0.44659, 0.45257,	*92.18, 93.41,*	*117*	72.75, 73.65,	*129*

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)***
			0.48621 *(0.462)*	*100.35 (95.3)*		74.94 *(73.8)*	
		3 (95)	0.43879, 0.40050 *(0.420)*	*90.57, 82.66 (86.6)*	*106*	**68.82, 57.29** *(63.1)*	*137*
		6 (181)	0.41493, 0.40827 (*0.412)*	*85.64, 84.27 (85.0)*	*104*	**63.05, 67.01** ***(65.0)***	*131*
		9 (271)	0.41863, 0.45460 *(0.437)*	*86.40, 93.83 (90.1)*	*111*	67.49, 71.18 *(69.3)*	*130*
		12 (376)	0.35097, 0.38796 *(0.369)*	*72.44, 80.07 (76.3)*	*94*	72.57, 71.21 *(71.9)*	*106*
		18 (528)	0.47231, 0.43306 *(0.453)*	*97.48, 89.38 (93.4)*	*1115*	72.85 (*72.9*[2])	*128*
		24 (742)	0.51688, 0.47690 *(0.497)*	*106.68, 98.43 (102.6)*	*126*	83.39, 79.66 *(81.5)*	*126*
		31 (944)	0.35502, 0.40079 *(0.378)*	*73.28, 82.72 (78.0)*	*96*	76.12, 65.22 *(70.7)*	*110*
Soya bean forage	Glyphosate	0	0.35722, 0.36537, 0.40999 *(0.378)*	*71.44, 73.07, 82.00 (75.5)*	*100*	71.44, 73.07, 82.00 *(75.5)*	*100*
		1 (27)	0.46264, 0.44040, 0.46341 *(0.455)*	*92.53, 88.08, 92.68 (91.1)*	*120*	81.73, 87.00, 82.59 *(83.8)*	*109*
		3 (92)	0.40712, 0.39804 *(0.403)*	*81.42, 79.61 (80.5)*	*107*	78.22, 76.18 *(77.2)*	*104*
		6 (182)	0.40604, 0.43011 *(0.418)*	*81.21, 86.02 (83.6)*	*111*	81.03, 79.18 *(80.1)*	*104*
		9 (274)	0.41352, 0.40875 *(0.411)*	*82.70, 81.75 (82.2)*	*109*	76.15, 79.04 *(77.6)*	*106*

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)***
		12 (379)	0.43363, 0.43193 *(0.433)*	*86.73, 86.39 (86.6)*	*114*	74.46, 75.08 *(74.8)*	*116*
		18 (531)	0.45009, 0.46203 *(0.456)*	*90.02, 92.41 (91.2)*	*121*	78.74, 81.66 *(80.2)*	*114*
		24 (743)	0.45469, 0.46251 *(0.459)*	*90.94, 92.50 (91.7)*	*121*	83.95, 79.52 *(81.7)*	*112*
		31 (958)	0.31134, 0.30924 *(0.310)*	***62.27, 61.85 (62.1)***	*82*	79.73, 80.82 *(80.3)*	*77*
	AMPA	0	0.32885, 0.33739, 0.35349 *(0.340)*	*67.88, 69.64, 72.96 (70.2)*	*100*	67.88, 69.64, 72.96 *(70.2)*	*100*
		1 (27)	0.38126, 0.36609, 0.41239 *(0.387)*	*78.69, 75.56, 85.12 (79.8)*	*114*	79.78, 81.93, 79.56 *(80.4)*	*99*
		3 (92)	0.36776, 0.36655 *(0.367)*	*75.91, 75.66 (75.8)*	*108*	79.95, 77.71 *(78.8)*	*96*
		6 (182)	0.33458, 0.36770 *(0.351)*	*69.06, 75.89 (72.5)*	*103*	81.65, 80.38 *(81.0)*	*89*
		9 (274)	0.29612, 0.29533 *(0.296)*	***61.12, 60.96 (61.0)***	*87*	75.85, 78.34 *(77.1)*	*79*
		12 (379)	0.32983, 0.32791 *(0.329)*	*68.08, 67.68 (67.9)*	*97*	72.66, 73.15 *(72.9)*	*93*
		18 (531)	0.34724, 0.34852 *(0.348)*	*71.67, 71.93 (71.8)*	*102*	77.72, 81.62 *(79.7)*	*90*
		24 (743)	0.35271, 0.36732 *(0.360)*	*72.80, 75.81 (74.3)*	*106*	93.70, 88.26 *(91.0)*	*82*
		31 (958)	0.22418 ,0.23118 *(0.228)*	***46.27, 47.72 (47.0)***	*67*	74.62, 82.48 *(78.6)*	*60*

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)***
Clover	Glyphosate	0	0.40570, 0.39971, 0.40461 *(0.403)*	*81.14, 79.94, 80.92 (80.7)*	*100*	81.14, 79.94, 80.92 *(80.7)*	*100*
		1 (28)	0.41917, 0.39365, 0.47824 *(0.430)*	*83.83, 78.73, 95.65 (86.1)*	*107*	88.33, 87.12, 80.38 *(85.3)*	*101*
		3 (96)	0.42359, 0.37282 *(0.398)*	*84.72, 74.56 (79.6)*	*99*	81.56, 80.44 *(81.0)*	*98*
		6 (183)	0.38336, 0.40914 *(0.396)*	*76.67, 81.83 (79.3)*	*98*	78.40, 76.65 *(77.5)*	*102*
		9 (273)	0.31237, 0.39254 *(0.352)*	*62.47, 78.51 (70.5)*	*87*	80.08, 75.81 *(77.9)*	*90*
		12 (376)	0.42592, 0.39795 *(0.412)*	*85.18, 79.59 (82.4)*	*102*	75.16, 71.24 *(73.2)*	*113*
		18 (530)	0.35954, 0.36234 *(0.361)*	*71.91, 72.47 (72.2)*	*89*	78.00, 77.76 *(77.9)*	*93*
		24 (741)	0.41715, 0.38619 *(0.402)*	*83.43, 77.24 (80.3)*	*100*	84.53, 82.93 *(83.7)*	*96*
		31 (944)	0.50409, 0.53311 *(0.519)*	*100.82, 106.62 (103.7)*	*129*	98.61, 97.80 *(98.2)*	*106*

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	0.41254, 0.35940, 0.42975 *(0.401)*	*85.15, 74.18, 88.70 (82.7)*	*99*	85.15, 74.18, 88.70 *(82.7)*	*100*
		1 (28)	0.35169, 0.34597, 0.34320 *(0.347)*	*72.59, 71.41, 70.84 (71.6)*	*86*	93.15, 85.58, 78.06 *(85.6)*	*84*
		3 (96)	0.32486, 0.28070 *(0.303)*	***67.05, 57.94 (62.5)***	*75*	71.69, 68.23 *(70.0)*	*89*
		6 (183)	0.32984, 0.33751 *(0.334)*	***68.08, 69.66 (68.9)***	*83*	84.84, 86.49 *(85.7)*	*80*
		9 (273)	0.22211, 0.26183 *(0.242)*	***45.84, 54.04 (49.9)***	*60*	79.48, 74.77 *(77.1)*	*65*
		12 (376)	0.27293, 0.26227 *(0.268)*	***56.33, 54.13 (55.2)***	*66*	74.34, 71.77 *(73.1)*	*76*
		18 (530)	0.23838, 0.24814 *(0.243)*	***49.20, 51.22 (50.2)***	*60*	79.42, 78.46 *(78.9)*	*64*
		24 (741)	0.28245, 0.27150 *(0.277)*	***58.30, 56.04 (57.2)***	*69*	84.95, 84.43 *(84.7)*	*68*
		31 (944)	0.25029, 0.22436 *(0.237)*	***51.66, 46.31 (49.0)***	*59*	89.55, 87.57 *(88.6)*	*55*
Tomatoes	Glyphosate	0	0.35886, 0.40693, 0.36576 *(0.377)*	*71.77, 81.39, 73.15 (75.4)*	*100*	71.77, 81.39, 73.15 *(75.4)*	*100*
		1 (34)	0.42468, 0.40448, 0.40871 *(0.413)*	*84.94, 80.90, 81.74 (82.5)*	*109*	83.59, 80.87, 70.76 *(78.4)*	*105*
		3 (91)	0.38455, 0.36591 *(0.375)*	*76.91, 73.18 (75.0)*	*100*	80.24, 76.42 *(78.3)*	*96*

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)*
		6 (188)	0.45147, 0.40152 *(0.426)*	*90.29, 80.30 (85.3)*	*113*	78.59, 83.40 *(81.0)*	*105*
		9 (272)	0.39191, 0.36684 *(0.379)*	*78.38, 73.37 (75.9)*	*101*	79.75, 78.30 *(79.0)*	*96*
		12 (379)	0.39571, 0.39024 *(0.393)*	*79.14, 78.05 (78.6)*	*104*	76.68, 78.61 *(77.6)*	*101*
		18 (528)	0.42761, 0.42144 *(0.425)*	*85.52, 84.29 (84.9)*	*113*	85.45, 85.27 *(85.4)*	*99*
		24 (735)	0.43059, 0.41645 *(0.424)*	*86.12, 83.29 (84.7)*	*112*	82.04, 81.54 *(81.8)*	*104*
		31 (938)	0.42877, 0.42601 *(0.427)*	*85.75, 85.20 (85.5)*	*113*	84.02, 79.00 *(81.5)*	*105*
	AMPA	0	0.31887, 0.34990, 0.31150 *(0.327)*	***65.81, 72.22, 64.29 (67.4)***	*100*	**65.81, 72.22, 64.29 *(67.4)***	*100*
		1 (34)	0.34341, 0.32634, 0.35006 *(0.340)*	*70.88, 67.36, 72.25 (70.2)*	*104*	**71.85, 67.39, 60.72 *(66.7)***	*105*
		3 (91)	0.35074, 0.32926 *(0.340)*	*72.39, 67.96 (70.2)*	*104*	73.42, 72.02 *(72.7)*	*97*
		6 (188)	0.41822, 0.39531 *(0.407)*	*86.32, 81.59 (84.0)*	*124*	67.22, 78.05 *(72.6)*	*116*
		9 (272)	0.34095, 0.31420 *(0.328)*	*70.37* ***64.85 (67.6)***	*100*	75.49, 73.69 *(74.6)*	*91*
		12 (379)	0.34038, 0.34142 *(0.341)*	*70.25, 70.47 (70.4)*	*104*	**68.30, 69.87 *(69.1)***	*102*
		18 (528)	0.38387, 0.37742 *(0.381)*	*79.23, 77.90 (78.6)*	*116*	84.16, 82.55 *(83.4)*	*94*

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)***
		24 (735)	0.36861, 0.35366 *(0.361)*	*76.08, 72.99 (74.5)*	*110*	83.11, 80.34 *(81.7)*	*91*
		31 (938)	0.35219, 0.36027 *(0.356)*	*72.69, 74.36 (73.5)*	*109*	75.27, 72.98 *(74.1)*	*99*
Sorghum stover/straw	Glyphosate	0	0.45551, 0.46855 *(0.462)*	*91.10, 93.71 (92.4)*	*100*	91.10, 93.71 *(92.4)*	*100*
		1 (29)	0.57284, 0.53433, 0.54969 *(0.552)*	*114.57, 106.87, 109.94 (110.5)*	*120*	79.29, 78.81, 77.09 *(78.4)*	*141*
		3 (92)	0.57924, 0.58437 *(0.582)*	*115.85, 116.87 (116.4)*	*126*	84.90, 120.59 *(102.7)*	*113*
		6 (182)	0.54267, 0.55417 *(0.548)*	*108.53, 110.83 (109.7)*	*119*	79.45, 76.84 *(78.1)*	*140*
		9 (274)	0.57775, 0.55989 *(0.569)*	*115.55, 111.98 (113.8)*	*123*	70.52, 78.56 *(74.5)*	*153*
		12 (377)	0.52466, 0.58576 *(0.555)*	*104.93, 117.15 (111.0)*	*120*	71.87, 75.47 *(73.7)*	*151*
		18 (531)	0.56104, 0.59172 *(0.576)*	*112.21, 118.34 (115.3)*	*125*	84.44, 83.90 *(84.2)*	*137*
		24 (743)	0.55589, 0.55884, 0.55845 *(0.558)*	*111.18, 111.77, 111.69 (111.5)*	*121*	85.79, 75.83 *(80.8)*	*138*
		31 (958)	0.48323, 0.60493 *(0.544)*	*96.65, 120.99 (108.8)*	*118*	75.58, 71.40 *(73.5)*	*148*
	AMPA	0	0.39501, 0.38633 *(0.391)*	*81.53, 79.74 (80.6)*	*100*	81.53, 79.74 *(80.6)*	*100*
		1 (29)	0.36957, 0.39679,	*76.28, 81.90,*	*100*	74.15, 74.22,	*110*

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)*
			0.40430 *(0.390)*	*83.45* *(80.5)*		71.51 *(73.3)*	
		3 (92)	0.40762, 0.39346 *(0.401)*	*84.13,* *81.21* *(82.7)*	*102*	78.54, 158.32 *(118.4)*	*70*
		6 (182)	0.32634, 0.32262 *(0.324)*	***67.36,*** ***66.59*** ***(67.0)***	*83*	82.86, 79.04 *(81.0)*	*83*
		9 (274)	0.33414, 0.33673 *(0.335)*	***68.97,*** ***69.50*** ***(69.2)***	*86*	73.89, 77.97 *(75.9)*	*91*
		12 (377)	0.27355, 0.29075 *(0.282)*	***56.46,*** ***60.01*** ***(58.2)***	*72*	68.05, 70.27 *(69.2)*	*84*
		18 (531)	0.30014, 0.30000 *(0.300)*	***61.95,*** ***61.92*** ***(61.9)***	*77*	82.36, 79.85 *(81.1)*	*76*
		24 (743)	0.32532, 0.28841, 0.32996 *(0.315)*	***67.15,*** ***59.53,*** ***68.10*** ***(64.9)***	*80*	82.37, 79.51 *(80.9)*	*80*
		31 (958)	0.19980, 0.22573 *(0.213)*	***41.24,*** ***46.59*** ***(43.9)***	*54*	72.64, 65.51 *(69.1)*	*64*

All newly calculated values are displayed in *italic*.

[1] Fortification level of 0.5 mg/kg for glyphosate and 0.4845 mg/kg 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.

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.
When single values, given in Appendix D of the study report, are assessed and the mean values are re-calculated, the results differ from the mean values in the report. Both mean values are presented in two separate tables in this summary. Notwithstanding, the above conclusion about the storage stability of glyphosate and AMPA in the analyzed commodities does not change and the stability for glyphosate and decline for AMPA at longer storage times can still clearly be seen.

Assessment and conclusion by RMS:
In this study storage stability of (incurred and fortified) glyphosate metabolite AMPA was investigated in different matrices.
Reported stability in incurred samples for both compounds cannot be accepted, since there is no measurement of residues at day 0 of storage. First reported measurement of residues is for most of the samples between 13 to 17 months (in some cases 2-4 months). Therefore, a potential decline in the first storage period cannot be determined. RMS does not accept an approach to set the first measurement as a "day 0" data for incurred residues.

Storage stability was also investigated after fortification with 0.5 mg/kg of glyphosate or AMPA. On request of RMS the applicant updated the evaluation with individual measurements for each replicate (Table B.7.1.2-11-3, based on Appendix D of the study report). **It is noted that conclusions were made on the updated data: Table B.7.1.2-11-3. However all data submitted by the applicant has been included for transparency.**

No conclusion can be drawn on stability of glyphosate in maize grain. After 6 months of storage recoveries were below acceptable ranges, however it is noted that fresh recoveries were also not acceptable, which suggest analytical method issues after 6, 9, 12 and 18 months of storage. After 24 and 31 months fresh recoveries were acceptable and at 31 months a clear decline of stability is observed.
Glyphosate was stable for 24 months (after 31 months decline was observed) in soybean forage, for 31 months in clover, tomatoes, sorghum stover (with stored recoveries on higher, but acceptable level).

No conclusion can be drawn on stability of AMPA in maize grain, since results were inconsistent through the study.
In soybean forage (high water matrix) a low level of recovered stability was measured already at the day 0 (70%). Taking this into account, no clear decline of more than 30% was observed up to 24 months, however it is noted that at 9 months of storage 61% residues were recovered. A decline of stability was observed after 31 months, where 47% was recovered with acceptable fresh recoveries.
In clover fresh recoveries were acceptable at all timepoints and a clear decline of stability was observed after 3 months of storage, where only 60.5% of residues were recovered. Decline was further observed up to 46.9% at 31 months. In tomato similar situation as in soybean forage was observed. Low fresh recoveries were measured, starting with day 0: 67.4%. Stored recoveries were also rather low, however no decline was observed in the time of the study. It could be concluded that AMPA was stable in tomatoes for 31 months.
In sorghum stover/straw AMPA is demonstrated unstable based on the reported stability recoveries. Already at 1 month of storage recovery was 65.8% and at 31 months 25.6%. Fresh recoveries were within acceptable ranges (with two values at 69%) at all timepoints.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.2.12. Study 12

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:	Conclusion applicant: Valid (Category 2a)

	Conclusion RMS: Acceptable, with remarks (see evaluation RMS below)

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 mg/kg glyphosate and AMPA, except for sorghum which was fortified at 5 mg/kg. 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 (see Volume 3, B-5). Samples were extracted with water. The extract was cleaned up using a cation exchange column, the analytes were converted to a f1uorescing 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 B.7.1.2.12-1: 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*

Table B.7.1.2.12-1: 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*
		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 (69.6)	*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: Storage stability of glyphosate and AMPA were investigated in the study.
In soybean (seed, straw) glyphosate and AMPA were stable for 24 months.
In sorghum grain both glyphosate and AMPA were stable for 48 month. Whereas in wheat grain glyphosate stability was demonstrated up to 24 months (no further measurements were done)
For AMPA a lower recovery can be seen at period of 12 months (69.6%). No freshly spiked procedural recoveries were analysed for all investigated matrices, therefore, it is not clear of there is a decline in stability of AMPA in wheat grain or an influence of method performance. Recoveries in day 0 can be considered as method performance test for the study, therefore it can be seen that there are still acceptable recoveries for AMPA in wheat grain at 24 months.
Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.3. Animals

B.7.1.3.1. Study 1

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	MSL-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:	Conclusion applicant: The study is acceptable (Category 2a) Conclusion RMS: The study is acceptable.

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. All tissues and eggs were purchased from local supermarkets and raw cow milk was obtained from Monsanto's Dardenne Farm for this storage stability study.
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

Fat and muscle (swine, cows, chicken) were fortified at 0.2 mg/kg glyphosate and 0.05 mg/kg of AMPA, liver (swine) was fortified with 0.8 mg/kg glyphosate and 0.1 mg/kg AMPA, liver (cows) was fortified with 4 mg/kg of glyphosate and 0.5 mg/kg AMPA, liver (chicken) was fortified with 2 mg/kg glyphosate and 0.25 mg/kg AMPA, kidney (swine, chicken) was fortified with 4 mg/kg glyphosate and 0.5 mg/kg AMPA, kidney (cows) was fortified with 6 mg/kg glyphosate and 1.5 mg/kg AMPA, milk and eggs were fortified with 0.2 mg/kg glyphosate and 0.05 mg/kg 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 (see Volume 3, B-5). 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 B.7.1.3.1-1: 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*	82, 83, 80 (81)	-
		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*
	AMPA	0	0.0416, 0.0376, 0.0392 (0.0395)	83, 75, 78 (79)	*100*	83, 84, 82 (83)	-
		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*	103, 103, 100 (102)	-
		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*

Table B.7.1.3.1-1: 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)***
		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*	100, 99, 94 (97)	-
		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*
Pig liver	Glyphosate	0	0.678, 0.639, 0.663 (0.660)	85, 80, 83 (83)	*100*	85, 84, 87 (85)	-
		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*	93, 92, 95 (95)	-
		14 (417)	0.0625, 0.0606 (0.0616)	63, 61 (62)	*69*	74, 81 (78)	*79*
		16 (468)	0.0758, 0.0788	76, 79 (77)	*87*	93, 94 (93)	*83*

Table B.7.1.3.1-1: 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)***
			(0.0773)				
		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*	100, 101, 98 (97)	-
		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*
	AMPA	0	0.505, 0.505, 0.504 (0.505)	101, 101, 101 (101)	*100*	99, 102, 96 (99)	-
		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*	83, 91, 93 (89)	-
		6 (175)	0.175, 0.165 (0.170)	88, 83 (85)	*99*	96, 95 (95)	*89*
		12	0.168,	84, 85 (85)	*99*	98, 100	*86*

Table B.7.1.3.1-1: 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)***
		(349)	0.170, (0.169)			(99)	
		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*	75, 85, 90 (83)	-
		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*
Cow muscle	Glyphosate	0	0.176, 0.163, 0.178 (0.172)	88, 82, 89 (86)	*100*	97, 90, 92 (93)	-
		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*	96, 90, 93 (93)	-
		6 (177)	0.0416, 0.0414 (0.0415)	83, 83 (83)	*97*	95, 100 (98)	*85*

Table B.7.1.3.1-1: 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)***
		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*	96, 97, 99 (97)	-
		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*
	AMPA	0	0.466, 0.463, 0.636 (0.522)	93, 93, 127 (104)	*100*	92, 95, 93 (93)	-
		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*	96, 95, 95 (95)	-
		6 (181)	5.221, 5.187	87, 86 (87)	*94*	94, 91 (93)	*94*

Table B.7.1.3.1-1: 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)***
			(5.204)				
		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*	93, 93, 92 (93)	-
		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*
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*	93, 94 (94)	-
		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*

Table B.7.1.3.1-1: 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)***
		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*	91, 94, 91 (92)	-
		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*
	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	87, 87, (87)	*99*	95, 96 (96)	*91*

Table B.7.1.3.1-1: 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)***
			(0.173)				
		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*
Chicken liver	Glyphosate	0	1.766, 1.618, 1.639 (1.674)	88, 81, 82 (84)	*100*	86, 91, 89 (89)	-
		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*	83, 81, 78 (81)	-
		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	0.213,	85, 86 (86)	*115*	109, 110	*79*

Table B.7.1.3.1-1: 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)***
		(474)	0.216 (0.215)			(109)	
		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*	94, 94, 95 (94)	-
		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*
	AMPA	0	0.508, 0.515, 0.506 (0.510)	102, 103, 101 (102)	*100*	102, 107, 104 (104)	-
		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*	86, 90, 91 (89)	-
		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*

Table B.7.1.3.1-1: 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	0.0494, 0.0448, 0.0479 (0.0474)	99, 90, 96 (95)	*100*	93, 99, 98 (97)	-
		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 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: The study is considered acceptable.

It is noted that a mixed solution of glyphosate and AMPA was used to fortify animal matrices.
Based on the study results it is concluded that glyphosate is stable in swine matrices for minimal 26 months, in ruminant matrices for 24 months, in milk for 16 months, for 25 months in chicken fat, muscle and liver and 13 months in kidney. Glyphosate is stable for maximum 14 months in eggs, since a significant decline is observed at later timepoints.
AMPA is considered stable in swine and ruminant tissues, except fat for 26 and 24 months, respectively. In chicken muscle and liver AMPA is stable for 25 months and in kidney for 13 months.
In swine fat a slow decline of stability has been observed and it is concluded that AMPA is stable in that matrix for maximum 15 months. In two other fat matrices (cow and chicken) single recoveries below the 70% were measured. No decline was observed, with storage recoveries at rather low level (70%) starting from 12 months timepoint. It is concluded that AMPA is demonstrated to be stable for 24/25 months in cow/chicken fat.

AMPA is stable for maximum 14 months in eggs, since a significant decline is observed at later timepoints.

Analytical method used in the study has been considered as acceptable for demonstrating storage stability (Volume 3, B-5).

B.7.1.3.2. Study 2 and Study 3

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	Storage stability part of the study was not evaluated in the RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant: Study is acceptable (Category 2a) Conclusion RMS: Acceptable for fortified samples, not acceptable for incurred residue samples.

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	Storage stability part of the study was not evaluated in the RAR (2015)_
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant: Study is acceptable (Category 2a) Conclusion RMS: Acceptable for fortified samples, not acceptable for incurred residue samples. It is noted that method is considered not acceptable.

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 [redacted] methods [redacted] 7-41 and [redacted]87-42 (see Volume 3, B-5). 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 B.7.1.3.2-1: 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 (0.50)	100, 98 (99)	*100*	-
		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*	-

Table B.7.1.3.2-1: 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 (%)
		328 (11)		0.52	104	*93*	
		671 (22)		0.41, 0.39, 0.40 (0.40)	82, 78, 80 (80)	*71*	92
	AMPA	0		0.22	109	*100*	-
		186 (6)	0.2	**0.26, 0.28, 0.23 (0.26)**	**130, 140, 115 (128)**	*118*	-
		328 (11)		0.20	101	*91*	-
		0		0.64	128	*100*	-
		186 (6)	0.5	**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
Cow muscle	Glyphosate	0		0.97, 0.96, 1.00 (0.98)	97, 96, 100 (98)	*100*	-
		182 (6)	1.0	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		0.86, 0.83, 0.86 (0.85)	86, 83, 86 (85)	*100*	-
		182 (6)	1.0	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		0.87, 0.93 0.90 (0.90)	87, 93, 90 (90)	*100*	-
		212 (7)	1.0	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		0.68, 0.93, 0.82 (0.81)	68, 93, 82 (81)	*100*	-
		212 (7)	1.0	1.16, 1.10, 1.10 (1.12)	116, 110, 110 (112)	*138*	-

Table B.7.1.3.2-1: 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 (%)
		687 (23)		1.00, 0.86, 0.86 (0.91)	100, 86, 86 (91)	*112*	90
Cow liver[3]	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*
		182 (6)	5	4.2; 4.5	85; 90	-	-
Cow kidney[3]	Glyphosate	0[3]	-	7.6	-	*100*	-
		604 (20)[3]	-	17.2, 16.5, 17.0 (16.9)	226, 217, 224 (222)	*222*	83
		1304 (43)[3]		17.1, 17.4, 17.2 (17.2)	225, 229, 226 (227)	*226*	94
	AMPA	0[3]	-	1.7	-	*100*	-
		604 (20)[3]	-	2.6, 1.9, 2.5 (2.3)	154, 109, 149 (137)	*135*	87
		1311 (44)[3]		2.9, 2.8, 2.8 (2.8)	170, 167, 166 (168)	*165*	97

[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:
Studies [redacted] 87-43 and [redacted] 87-44 investigate magnitude of glyphosate and AMPA in animal matrices. Part of this study was determination of storage stability in animal tissues: chicken eggs, cow milk and tissues (liver, fat, muscle, kidney). It is noted that storage stability part of the study has not been evaluated in the RAR (2015) as reported by the applicant.
Two sets of storage stability experiments were performed: with fortified and incurred residues.
Samples of eggs, milk, cow muscle and fat were fortified with known concentration of glyphosate and AMPA and were stored up to 23 months. No storage conditions were reported. No freshly fortified samples were measured at 0 and 6 months. However, taking into account that at the day 0 all recoveries were in acceptable range a possible decline could still be determined.
No decline of glyphosate concentration in stored samples has been observed in eggs, cow muscle, cow fat and milk. Stability in those tissues was demonstrated for 22-23 months. It is noted that in milk fortified with 0.2 mg/kg single recoveries at day 0 and 6 months are high (110-128%), however for the second fortification level all recoveries were within acceptable ranges and no decline was observed.

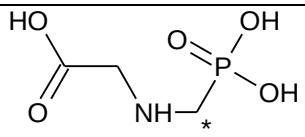
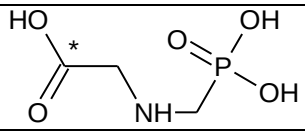
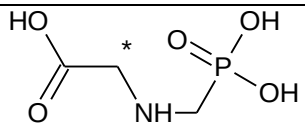
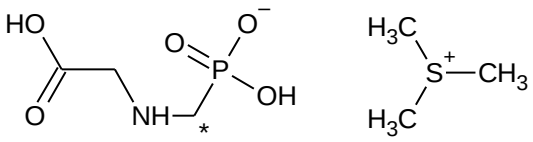
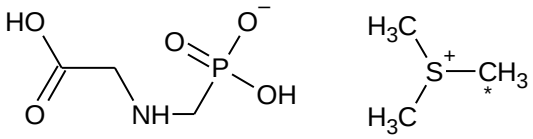
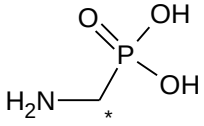
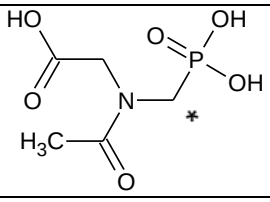
Stability of AMPA in eggs cannot be determined, since at each time point a different fortification concentration was used and therefore results are not comparable.
Stability results of AMPA in milk and liver are not conclusive, since it seems that method performance was not optimal. In milk already at day 0 for 0.5 mg/kg fortification level, recovery was 128%, and at 6 months 64% and acceptable after 11 and 22 months. In cow liver at day 0 also a very low recovery of 59% and therefore proper comparison of results cannot be done.

AMPA is demonstrated to be stable in cow muscle and fat for 23 months.
Stability was also measured for glyphosate in incurred samples of cow liver and kidney after 20 and 44 months of storage and for AMPA in cow kidney for 20 and 44 months. For both analytes storage recoveries in all timepoint are much above the acceptable ranges (166-294%). No fresh recoveries were reported to verify the method performance. Results from the incurred storage samples are consider not acceptable.

It is noted that analytical method used in the studies is not considered acceptable (Volume 3, B-5). The acceptability of the method was not assessed as fit-for-purpose for demonstration of storage stability.

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

Several metabolism studies are available for non-tolerant/conventional plants and tolerant/genetically modified plants. 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. In addition, 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 on the next page. As the naming of labels differs in the different metabolism studies a common naming is used in the summary of this section. The 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-MCX20		
^{14}C-*N*-acetyl glyphosate		• ***N*-acetyl-*N*-(phosphono-^{14}C-methyl)glycine** • [^{14}C]-*N*-acetyl glyphosate

B.7.2.1. Plants

B.7.2.1.1. Non-tolerant plants, fruit crops

B.7.2.1.1.1. Citrus 1

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	Conclusion applicant: supportive (Category 2a) Conclusion RMS: supportive only

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.

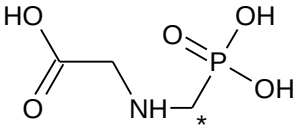
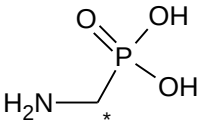
Hydroponic uptake was investigated in a hydroponic solution at 10 mg test substance/kg with ^{14}C-glyphosate or ^{14}C-AMPA.

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 % 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

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

^{14}C-glyphosate and ^{14}C-AMPA 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 x 10^8 dpm (9.88 MBq) applied radioactivity for ^{14}C-glyphosate or 5.6 mg and 1 x 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 x 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 x 10^8 dpm (7.33 MBq), chambers no. 1 and no. 2 or ^{14}C-AMPA: 8.0 x 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 x 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 x 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 x 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 as well as molecular sieve type supports.
The quantification and identification of the residues in the extracts was performed following derivatisation to N-trifluoroacetyl-butyl-esters by GC/MS.
^{13}C NMR spectra of the residues in the purified extracts were obtained.
High voltage electrophoresis was used for characterisation of the extract residues.

Standards of unlabelled glyphosate and AMPA 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 B.7.2.1.1.1-1: 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 (soil), initial: 5.93 x 10^8 dpm (5.6 mg)											
% 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
[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 lst and 2nd week. The decrease of radioactivity in roots between the lst 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 lst week. The removal of ^{14}C-AMPA from the nutrient solution in chamber no. 4 after the lst 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 B.7.2.1.1.1-2: 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 108 dpm	**Chamber 2 (2 weeks) ^{14}C-glyphosate (10 mg/L) 4.4 x 108 dpm**	**Chamber 3 (1 week) ^{14}C-AMPA (10 mg/L) 8.0 x 108 dpm**	**Chamber 4 (2 weeks) ^{14}C-AMPA (10 mg/L) 8.0 x 108 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}CO2				
1 week	2.1	2.9	1.4	0.8
2 weeks	-	0.3	-	0.2
Nutrient ^{14}CO2				
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	-	2.31	-	3.31
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 B.7.2.1.1.1-3: 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	-	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 B.7.2.1.1.1-4: 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 x 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 B.7.2.1.1.1-5: 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 x 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 x 10^6 dpm). The extract from leaves of treated plants contained 1.84 x 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 in Vol. 1, 2.7.2.

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], 1994, CA 6.2.1/022). Additional detailed information on storage stability of glyphosate and its metabolites is available under B.7.1). 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:

The RMS largely agrees with the assessment of the applicant. It is indeed considered as major deficit that hardly any recalculation into quantitative levels of mg/kg was possible. In addition, the unextracted residues should have been further investigated. Therefore, the study is considered to provide only qualitative information on the metabolism of glyphosate after soil, foliar and hydroponic application. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Storage stability of glyphosate and AMPA in orange has been demonstrated for 24 months, thus covering the storage time period in the current study. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which

covers the storage period of max. 21 months of the leaves and stems. Storage stability of AMPA in watery crops is demonstrated for 18 months, which is slightly shorter than the possible max. storage period of 21 months. However, it is not expected that these additional 3 months will strongly impact on the storage stability. And regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. On the other hand, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is not covering the time period of the current study. However, it's not expected that this influences the reliability of the study to a large extent, since the interest of the study is not so much into the roots of the citrus tree. In addition, in several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue. Altogether, storage stability is considered to not impact this metabolism study to a large extent. The observation that no quantitative information can be derived for fruits (being the edible part of the plant), and that the fruits were not even sampled in the transpiration study are also considered important in concluding on this study as 'supportive only'.

B.7.2.1.1.2. Citrus 2

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) RMS: the study cannot be retrieved from the RAR, therefore, it is unclear why the study would not have been accepted.
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Conclusion applicant: valid (Category 3a) Conclusion RMS: acceptable

2. Full summary of the study according to OECD format

Executive summary

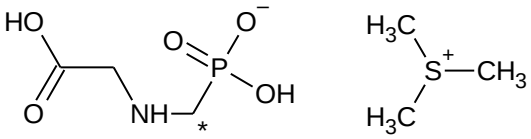
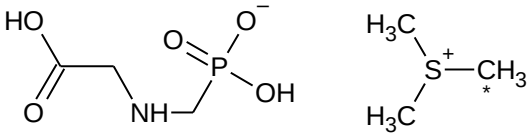
This metabolism study was designed to determine the nature and magnitude of glyphosate-derived residues, resulting from soil uptake after application of 3.918 kg glyphosate acid equivalents/ha to bare soil containing lemon trees. In total 5 lemon trees were used and maintained in large pots throughout 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, characterization 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

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 ^{14}C-PMG-equivalents or ^{14}C-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 characterization.

Table B.7.2.1.1.2-1: 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*	Leachate#	Soil*	Leachate#
		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	---
	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

Table B.7.2.1.1.2-1: 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*	Leachate[#]	Soil*	Leachate[#]
		mg/kg	% ^{14}C	mg/kg	% ^{14}C

DAT days after treatment
* 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.
[#] Leachate is presented as percent of the total ^{14}C applied.

Table B.7.2.1.1.2-2: 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	---	---
	Peel	---	0.009	0.009
	Pulp	---	0.006	0.010
	Juice	---	0.004	0.001
	Leaves	0.003	0.035	0.030

Table B.7.2.1.1.2-3: 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 fruits 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:

RMS agrees that the deficits mentioned by the applicant can be considered minor, and are not expected to have any influence on the study results. No identification took place; although residue levels in leaves are considered sufficiently high for identification, this matrix is not required to be analysed according to OECD guideline 501; it could be discussed whether some lemon fruit samples with residues around 0.01 m/kg could have been further investigated for identification, but this is border-line. Overall, the study provides reliable qualitative information on the metabolism of glyphosate in lemons after soil application. The study is considered acceptable.

B.7.2.1.1.3. Tree nuts

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	Conclusion applicant: supportive (Category 2a) Conclusion RMS: supportive only

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.

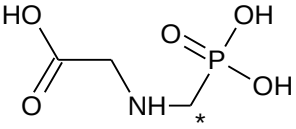
In the soil experiment ^{14}C-glyphosate was applied as an aqueous solution at the rate of 5.07 kg a.s./ha for pecan and walnut and at the rate of 2.43 kg a.s./ha for almonds. In the foliar application experiments, ^{14}C-glyphosate was formulated to simulate the commercial Roundup® formulation and applied at the rate of 100 µg ^{14}C-glyphosate (1.2x 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 % [a]
Specific activity:	1.98 MBq/mg (9.07 mCi/mmol) for foliar application 0.41 MBq/mg (1.87 mCi/mmol) for soil application

a) 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 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 (1.2x 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, lyophilized, and ground.

3. Analytical procedures

Dry plant powders were quantitatively oxidized 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 B.7.2.1.1.3-1: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	% AR
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 [a]	14	60.5	45.0
Walnut, other tops [a]	14	7.3	6.2
Walnut tops (control) [a]	14	(0.02)	(0.04)
Walnut, roots [a]	14	10.4	23.5
Walnut roots (control) [a]	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)	-

[a] 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.
[b] 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 B.7.2.1.1.3-2: 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 characterization 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 derivatization to ^{14}C-glyhosate-N-trifluoroacetyl-trimethyl ester and subsequent GC/MS analysis.

Table B.7.2.1.1.3-3: 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 [a]	14	103
Walnut, other tops [a]	14	85
Walnut, roots [a]	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

[a] 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 B.7.2.1.1.3-4: Distribution of the radioactive residues and characterization 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**
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 characterized	***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 are given in *italics*.
DALT days after treatment
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

Table B.7.2.1.1.3-5: Distribution of the radioactive residues and characterization 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**
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 characterized	*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 finalization 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 ([redacted] MSL_9810).
Therefore, the storage stability is covered.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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 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 finalization 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 [redacted] 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:

RMS considers that there are quite a large amount of deficits in this study, which are already presented by the applicant in the box above. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which covers the storage period of max. 10 months of the leaves and tops. Storage

stability of AMPA in watery crops is demonstrated for 18 months, thereby covering the max. storage period of 10 months. Regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. Storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is also covering the time period of the current study. In addition, in several other metabolism studies, it was shown that degradation of radioactive residues over time was not an issue. Altogether, storage stability is considered to not impact this metabolism study to a large extent. In addition, the finding of significant residues in the control samples of the first experiment, although a reasonable explanation has been provided, further influences the acceptability of the study. However, this study is still considered to provide qualitative information on the metabolism of glyphosate in tree nuts. Overall, the study is considered supportive only.

B.7.2.1.1.4. Apple

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	Conclusion applicant: supportive (Category 2a) Conclusion RMS: supportive only

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.

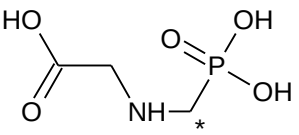
Extractabilities in apple tree commodities after foliar treatment ranged from 71.51 % of the TRR (0.029 mg/kg) in roots and 79.47 % (0.018 mg/kg) in trunk and branches, respectively, at 28 days after application, to 102.59 % TRR in new growth above at day 21. The aqueous extractability of the stem and 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:	
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 % 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 (1.76 MBq/mg)*, 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)

*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 [redacted], 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 % [redacted] and 1.7 % [redacted]. 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 mL (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 B.7.2.1.1.4-1: 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.

Soil uptake experiment:
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.

Trunk uptake experiment:
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.

Foliar uptake experiments:
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.

Isotopic 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 B.7.2.1.1.4-2: 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 B.7.2.1.1.4-3: 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 B.7.2.1.1.4-4: 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

Table B.7.2.1.1.4-4: 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
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	-	2.31	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	*144.31*	*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.

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] 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 B.7.2.1.1.4-5: 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 B.7.2.1.1.4-6: 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 B.7.2.1.1.4-7: 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.[1]**	**n r.[1]**		**n.r.[1]**	**n r.[1]**		**n.r.[1]**	**n.r.[1]**
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
[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 B.7.2.1.1.4-8: 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 B.7.2.1.1.4-9: 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.[1]**	**n.r.[1]**			**n r.[1]**	**n r.[1]**			**n r.[1]**	**n.r.[1]**
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
[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 B.7.2.1.1.4-10: 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 B.7.2.1.1.4-11: 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 in Vol. 1, 2.7.2.

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] 1994, CA 6.2.1/024). Additional detailed information on storage stability of glyphosate and its metabolites is available under B.7.1.
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:
RMS agrees with the assessment of the applicant. The study provides useful information on the metabolism of glyphosate after soil, trunk and foliar application, however, the study has quite some deficits. The observation that the apples (being the edible part of the plant) were not even sampled, and that recoveries and accountabilities were low are considered important in concluding on this study as 'supportive only'. The study only provides qualitative information on glyphosate metabolism.

B.7.2.1.1.5. Grape vines 1

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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

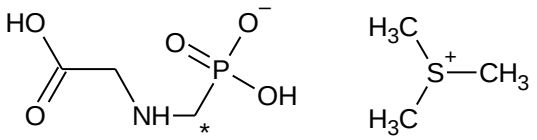
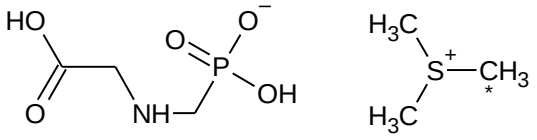
around mature vines 14 days or one year prior to sampling. In an additional experiment, ten bunches of grapes were deliberately oversprayed with ^{14}C-PMG and ^{14}C-TMS-labelled glyphosate-trimesium, at amounts of 14.3 mg and 13.2 mg (9.9 mg and 9.1 mg expressed as glyphosate equivalents), respectively, 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)

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.
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.
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 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

Bad grapes were not analysed. 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 □-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 B.7.2.1.1.5-1: 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 B.7.2.1.1.5-2: 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 B.7.2.1.1.5-3: 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 B.7.2.1.1.5-4: 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 in Vol. 1, 2.7.2.

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:
RMS largely agrees with the assessment of the applicant. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Storage stability of glyphosate and AMPA in orange has been demonstrated for 24 months, thus covering the storage time period in the current study. However, no other storage stability studies in high acid crops are available, which would be required to extrapolate the findings in orange to the whole group of crops with acid matrix including grapes. But since about 80% of the TRR has been identified as glyphosate or AMPA, the deficits on sampling storage in the current metabolism study are not considered to have an impact on the study results. The study is considered acceptable, and considered to provide quantitative information on the metabolism of glyphosate in grape vines.

B.7.2.1.1.6. Grape vines 2

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

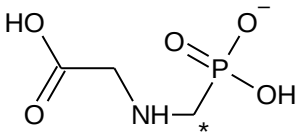
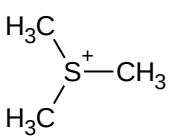
2. Full summary of the study according to OECD format

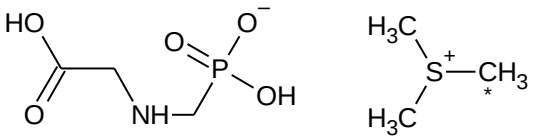
Executive summary

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

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 l.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 trimethylsulfonium (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 tables below).

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 B.7.2.1.1.6-1: 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 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 B.7.2.1.1.6-2: 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 B.7.2.1.1.6-3: 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 in Vol. 1, 2.7.2.

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:
The RMS considers the study as acceptable. Although indeed there were some deviations according to guidelines, these deviations are not expected to influence the study results, as also explained by the applicant. Another argument to conclude that the deviation regarding the non-extraction and no characterization of grape vine leaves, stems and stalks is less relevant, is the fact that these RACs are not required to be analysed when grape metabolism is being studied. In grape metabolism studies, the RAC to be analysed is the fruit. However (not impacting the study results and conclusion), the RMS considers that the TRR in grapes should be calculated differently. Since significant results were obtained in the methanol fractions of the fruit from both labels, the TRR should be calculated as **0.006** mg/kg for the PMG-label, and **0.003** mg/kg for the TMS-label (so the <-symbol should not be used).

B.7.2.1.1.7. Grape vines 3

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: supportive only

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

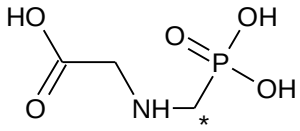
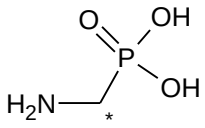
4.7 and 18.7 % of the applied ^{14}C-activity (0.83 – 4.10 mg/kg) was associated with the roots. Markedly less activity was observed in the aerial portions of the grapevines; the maximum uptake (sum of trunk, stem and leaf) at 10, 21, and 42 days was 0.26, 0.43 and 0.67 % of the applied radioactivity,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

[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 0.40 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 ^{l4}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 B.7.2.1.1.7-1: 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
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

Table B.7.2.1.1.7-1: Overview on soil, trunk, hydroponic and foliar application experiments in grape

Experiment		Grape variety	Duration of experiment (days)	Sampling (days)
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 B.7.2.1.1.7-2: 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*	-	-	-
Vine (Sauvignon Blanc)	*0.139*	*0.103*	*0.091*	*0.065*	*0.078*[3]	*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

Table B.7.2.1.1.7-2: 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**

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 B.7.2.1.1.7-3: 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)**
5 mg/L in hydroponic solution						
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	-

Table B.7.2.1.1.7-3: 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)**
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-equivalents.
Values in *italics* were calculated upon dossier compilation

Table B.7.2.1.1.7-4: 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 B.7.2.1.1.7-5: 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 B.7.2.1.1.7-6: 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 B.7.2.1.1.7-7: 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 plants 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 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 [redacted] 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 B.7.2.1.1.7-8: 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	-	-

Table B.7.2.1.1.7-8: 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**
Total identified	*78.7*	*74.3*	*72.0*	*78.1*	*83.2*	*100.5*	*87.6*
Others	-	3.27	-	4.15	-	1.0	-
Total characterised	-	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 B.7.2.1.1.7-9: 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 B.7.2.1.1.7-10: 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 B.7.2.1.1.7-11: 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 B.7.2.1.1.7-12: 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 B.7.2.1.1.7-13: 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 B.7.2.1.1.7-14: 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 B.7.2.1.1.7-15: 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 in Vol. 1, 2.7.2.

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 in grapes (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 [redacted] 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], 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], 2012, CA 6.1/002). Additional detailed information on storage stability of glyphosate and its metabolites is available under B.7.1.

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:

The applicant mentions some deficits of the study in the box above. With regard to the lack of storage stability information, the assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Storage stability of glyphosate and AMPA in orange has been demonstrated for 24 months, thus covering the storage time period in the current study. However, no other storage stability studies in high acid crops are available, which would be required to extrapolate the findings in orange to the whole group of crops with acid matrix including grapes. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which covers the storage period of max. 10 months of the leaves, trunk and stems. Storage stability of AMPA in watery crops is demonstrated for 18 months. And regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. Storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is also covering the time period of the current study. In addition, in several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue. Altogether, storage stability is considered to not impact this metabolism study to a large extent.

The observation that the radioactive residues are only expressed in %AR is relevant for the experiments with foliar treatment, since for all other experiments recalculation into mg/kg was possible. This is considered as a major deficit, however, still qualitative conclusions can be drawn from these foliar experiments.

The observed deficit that no characterization or identification took place for the samples after soil, trunk or hydroponic treatment is mentioned. The only RAC for grape metabolism studies that is required to be analysed according to the guidelines is the fruit. In several occasions the residues in grapes were >0.01 mg/kg, which is the trigger for further investigations. Therefore, more characterization or identification would be required for these grape samples. In addition, in several experiments, even no grapes were sampled, while grapes are the edible part (and therefore the most important part) of the plant to be analysed.

In addition, in the foliar experiments, where identification/characterization took place, the unextracted residues have not been quantified, nor investigated further.

Altogether, due to the many deficits, the RMS considers the study as supportive only. However, the study provides qualitative information on the metabolism of glyphosate.

B.7.2.1.2. Non-tolerant plants, root and tuber vegetables

B.7.2.1.2.1. Potatoes

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 dry 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, considered as additional information in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Conclusion applicant: invalid (Category 2b) Conclusion RMS: not acceptable

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.

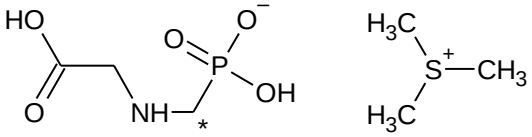
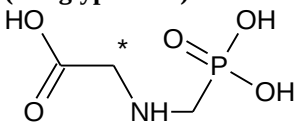
The nature of residues resulting from foliar uptake was investigated by a post-emergence treatment, involving a single application of 100 µL application solution containing ^{14}C-glyphosate (108 µg, 1.28×10^7 dpm) applied 40 days after planting (estimated approx. BBCH 50). For the foliar application 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.

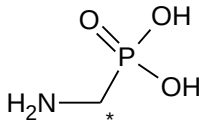
Analysis of soils after harvesting the plants indicated that in the case of the glyphosate treatments only 22 % of the radioactivity remained and in the case of the AMPA treatments 51 % of the starting radioactivity remained in the soil. The only identifiable soil metabolite was AMPA. [redacted], which 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 (9.07 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)

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 soil are summarised in the table below. 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/mg 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 B.7.2.1.2.1.-1: 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 the tables below. Radioactivity of tuber samples of the soil propensity experiments following soil application, was approx. 50 % extractable for glyphosate and AMPA (see table below). Tuber that had grown in soil containing no ^{14}C was spiked with ^{14}C standards gave high recovery rates (≥ 80 %).

Table B.7.2.1.2.1-2: 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, [redacted], 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 B.7.2.1.2.1-3: 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

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 B.7.2.1.2.1-4: 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

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 B.7.2.1.2.1-5: 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

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 B.7.2.1.2.1-6: 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
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 B.7.2.1.2.1-7: 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 B.7.2.1.2.1-8: 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 B.7.2.1.2.1-9: 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 in Vol. 1, 2.7.2.

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, [redacted], 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] 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] 1994, CA 6.2.1-20, [redacted] 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:
The RMS largely agrees with all the described shortcomings of this metabolism study. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which covers the storage period of max. 11 months of the foliage and tops. Storage stability of AMPA in watery crops is demonstrated for 18 months, which covers the possible max. storage period of 11 months. And regarding the storage period of the roots and tubers, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. On the other hand, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is just covering the time period of the current study. In addition, in several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue. Altogether, storage stability is considered to not impact this metabolism study to a large extent. Particularly, the observations that no quantitative levels were available for tubers and leaves; that the percentage extracted was often very low (40-50% TRR); that only in some extracts identification took place, are considered important to assess the study as not acceptable. There is hardly any useful and reliable information in this study, which can be used for the evaluation of the metabolism of glyphosate.

B.7.2.1.2.2. Sugar beets

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	Conclusion applicant: supportive (Category 2a) Conclusion RMS: supportive only

2. Full summary of the study according to OECD format

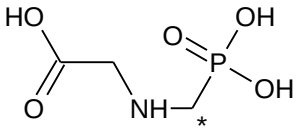
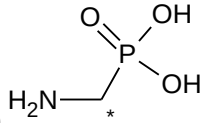
Executive summary

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)

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 B.7.2.1.2.2-1: 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

Table B.7.2.1.2.2-1: 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-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 in Vol. 1, 2.7.2.

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] 1994, CA 6.2.1/022). The storage duration is also well covered by storage stability studies of glyphosate and its metabolites available under B.7.1.
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:
The RMS largely agrees with the assessment of the applicant. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which covers the storage period of max. 15 months of the leaves. Storage stability of AMPA in watery crops is demonstrated for 18 months, which covers the possible max. storage period of 15 months. And regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. On the other hand, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is not covering the time period of the current study. Although there is only 3-5 months difference between the demonstrated storage stability period and the max. period of sample storage, results of AMPA in roots are considered less reliable, since in particular in sugar beets roots a decline was observed in AMPA levels after 12 months. In several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue. Altogether, storage stability is considered to not impact this metabolism study to a large extent, except for the results of AMPA in roots. The findings that no quantitative information can be derived, and that the 'neutral material' was only characterized while the proportions were large, lead to the conclusion that only qualitative information can be drawn from this metabolism study. Therefore, the study is concluded to be 'supportive only'.

B.7.2.1.3. Non-tolerant plants, cereals

B.7.2.1.3.1. Wheat

1. Information on the study

Data point:	CA 6.2.1/010
Report author	[redacted]
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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

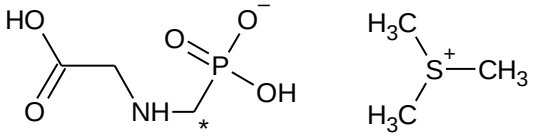
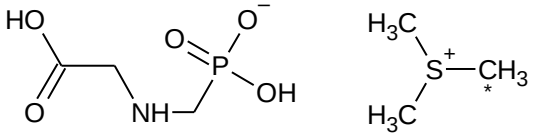
The extraction of samples was performed with water for the ^{14}C-PMG-label and with methanol followed by water for the ^{14}C-TMS-label.

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

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 four 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 B.7.2.1.3.1-1: 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 B.7.2.1.3.1-2: 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***

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 B.7.2.1.3.1-3: 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

Table B.7.2.1.3.1-3: 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**
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 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 B.7.2.1.3.1-4: 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 B.7.2.1.3.1-5: 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 in Vol. 1, 2.7.2.

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] 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] 1994, CA 6.2.1/022, [redacted] 1990, CA 6.6.2/003).
Additional detailed information on storage stability of glyphosate and its metabolites is available under B.7.1.
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:

The study indeed hardly has any deficits compared with the guidelines. The metabolism study provides quantitative information. However, it could be discussed whether the fractions with residual radioactive residues (RRR) should have been further investigated, since in all these fractions residue levels were >0.05 mg/kg (although sometimes <10% TRR). The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Regarding the storage period of the grain, chaff and straw, glyphosate is considered stable for 24 months in starch containing crops, and dry crops, which covers the max. possible storage time in this study. On the other hand, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is just covering the time period of the current study. And storage stability of AMPA in dry crops is variable, making it difficult to draw a general conclusion. Therefore, results on AMPA in straw and chaff should be considered with caution, since the levels might be underestimated. In addition, in several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue. Overall, the study is considered acceptable.

B.7.2.1.3.2. Barley, oats, rice and sorghum

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: supportive only

2. Full summary of the study according to OECD format

Executive summary

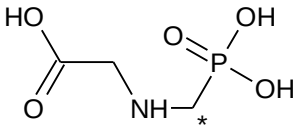
The uptake and nature of the residues in plants following the use of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) was studied in cereal grains (barley, oats, rice and sorghum).

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)

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 nutrients (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 B.7.2.1.3.2-1: 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
	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 B.7.2.1.3.2-2: 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**
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 B.7.2.1.3.2-3: 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 B.7.2.1.3.2-4: 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***
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

Table B.7.2.1.3.2-4: 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

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 in Vol. 1, 2.7.2.

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] 1994, CA 6.2.1/022). Additional detailed information on storage stability of glyphosate and its metabolites is available under B.7.1.

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:
The RMS considers it a major deficit that no sampling of grains took place. This could be due to the fact that the plants were not mature yet, and therefore, no grains had developed yet. This is accompanied by the observation that the information on the exact growth stage of the plants is also missing. Therefore, the sampled RACs are indeed not appropriate. Furthermore, no characterization or identification took place in the soil uptake experiment. In addition, fractions with residual radioactive residues (RRR) should have been further investigated, since in several fractions residue levels were >0.05 mg/kg. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate is shown to be stable in watery matrix (including tops) for approximately 24 months, however, without knowing anything about the storage period, it is not possible to draw any conclusion whether or not the max. possible storage time in this study is covered. Similarly, storage stability of AMPA in watery crops is demonstrated for 18 months, but no conclusion can be drawn. Regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, however, as for the tops, it is not possible to draw any conclusion whether or not the max. possible storage time in this study is covered. Similarly, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, but cannot be concluded on. Therefore, results should be considered with caution, since the levels might be underestimated. In addition, in several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue. The study provides quantitative information on glyphosate metabolism in cereals, however, since the appropriate RACs have not been sampled, in combination with the finding that in several RACs the RRR have not been further investigated, the RMS evaluates the study as supportive only.

B.7.2.1.3.3. Soybeans, cotton, wheat and corn

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: supportive only

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.

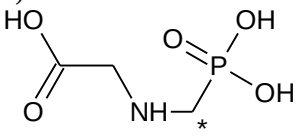
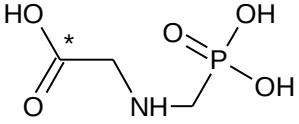
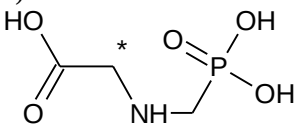
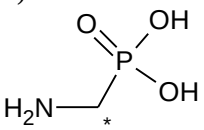
Plants (aerial parts and roots) after hydroponic uptake were extracted and the residues were analysed further. With the exception of maize forage, the major ^{14}C-containing component in the aqueous extracts in all cases was parent glyphosate in aerial parts and in roots; in maize forage, comparable 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/□-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

Test system:

Crop:	Winter varieties Soybean (Clark) Maize (DeKalb KL-45) Wheat (Thacher) Cotton (Stoneville)
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) (8-12 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 7.50×10^8 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 B.7.2.1.3.3-1: 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	All crops	8 weeks	4, 6, 8 weeks

Table B.7.2.1.3.3-1: Overview on soil and hydroponic uptake experiments in soybean, cotton, maize and wheat

	Experiment	Type of plant	Duration of the experiment (days)	Sampling (days)
	7.50 x 10^8 dpm, equivalent to 1.7 kg/ha (corresponding to 2.6 kg glyphosate equiv./ha)			
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 109 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 109 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 109 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 109 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 108 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 108 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 109 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 108 dpm)	Soybean	26	Solution: 1, 6, 12, 20, 26 Plants: 6 (8 plants), 12 (8 plants), 20 (4 plants),

Table B.7.2.1.3.3-1: Overview on soil and hydroponic uptake experiments in soybean, cotton, maize and wheat

	Experiment	Type of plant	Duration of the experiment (days)	Sampling (days)
				26 (178 plants)
10	24 plants in 5 L hydroponic solution, 11.85 mg N-(phosphono-^{14}C-methyl)glycine (1.24 x 109 dpm), pulse treatment for first 6 days only	Soybean	56	Solution: 1, 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 □-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 □-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 B.7.2.1.3.3-2: 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
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 B.7.2.1.3.3-3: 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*

Table B.7.2.1.3.3-3: 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
	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 B.7.2.1.3.3-4: 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 B.7.2.1.3.3-5: 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 B.7.2.1.3.3-6: 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

Table B.7.2.1.3.3-6: Uptake of ^{14}C-glyphosate in soybean plants grown in hydroponic solution

Time (days)	Matrix	% AR
20	Hydroponic solution	67.20
	Aerial parts	2.00
	Roots	8.56
25	Hydroponic solution	57.36
	Aerial parts	2.09
	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 B.7.2.1.3.3-7: 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

Table B.7.2.1.3.3-7: Uptake of ^{14}C-glyphosate in maize, wheat and cotton plants grown in hydroponic solution

Time (days)	Matrix	% AR recovered
	Roots	8.43
20	Hydroponic solution	56.93
	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 B.7.2.1.3.3-8: 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 B.7.2.1.3.3-9: 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 B.7.2.1.3.3-10: 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

Table B.7.2.1.3.3-10: 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**			
	% 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 B.7.2.1.3.3-11: 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	99.5	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**	**99.5**	**88.5**
RRR[1]	***29.4***	***27.0***	***28.6***	***3.4***	***31.4***	***33.3***	***0.5***	***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 B.7.2.1.3.3-12: 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 B.7.2.1.3.3-13: 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 B.7.2.1.3.3-14: 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 B.7.2.1.3.3-15: 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/□-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 B.7.2.1.3.3-16: 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 identified	***-***	***70.8***	***82.3***	***75.4***	***72.3***	***32.6***	***59.7***	***33.7***	***45.7***	***39.0***
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 B.7.2.1.3.3-17: 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***

Table B.7.2.1.3.3-17: 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**							
Origin	-	-	-	-	-	-	-	-
Unknown	-	-	2.4	-	-	-	-	5.0
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 B.7.2.1.3.3-18: 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 B.7.2.1.3.3-19: 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 B.7.2.1.3.3-20: 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
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

Table B.7.2.1.3.3-20: 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 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 B.7.2.1.3.3-21: 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 B.7.2.1.3.3-22: 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

Table B.7.2.1.3.3-22: 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

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 B.7.2.1.3.3-23: 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
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 B.7.2.1.3.3-24: 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	-	-

Table B.7.2.1.3.3-24: 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	
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[1]	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] 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 B.7.2.1.3.3-25: 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**

Table B.7.2.1.3.3-25: 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											
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*
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***	***42.5***	***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 B.7.2.1.3.3-26: 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

Table B.7.2.1.3.3-26: 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

	% 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*	-	-	-	-
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 B.7.2.1.3.3-27: 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	-	-	-	-
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 in Vol. 1, 2.7.2.

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] 1994, CA 6.2.1/022). Additional detailed information on storage stability of glyphosate and its metabolites is available under B.7.1.

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:
The RMS considers it a major deficit that no sampling of the edible part of the plants took place. This could be due to the fact that the plants were not mature yet, and therefore, no grains/seeds had developed yet. This is accompanied by the observation that the information on the exact growth stage of the plants is also missing. Therefore, the sampled RACs are indeed not appropriate.
Furthermore, no characterization or identification took place in the soil uptake experiment, while residues were higher than 0.01 mg/kg in the whole crop samples. In addition, fractions with residual radioactive residues (RRR) should have been further investigated, since in several fractions residue levels were >10% TRR (unclear what the levels in mg/kg were). The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate is shown to be stable in watery matrix (including tops) for approximately 24 months, which covers the max. possible storage time in this study. Storage stability of AMPA in watery crops is demonstrated for 18 months, which is less than the max. estimated storage period in this study. However, there are no reasons to expect that AMPA will degrade after 18 months based on the storage stability studies. Regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, thereby covering the max. storage time period in this study. Storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is not covering the time period of the current study. There is a possible 12 months difference between the demonstrated storage stability period and the max. period of sample storage. Results of AMPA in roots are considered less reliable. Therefore, AMPA results in roots should be considered with caution, since the levels might be underestimated. In addition, in several other metabolism studies (see also references in assessment of applicant), it was shown that degradation of radioactive residues was not an issue.
The study provides mainly qualitative information on glyphosate metabolism in cereals and oilseeds. Furthermore, since the appropriate RACs have not been sampled, in combination with the finding that in several RACs the RRR have not been further investigated, the RMS evaluates the study as supportive only.

B.7.2.1.3.4. Pasture crops

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	Conclusion applicant: supportive (Category 2a) Conclusion RMS: supportive only

2. Full summary of the study according to OECD format

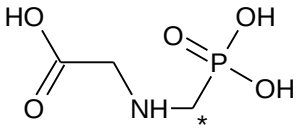
Executive summary

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)
[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.

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 B.7.2.1.3.4-1: 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

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 B.7.2.1.3.4-2: 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 B.7.2.1.3.4-3: 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 overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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-MS. 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:
Indeed, the study almost only provides qualitative information on glyphosate metabolism in pasture crops. For one experiment, TRRs in mg/kg could be calculated. These TRRs show that, although only maximally 0.1% of the AR is recovered in the treated plants, quantitatively these amounts are not negligible (i.e. up to 5.67 mg/kg in treated clover). In addition, hardly any investigation on identification took place, and if it was conducted, it was only qualitatively. Therefore, the study is considered supportive only.

B.7.2.1.4. Non-tolerant plants, pulses and oilseeds

B.7.2.1.4.1. Soybeans

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

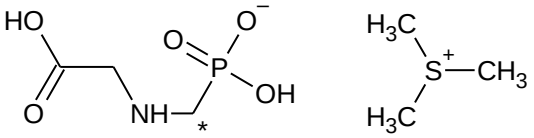
Within extracts of forage, straw, hulls and yellow seeds PMG and its metabolite AMPA were identified accounting for 0.57 - 4.10 and 1.50 - 5.70 % of the TRR, respectively.

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)

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 acids) 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 B.7.2.1.4.1-1. 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. The control plants contained approximately 10% ^{14}C as much as the treated plants, which is presumably due to uptake of $^{14}CO_2$.

Table B.7.2.1.4.1-1: 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 tables below. Large scale-extractions of soybean green and yellow seed are summarised in a separate table and the results of identification of the radioactive residues are presented below.

Table B.7.2.1.4.1-2: 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 B.7.2.1.4.1-3: 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 B.7.2.1.4.1-4: 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 B.7.2.1.4.1-4: 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 HCI 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 B.7.2.1.4.1-5: 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

Table B.7.2.1.4.1-5: 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**
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	-	-
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**

Table B.7.2.1.4.1-5: 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**
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.
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 B.7.2.1.4.1-6: 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 B.7.2.1.4.1-7: 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 B.7.2.1.4.1-8 and Table B.7.2.1.4.1-9. There did not appear to be any degradation over the storage span during the study.

Table B.7.2.1.4.1-8: 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 B.7.2.1.4.1-9: 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

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 in Vol. 1, 2.7.2.

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:
The RMS considers the metabolism study in soybeans as an acceptable study. Quantitative information can be retrieved from the samples (although sometimes calculation was required from reported values in the study report, since the mg/kg-levels were not always available in the report), and extensive characterization took place of the both the extractable as well as the unextractable fractions.

B.7.2.1.5. Non-tolerant plants, miscellaneous

B.7.2.1.5.1. Coffee plants

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

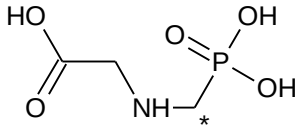
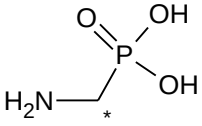
For the uptake via the stem a ^{14}C-glyphosate solution was applied to the stem. The plant was kept in a hydroponic solution for 5 weeks. After this timeframe samples of leaves, untreated stems, treated stems and roots were collected.

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) 97.7 % (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

[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 (1:1) 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 B.7.2.1.5.1-1: 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 B.7.2.1.5.1-2: 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 number of beans that developed on this tree.

Table B.7.2.1.5.1-3: 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*
^{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*

Table B.7.2.1.5.1-3: 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
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 B.7.2.1.5.1-4: 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 the tables below.

Table B.7.2.1.5.1-5: 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 B.7.2.1.5.1-6: 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.

Table B.7.2.1.5.1-6: 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***

1) N-methyl-AMPA named as CP-70948 in the report
TRR Total radioactive residue
ERR Extractable radioactive residue
RRR Residual radioactive residue

Table B.7.2.1.5.1-7: 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 B.7.2.1.5.1-8: 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*

Table B.7.2.1.5.1-8: Distribution of the radioactive residues and characterisation of the nature of the residues of glyphosate in roots of coffee trees following hydroponic treatment

RRR	***10***	*0.63*	***13***	*1.58*	***62***	*18.38*
Total sum	***100***	*6.27*	***100***	*12.19*	***100***	*29.64*

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) ([redacted] MSL_13318.)
Storage stability of frozen samples of high water content in metabolism study has been shown in carrot tops for 15 months ([redacted] MSL_9810).
Therefore, the storage stability is covered.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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]_MSL_13318). Storage stability of frozen samples of high water content has been shown in carrot tops for 15 months [redacted]_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:
The RMS agrees on the assessment of the applicant. The study provides qualitative as well as quantitative information on the metabolism of glyphosate in coffee plants. Indeed, in some experiments further investigation of the RRR could be required. However, for the relevant matrices after foliar treatment, the RRR was always <10% of the TRR; and importantly, in bean matrices, the RRR was also still below the trigger of 0.01 mg/kg. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate has been demonstrated to be stable for 18 months in high oil commodities, covering the max. storage time of the coffee beans. Storage stability of AMPA in high oil content crops was shown for at least 12 months, which is shorter than the possible max. storage time of 15 months of the samples. However, it is not expected that these additional 3 months will strongly impact on the storage stability. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which covers the storage period of max. 15 months of the leaves and stems. Storage stability of AMPA in watery crops is demonstrated for 18 months, which covers the max. possible storage period. And regarding the storage period of the roots, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. On the other hand, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is not covering the time period of the current study. However, it's not expected that this influences the reliability of the study to a large extent, since the interest of the study is not so much into the roots of the trees. The study is considered acceptable.

B.7.2.1.5.2. Sugarcane

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	Conclusion applicant: supportive (Category 2a) Conclusion RMS: supportive only

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.

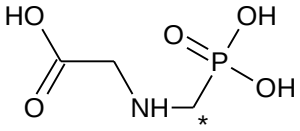
These results were confirmed after processing of sugar with an aqueous solution of labelled and unlabelled glyphosate into refined sugar. During processing the main part of the radioactivity remained in the bone char (84 %). Further 6 % were recovered in the filter cake from liming, 3 % in refined molasses (<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]) (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

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 B.7.2.1.5.2-1: 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 B.7.2.1.5.2-2: 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 B.7.2.1.5.2-3: 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

Table B.7.2.1.5.2-3: 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

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
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 B.7.2.1.5.2-4: 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 B.7.2.1.5.2-5: 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	0.85
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 B.7.2.1.5.2-6: 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
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

Table B.7.2.1.5.2-6: 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**
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

Please refer to the overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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:
The RMS largely agrees with the assessment of the applicant. Several of the experiments are of limited relevance, since they are not investigating plant metabolism. In addition, in the experiments in which glyphosate metabolism has been investigated to a minor extent, several short comings have been identified. Altogether, the study is considered to only provide some qualitative information, and therefore, it is considered as supportive only.

B.7.2.1.6. Genetically modified plants, CP4 EPSPS and GOX modification, root and tuber vegetables

B.7.2.1.6.1. Sugarbeet

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

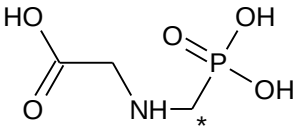
Trace levels of glyphosate/AMPA acetylated conjugates (0.80 % of the TRR (0.03 mg/kg) in tops and 0.55 % of the TRR (0.01 mg/kg) in roots) and small amounts of ^{14}C-labelled natural products (1.38 % of the TRR (0.05 mg/kg) in tops and 1.22 % of the TRR (0.02 mg/kg) in roots) were also found after post-emergent 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)

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 HME 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 ^{12}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 B.7.2.1.6.1-1. 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 B.7.2.1.6.1-1: 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 B.7.2.1.6.1-2 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 ([redacted], 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 B.7.2.1.6.1-2: 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
Eluate 1 (Conjugate fractions # 5-6)							0.011	0.81

Table B.7.2.1.6.1-2: 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 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 B.7.2.1.6.1-3: 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		

Table B.7.2.1.6.1-3: 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**
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
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 B.7.2.1.6.1-4: 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**

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 B.7.2.1.6.1-5: 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	0.756	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 in Vol. 1, 2.7.2.

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:

Although genetically modified crops are not within the intended use of the renewal of glyphosate, this metabolism study with glyphosate-tolerant CP4 EPSPS sugar beets has been evaluated. Indeed, in some instances the radioactive balance was <90%. However, as also described by the applicant, this is not considered to influence the reliability of the study to a large extent. The study is considered acceptable.

B.7.2.1.7. Genetically modified plants, CP4 EPSPS and GOX modification, cereals

B.7.2.1.7.1. Wheat

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	Conclusion applicant: valid (Category 1) Conclusion RMS: acceptable

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.

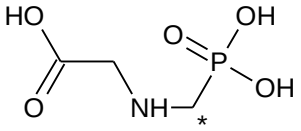
AMPA, a well-known plant metabolite of glyphosate, was found to be the most significant metabolite of glyphosate in wheat matrices ranging between 0.15 and 1.77 mg/kg (0.76 – 10.77 % TRR).

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)

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 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 B.7.2.1.7.1-1. 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 B.7.2.1.7.1-1: 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 B.7.2.1.7.1-2 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 B.7.2.1.7.1-2: 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
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

Table B.7.2.1.7.1-2: 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**
RRR	0.49	2.45	1.07	3.86	2.81	8.06	1.74	14.32
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 B.7.2.1.7.1-3: 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
Concentration, centrifugation		
Supernatant	*10.26*	84.64

Table B.7.2.1.7.1-3: 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**
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 B.7.2.1.7.1-4: 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
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

Table B.7.2.1.7.1-4: 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**
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 B.7.2.1.7.1-5: 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 in Vol. 1, 2.7.2.

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:
This metabolism study has not been previously evaluated at EU level, while the year of publication was already in 2000. Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant CP4 EPSPS wheat has been evaluated. Although less than 10% TRR was residual radioactive residue (RRR), still quantitatively these residue levels of the RRR were rather high. It would have been desirable if further examination of these fractions was conducted. For the grain fraction, additional exhaustive extraction took place, but still 0.42 mg/kg remained unextracted. Overall, the study is considered to acceptably investigate the metabolism of glyphosate in glyphosate-tolerant CP4 EPSPS wheat.

B.7.2.1.7.2. Corn

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

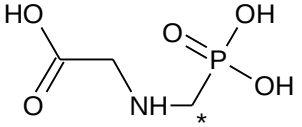
In addition to this, aqueous extracts contained ^{14}C-labelled natural products (<3.6 % of the TRR). The radioactive natural products were derived from the incorporation of $^{14}CO_2$ and other one carbon fragments from N-(phosphono-^{14}C methyl)glycine into plant constituents.

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

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 19, 4 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 2.25 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 37, 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 B.7.2.1.7.2-1: 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.
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 B.7.2.1.7.2-2: 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
Minor deviations may occur due to rounding

Table B.7.2.1.7.2-3: 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
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 contain 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 B.7.2.1.7.2-4: 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)				

Table B.7.2.1.7.2-4: 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**
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
(1): 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.
(2): 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.
(3): 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).
(4): 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.
(5): 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.
(6): 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 B.7.2.1.7.2-5: 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 B.7.2.1.7.2-6: 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

Table B.7.2.1.7.2-6: 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**

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 B.7.2.1.7.2-7: 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 in Vol. 1, 2.7.2.

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

Assessment and conclusion by applicant:

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.

Assessment and conclusion by RMS:

Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant wheat, expressing CP4 EPSPS and GOX proteins, has been evaluated. Although the residual radioactive residues (RRR) in forage, silage and fodder were below 10 % TRR, the quantitative levels are considered high (>0.307 mg/kg). It would have been desirable, if further attempts were made to investigate the RRR, like it has been done for corn grain. No further remarks for this metabolism study. The study is considered acceptable.

B.7.2.1.8. Genetically modified plants, CP4 EPSPS and GOX modification, pulses and oilseeds

B.7.2.1.8.1. Canola

1. Information on the study

Data point:	CA 6.2.1/021
Report author	[redacted]
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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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 of 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.

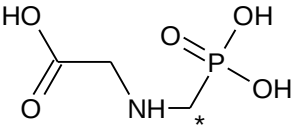
Extraction of canola seeds after early post-emergence application with hexane and water yielded 25.5 % TRR (0.123 mg/kg). In the aqueous fraction of canola seeds after early post-emergence application 7.7, 3.4, 0.9 and <2 % TRR (0.037, 0.017, 0.004 and <0.01 mg/kg) AMPA, N-glyceryl-AMPA, N-acetyl-AMPA 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)

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). During application to the foliage, the soil surface of each pot was shielded.

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 B.7.2.1.8.1-1: 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), so all radioactivity was in the ether fraction. 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.l 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 B.7.2.1.8.1-2: 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 B.7.2.1.8.1-3: 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				

Table B.7.2.1.8.1-3: 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
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	-	-
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 B.7.2.1.8.1-4: 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 overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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:
Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant canola, expressing CP4 EPSPS and GOX proteins, has been evaluated. Extensive investigations have been conducted to characterize/identify the non-extracted residues in canola seeds. Therefore, although these experiments did not lead to quantitative residue levels below 0.01 mg/kg, based on the observation that several attempts were undertaken to understand what is in the none-extracted residues, the study is considered acceptable.

B.7.2.1.8.2. Soybean

1. Information on the study

Data point:	CA 6.2.1/022
Report author	[redacted]
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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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 following 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 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.

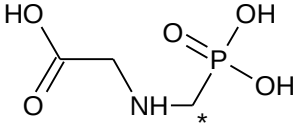
The total radioactive residue (TRR) in soybean forage, hay and seeds after sequential post-emergence treatment amounted to 23.651, 10.416 and 17.459 mg/kg in forage, hay and seeds, respectively. Early post-emergence treatment resulted in the TRRs of 0.863, 0.546 and 0.406 mg/kg in forage, hay and seeds 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 TRR 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)

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 following 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). During the post-emergence application, the soil surface of each pot was protected. 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 $^{14}CO_2$ 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 were 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 extracted, 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 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 B.7.2.1.8.2-1: 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 seeds 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 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 B.7.2.1.8.2-2: 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 B.7.2.1.8.2-3: 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

Table B.7.2.1.8.2-3: 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**	
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 B.7.2.1.8.2-4: 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.

Table B.7.2.1.8.2-4: 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	
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 B.7.2.1.8.2-5: 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		

Table B.7.2.1.8.2-5: 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**	
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 B.7.2.1.8.2-6: 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 products	0.013	1.5	0.618	2.6	0.015	2.7	0.274	2.6
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 B.7.2.1.8.2-7: 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	1.863	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.

Table B.7.2.1.8.2-8: 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 overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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 following 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 cellulase, 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:
Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant soybean, expressing CP4 EPSPS proteins, has been evaluated. As already mentioned by the applicant, the residual radioactive residue (RRR) was often too high, in particular for seeds after sequential post-emergence treatment (up to 2.020 mg/kg). For some samples, several additional attempts were conducted to further analyse the residual residues, indicating incorporation of radioactive residues into natural compounds. Therefore, overall, the study is considered acceptable.

B.7.2.1.8.3. Cotton

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

2. Full summary of the study according to OECD format

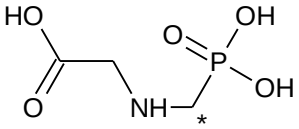
Executive summary

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)

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 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 extracted, 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 B.7.2.1.8.3-1: 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 B.7.2.1.8.3-2: 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 B.7.2.1.8.3-3: 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**	**-**	**-**

Table B.7.2.1.8.3-3: 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
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 B.7.2.1.8.3-4: 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)

Table B.7.2.1.8.3-4: 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	

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.

B.7.2.1.8.3-5: 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 overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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:
Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant cotton plants, expressing CP4 EPSPS proteins, has been evaluated. Although >90% TRR in forage has been identified, still the quantitative levels of the residual residues is considered high (0.447-0.708 mg/kg). In addition, as already mentioned by the applicant, relatively high quantities of residual radioactive residues (RRR) were observed in seeds. However, several attempts were made to further characterize these RRR, and a similar situation can be observed in the ^{12}C treated plants. Therefore, the study is considered acceptable.

B.7.2.1.9. Genetically modified plants, GAT modification, cereal/grass crops

B.7.2.1.9.1. Corn

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	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

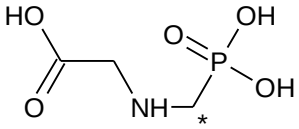
At maturity, the majority of the TRR was present in stover (12.255 mg/kg), with 0.686 mg/kg in cobs, and 0.275 mg/kg in grain. The majority of the radioactivity in the mature maize fractions was extracted using 0.1 % formic acid:methanol (96:4 v/v) and α-amylase (69.3-85.0 % and 11.1-20.5 % TRR, respectively). 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)

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 V5, 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 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 B.7.2.1.9.1-1: 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 B.7.2.1.9.1-2: 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 B.7.2.1.9.1-3: 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*

Table B.7.2.1.9.1-3: 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

RRR	0.031	0.9
Total	*3.652*	100.0

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 B.7.2.1.9.1-4: 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

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

Table B.7.2.1.9.1-4: 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

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 B.7.2.1.9.1-5: 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-acetyl glyphosate	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 in Vol. 1, 2.7.2.

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:
Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant corn, expressing GAT genes, has been evaluated. The study complies with the guidelines. Also residual radioactive residues are <0.05 mg/kg, except for stover (0.110 mg/kg), however, 97.4 % TRR has been identified in stover. Furthermore, individual unidentified components were maximally 0.025 mg/kg. Although unidentified components in different fractions could possibly be the same compound, it is considered that sufficient attempts have been undertaken. The study is considered acceptable.

B.7.2.1.10. Genetically modified plants, GAT modification, pulses and oilseeds

B.7.2.1.10.1. Canola

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:	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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 after 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), 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.

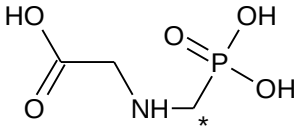
TRR in the immature foliage from the first harvest was 5.979 mg/kg and the majority of the radioactivity was extracted using aqueous medium (97.3 % of the TRR, 5.818 mg/kg). *N*-Acetyl glyphosate was the major extractable radioactive component accounting for 89.5 % of the TRR (5.351 mg/kg). Glyphosate, *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)

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), 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 cellulase 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 B.7.2.1.10.1-1: 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	% TRR	-	0.5	-

Table B.7.2.1.10.1-1: 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**
cellulase extract	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 B.7.2.1.10.1-2: 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 B.7.2.1.10.1-3: 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 B.7.2.1.10.1-4: 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).

Table B.7.2.1.10.1-4: 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**

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 B.7.2.1.10.1-5: 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)

Table B.7.2.1.10.1-5: 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**

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 B.7.2.1.10.1-6: 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 AMPA	0.203	3.4	n.d	n.d	n.d	n.d	0.316	14.7
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 in Vol. 1, 2.7.2.

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] 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] 1994, CA 6.2.1/022 and [redacted]., 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:

Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant canola, expressing GAT genes, has been evaluated. The study largely complies with the guidelines. The residual radioactive residues (RRR) in pre-mature pods (with seed) could have been further investigated, since its level was 0.26 mg/kg. However, since the mature seeds have been investigated intensively with several extraction methods, finally leading to an RRR of 0.075 mg/kg and a maximum individual unidentified component of 0.022 mg/kg, this is considered acceptable. The assessment

of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. However, since samples were stored for max. 70 days, no additional storage stability argumentation is required. The study is considered acceptable.

B.7.2.1.10.2. Soybean

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:	Conclusion applicant: valid (Category 2a) Conclusion RMS: acceptable

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.

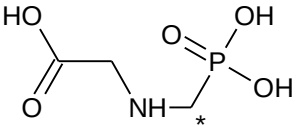
TRR in grain, pods, and foliage collected at maturity (14 days PHI) contained 3.142 mg/kg, 17.751 mg/kg, and 22.087 mg/kg, respectively. *N*-Acetylglyphosate was the major radioactive component detected in the mature grain accounting for 56.9 % of the TRR, 1.788 mg/kg. Glyphosate (3.2 % of the TRR, 0.102 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)

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 cellulase. 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 B.7.2.1.10.2-1: 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

Characterisation and identification of residues in soybean forage

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 B.7.2.1.10.2-2: 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 B.7.2.1.10.2-3: 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 B.7.2.1.10.2-4: 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**
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)

Table B.7.2.1.10.2-4: 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**

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 B.7.2.1.10.2-5: 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

Table B.7.2.1.10.2-5: 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

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**

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.

Table B.7.2.1.10.2-5: 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

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 B.7.2.1.10.2-6: 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 B.7.2.1.10.2-7: 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.

D. Degradation pathway

Please refer to the overall pathway of glyphosate in plants in Vol. 1, 2.7.2.

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] 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] 1994, CA 6.2.1/22 and [redacted]., 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:

Genetically modified crops are not within the intended use of the renewal of glyphosate, however, this metabolism study with glyphosate-tolerant soybean, expressing GAT genes, has been evaluated. The unextracted residue was almost always >0.05 mg/kg. However, since extensive attempts have been conducted to characterize/identify the residues, this is considered acceptable. The assessment of the applicant on storage stability should be considered in the light of the evaluation of the RMS in Vol. 1, 2.7.1. Glyphosate is shown to be stable in watery matrix for approximately 24 months, which covers the storage period of max. 11 months of the forage, foliage and pods. Storage stability of AMPA in watery crops is demonstrated for 18 months, which also covers the possible max. storage period of 11 months. And regarding the storage period of the grain, glyphosate is considered stable for 24 months in starch containing crops, which covers the max. possible storage time in this study. On the other hand, storage of AMPA in crops with a high starch content is demonstrated for max. 10-12 months, which is also covering the time period of the current study. Regarding stability in hay, glyphosate has been shown to be stable for at least 12 months in soybean hay and other dry commodities, thereby covering the max. storage period of the current study. Storage stability of AMPA in dry commodities is less straight forward. However, since AMPA was shown to be stable for at least 9 months in soybean hay, it is considered that the possible additional 2 months of storage of the soybean hay in the current study won't influence the stability of AMPA. The study is considered acceptable.

B.7.2.2. Poultry

B.7.2.2.1. Study 1

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	Conclusion applicant: Supportive (Category 2a) Conclusion RMS: Supportive

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.

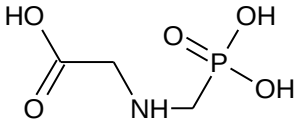
I. Materials and methods

A. Materials

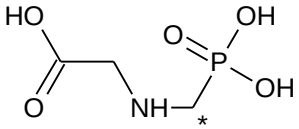
Test material

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

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: [redacted]

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 (Group A) 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 by extraction and metabolite profiling.
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 B.7.2.2-1: 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 B.7.2.2-2: 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 B.7.2.2-3: 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 B.7.2.2-4: 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 B.7.2.2-5: 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*)[3]**
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

[3] The recovery of radioactivity after acididfied aqueous extraction of a pooled sample of skeletal muscle was ca. 81%. Procedural loses subsequently reduces efficiencies to 43.7%.

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 B.7.2.2-6: 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 B.7.2.2-7: 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	*97.81/0.904*	*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 B.7.2.2-7: 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 in Volume 1, Section 2.7.2

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:
RMS agrees with the assessment of the applicant. The study investigates metabolism of glyphosate in poultry (laying hens). Based on the reported results it can be concluded that glyphosate was not extensive metabolised in laying hens. Glyphosate was rapidly excreted (as unchanged parent compound) resulting in low residue levels in tissues and eggs, where also unchanged glyphosate was the primary residue. The study provides qualitative information on the metabolism of glyphosate in poultry, however, taking into account reported deficiencies regarding extraction efficiency for some matrices (muscle, egg white) no robust quantification of residues could be performed. The study is considered as supportive information.

B.7.2.2.2. Study 2 and Study 3

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	Conclusion applicant: Supportive (Category 2a) Conclusion RMS: Study is considered acceptable

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.

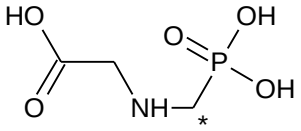
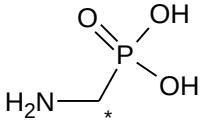
The radioactivity level in tissues of the depuration group were generally lower; the liver had the highest level (0.079 mg equiv./kg).

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 (2.4-16.2 % 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)

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 [redacted] (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 B.7.2.2-8: 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 ([redacted]). After determination of radioactivity in the samples, they were sent to [redacted] (site of analysis) on dry ice via overnight freight. The samples were received on May 7, 1987 and stored at -20 °C at [redacted] Sample analysis were conducted between 11 May to 20 August 1987.
Comparison of the results from the analysis of excreta sample at the beginning and the end of the study was used to assess the storage stability of samples.

3. Analytical procedures

Radioanalysis (Quantitation and distribution of Total Recovered Radioactivity)
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.

Extraction and fractionation of Radioactivity
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. Most of the 14C residues in tissues were extracted into water, which is consistent with solubility of glyphosate and AMPA in water. 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) contained higher levels of radioactivity than in other tissues and were characterised further 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. A standard mixture of radiolabelled glyphosate and AMPA was always analysed prior to the HPLC analysis of the tissue extracts.

For identification of residues in the extracts, HPLC fractions corresponding to glyphosate and AMPA were isolated from kidney extracts using ion pair HPLC. Only kidney extracts contained sufficient levels of glyphosate and AMPA to permit purification and mass spectral identification.
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 B.7.2.2-9: 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	90.5

[1] % AR = percent of administered radioactivity

Table B.7.2.2-10: 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 B.7.2.2-11: 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 B.7.2.2-12: 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 B.7.2.2-13 : Distribution of Radioactivity in the tissue Extracts of Chickens (Total Extraction Rates)

Treatment group	Kidney	Liver	Gizzard	Fat	Thight Muscle	Breast Muscle	Pooled egg yolk
Group 2							
Chloroform	0.1	0.5	0.1	17.2	2.2	1.2	10.0
Water	98.4	97.9	98.4	82.8	97.8	98.9	80.2
Unextracted	1.4	1.8	1.5	0.0	NA	NA	9.7
Group 3							
Chloroform	0.1	0.5	0.2	16.2	1.8	0.8	10.3
Water	97.3	96.9	97.1	83.9	98.2	99.2	74.0
Unextracted	2.6	2.7	2.8	0.0	NA	NA	15.0
Group 4							
Chloroform	0.1	0.6	.0.	12.0	1.3	1.4	8.8
Water	97.9	96.8	97.6	88.0	98.7	98.7	77.2
Unextracted	2.1	2.7	2.4	0.0	NA	NA	14
Group 5							
Chloroform	0.3	0.0	0.5	57.2	1.5	6	9.5
Water	95.4	97.4	86.2	42.7	98.4	94.0	71.9
Unextracted	4.4	2.6	13.4	0.0	NA	NA	18.7

Table B.7.2.2-13: 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 B.7.2.2-14: 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 B.7.2.2-15: 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 B.7.2.2-16: 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 B.7.2.2-17: 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 B.7.2.2-18: 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 B.7.2.2-19: 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 B.7.2.2-20: 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 B.7.2.2-21: 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 B.7.2.2-22: 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 B.7.2.2-23: 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 B.7.2.2-24: 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 B.7.2.2-25: 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 B.7.2.2-26: 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.

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:
RMS agreed with the study evaluation and considered the studies as acceptable. The deviations reported by the applicant are noted, however RMS is of the opinion that based on the available data metabolism pattern of glyphosate and AMPA in poultry has been sufficiently addressed.
Most of the administrated radioactivity was found in excreta (81-90.5% AR). In eggs yolk, eggs white and tissues only very low amounts of administrated recovery was measured (up to 0.02% AR). No significant degradation of glyphosate was observed, with glyphosate and AMPA being the most relevant residues.
The duration of sample storage was maximum four months, which is covered by the available stability studies.

B.7.2.2.3. Study 4

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	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Acceptable

2. Full summary of the study according to OECD format

Executive Summary

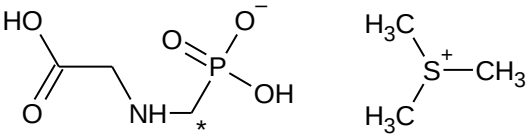
N-(phosphono-^{14}C-methyl)glycine trimesium salt (^{14}C-PMG-labeled glyphosate-trimesium) was administered orally for 10 days to 10 laying hens.
The target dose level was 90 mg ^{14}C-PMG-labeled glyphosate-trimesium per kg feed consumed (62 mg phosphonomethyl-glycine (PMG) per kg feed consumed). The 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 (hens received gelatin capsules containing powdered cellulose). The hens were sacrificed 12 to 15 hours after the last dosing.
103.9.0 % 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 accounted in sum for 0.13% (0.04% eggs and 0.09% tissue).
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)

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 [redacted] and shipped to [redacted] 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 [redacted]. All tissue, eggs, excreta and cage rinse were sent frozen on dry ice to [redacted]. The samples were stored at -20°C at [redacted]

All tissues were extracted and analysed within 4 to 6 months of sacrifice, except for egg white, which was extracted at 6.5 months.

3. Analytical procedures

The total radioactive residue (TRR) in all tissues, eggs and excreta was determined by combustion and radioassay at [redacted]. 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.

[redacted]s, eggs, cage rinses and excreta were send to the [redacted] The total ^{14}C-residue in tissues determined at [redacted] was confirmed by combustion/liquid scintillation counting (LSC) at [redacted]. Except kidney and blood; those samples were not extracted/analysed at [redacted] GI tract samples were not recombusted nor extracted/analysed at [redacted]

All characterisation and identification of radioactive residues in edible tissues (thigh muscle, breast muscle, liver, fat) and eggs were performed at [redacted]. Tissues were extracted with 0.1 M HCl/chloroform and the extracts were purified with a Chelex 100 iron form column and analysed by HPLC and TLC. Post-extraction solids, in which % of TRR exceeded 10%, were hydrolysed with acid and the soluble products were analysed. Metabolites from representative tissues were also derivatized and id

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. If the PES residues was ≥10% and ≥0.01 mg/kg, the PES was acid hydrolysed and the hydrolysate analysed.

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 0.13 %.

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 B.7.2.2-27: Distribution of radioactive residues in tissues, excreta and eggs of laying hens after treatment with ^{14}C-PMG-labeled glyphosate-trimesium

Matrix	% AD (% 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 B.7.2.2-28: 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 [redacted]
For liver, thigh muscle, breast muscle, fat, egg white and egg yolk the TRR was further confirmed by re-combustion at [redacted] 0 4402, 0 0401, 0 0191, 0 0293, 0 0189 and 0 2400 mg/kg were found, respectively.

Table B.7.2.2-29: Radioactive residues in egg white and egg yolk of laying hens after treatment with ^{14}C-PMG-labeled glyphosate-trimesium (in-life study, [redacted])

	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 B.7.2.2-30: 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 [redacted] 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 B.7.2.2-31: 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 B.7.2.2-32: 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 B.7.2.2-32: 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 % TTR 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 B.7.2.2-33: 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..

[6] Characterised by extraction and/or chromatographic behaviour.

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 B.7.2.2-34: 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.
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:

RMS agreed with the study evaluation and considered the study as acceptable. Based on the available data, metabolism pattern of glyphosate and AMPA in poultry can be adressed.
Most of the administrated radioactivity was excreted (>99%). In eggs yolk, eggs white and tissues low amounts of administrated recovery was measured (up to 0.13% AR). No significant degradation of glyphosate was observed, with glyphosate (PMG) and AMPA being the most relevant residues.

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. Storage time for all tissues is covered by the available stability data.

B.7.2.2.4. Study 5

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	Applicant: Supportive (Category 2a) RMS: The study is considered acceptable.

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.

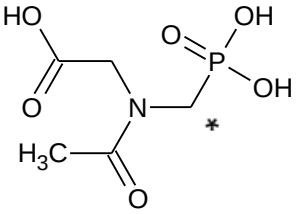
The HPLC-profile of the muscle extract contained eight radiolabelled components, the most abundant was *N*-acetylglyphosate, and accounted for 25.22 % TRR (0.009 mg/kg). AMPA, glyphosate, and *N*-acetyl AMPA, were detected and accounted for 16.69 % TRR (0.005 mg/kg), 7.19 % TRR (0.002 mg/kg), and 1.89 % 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 [redacted]
Specific activity:	0.51 MBq/mg (13.83 µCi/mg)
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 [redacted] 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 B.7.2.2-35: Distribution and concentration of total 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	≤0.05	0.039	*0.031*
Abdominal fat	≤0.05	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 B.7.2.2-36: 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 B.7.2.2-37: 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 B.7.2.2-38: 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 B.7.2.2-39: 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***

Table B.7.2.2-39: 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])*
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***
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 B.7.2.2-40: 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:
RMS agreed with the study evaluation and considered the study as acceptable. The reported deficiencies are noted, however, it has been concluded that based on the available data, metabolism pattern of glyphosate and AMPA in poultry can be sufficiently addressed.

It is reported by the applicant that in balance of components in matrices egg white and muscle, there are losses of up to 34.21% TRR (0.001 mg/kg) in egg white and 21.6% TRR (0.005 mg/kg) in muscle. This observation holds true for balance of ERR. On the other hand it has been concluded in the evaluation that for example for muscle extract subsequent concentration 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. RMS agrees with this last conclusion and find the data acceptable, especially taking into account low absolute concentrations in those two matrices.

Most of the administrated radioactivity was excreted (>90%). In eggs yolk, eggs white and tissues low amounts of administrated recovery was measured (up to ≤0.05 % of the administrated dose). Most significant residues (>10% TRR) were N-acetylglyphosate, AMPA, glyphosate and N-acetyl AMPA.

Samples were analysed within 4 months of collection, which is covered by the available stability data.

B.7.2.3. Lactating ruminants

B.7.2.3.1. Study 1

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	Conclusion applicant: Supportive (Category 2a) Conclusion RMS: Supportive information

2. Full summary of the study according to OECD format

Executive Summary

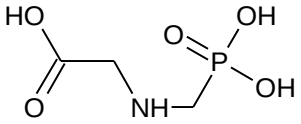
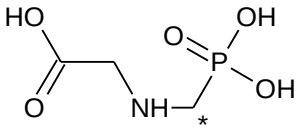
The absorption, distribution, metabolism and excretion of radioactive residues have been studied following repeated oral administration of N-(phosphono-^{14}C-methyl)glycine (^{14}C-glyphosate) to lactating goats twice daily for five (Goat 1) or three consecutive days (Goat 2), respectively. The nominal 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 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), 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:	[redacted]
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 B.7.2.3-1: 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. 0.064 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 B.7.2.3-2: 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 7.2.3-3: 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 B.7.2.3-4: 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 B.7.2.3-5: 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 B.7.2.3-6: 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] 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 B.7.2.3-7: 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.

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: RMS agrees with the assessment of the applicant. The study investigates metabolism of glyphosate in ruminants (lactating goats). Based on the reported results it can be concluded that glyphosate was not extensive metabolised in lactating goats. Glyphosate was rapidly excreted (as unchanged parent compound) resulting in low residue levels in tissues and milk, where also unchanged glyphosate was the primary residue. The study provides qualitative information on the metabolism of glyphosate in ruminants, however, taking into account all the above reported deficiencies, especially regarding extraction efficiency for some matrices (liver, kidney) no robust quantification of residues could be performed. Additionally no residue profile was investigated in milk and muscle. The study is considered as supportive information.

B.7.2.3.2. Study 2

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	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Study is acceptable

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).

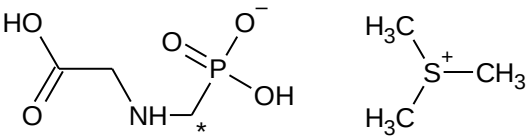
Of the relevant edible matrices of the goat, highest total radioactive residues were found in kidneys (5.58 mg/kg) and liver (0.234 mg/kg). In muscle and fat 0.0256 and 0.0175 mg/kg were found, respectively. For whole milk samples, a plateau was reached after 4 days.

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 aegarus 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 [redacted] and shipped to [redacted] 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 [redacted]. All tissue, milk and excreta were sent frozen on dry ice to [redacted]. The samples were stored at -20°C at [redacted]

3. Analytical procedures

Processing of Tissues for Determination of Total Radioactive Residues (TRR)
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.

Extraction

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 B.7.2.3-8: 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] Gl 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 B.7.2.3-9: 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 [redacted]
[2] The TRR values determined by combustion analysis at [redacted] 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 [redacted] was set as 100 %, as a larger sample (8 g) were analysed at [redacted] compared to combustion analysis at [redacted] (approximately 0.1 g).
[3] For muscle and milk (day 7) the TRR was further determined by combustion at [redacted] 0.022 and 0.024 mg/kg were found, respectively.

Table B.7.2.3-10: 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 B.7.2.3-11: 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 [redacted]. The TRR values determined by combustion analysis at [redacted] 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 [redacted] was set as 100 %, as a larger sample (8 g) were analysed at [redacted] compared to combustion analysis at [redacted] (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 B.7.2.3-12: 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 B.7.2.3-13: 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 B.7.2.3-14: 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 in Volume 1.

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:
RMS agrees with the study evaluation and considered the study as acceptable. Based on the available data metabolism pattern of glyphosate in ruminants can be sufficiently addressed.
Most of the administrated radioactivity was found in excreta (90% AR). In milk and tissues only low amounts of administrated recovery was measured (up to 0.09% AR in kidney). No significant degradation of glyphosate was observed, with glyphosate being the most relevant residues. AMPA was also detected in tissues, however in most of matrices as a minor residue and in liver up to 21% TR (0.05 mg/kg).
The duration of sample storage was estimated as maximum of 9 months, which is covered by the available stability data.

B.7.2.3.3. Study 3 and Study 4

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	Conclusion applicant: Supportive (Category 2a) Conclusion RMS: Study is considered acceptable

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% (mean of two goats) and 86.7 % of the administered dose was recovered. The main part was excreted, accounting in sum (urine, faeces plus pan rinse) for 75 – 86.46 %. Total milk production contained less than 0.01% of the total dose. The remainder of the radioactivity from 0.03% to 0.17% of the total dose, was recovered in tissues that were examined.

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. For the test 2 treatment, results are reported as a mean of the two goats.

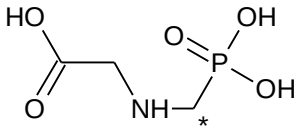
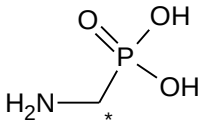
In milk the radioactive residue ranged between 0.019 and 0.060 mg/kg during the dosing period. After the 5 day depuration the radioactive residue decreased to 0.006 mg/kg.

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 B.7.2.3-15: 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 [redacted] (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 milking 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 [redacted] After determination of radioactivity in the samples, they were sent to [redacted] (site of analysis) on dry ice via overnight freight. The samples were stored at -20 °C at [redacted]

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. Samples were combusted in a sample oxidizer and radioactivity was analysed by LSC

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 B.7.2.3-16: 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	58.3	47.3	*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 B.7.2.3-17: 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 B.7.2.3-18: 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 B.7.2.3-19: 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 B.7.2.3-20: 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 B.7.2.3-21: 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 B.7.2.3-22: 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 B.7.2.3-23: 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 B.7.2.3-24: 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:								
AMPA[2]	*0.417*	*4.0*	*0.305*	*8.7*	*0.361*	*6.3*	*0.773*	*15.3*

Table B.7.2.3-24: 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
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 B.7.2.3-25: 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*	*94.2*	*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 B.7.2.3-26: 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 B.7.2.3-27: 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 B.7.2.3-28: 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 were 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 in the Volume 1, point 2.7.2.

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:
RMS agreed with the studies evaluation and considered the studies as acceptable. The deviations reported by the applicant are noted, however RMS is of the opinion that based on the available data metabolism pattern of glyphosate and AMPA in ruminants has been sufficiently addressed
For some of the reported results a clarification was added, where mean values of two goats were used to report the data (test 2).
Most of the administrated radioactivity was found in excreta (75-86.5% AR). In milk and tissues only low amounts of administrated recovery was measured (up to 0.17% AR). No significant degradation of glyphosate was observed, with glyphosate and AMPA being the most relevant residues. In milk an unknown metabolite

was detected and further characterised as low molecular weight proteins or glycoproteins. The duration of sample storage was estimated as seven months (based on the study report data), which is covered by the available stability data.

B.7.2.3.4. Study 5

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	Conclusion applicant: Supportive (Category 2a) Conclusion RMS: Acceptable

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*-acteylglyphosate, 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).

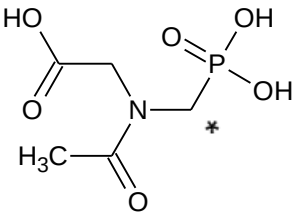
N-acetylglyphosate was metabolised in the goat by de-acetylation to form glyphosate. *N*-acetylglyphosate and glyphosate were metabolised to form *N*-acetyl AMPA and AMPA, respectively. *N*-acetyl AMPA may also have undergone de-acetylation to form AMPA.

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 [redacted])
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 after combustion analysis. 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 B.7.2.3-29: 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*
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 B.7.2.3-30: 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 B.7.2.3-31: 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 B.7.2.3-32: 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[6]	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).
* TRR = ERR+RRR
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 B.7.2.3-33: 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 B.7.2.3-34: 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 B.7.2.3-35: 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*
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***

Table B.7.2.3-35: 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])*
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:
RMS agreed with the study evaluation and considered the study as acceptable. The reported deficiencies were noted and evaluated, however, it has been concluded that based on the available data metabolism pattern of glyphosate and AMPA in ruminants can be sufficiently addressed.

It is reported by the applicant that in balance of components there are losses of up to 29.93% TRR or up to 0.338 mg/kg. This observation holds true for balance of ERR. However, in most of the tissues loses are <10% and/or <0.01 mg/kg. Estimated portion of 29.9% TRR has been calculated for milk, however, taking into account absolute values it correspond to 0.008 mg/kg. Moreover, more than 50% of residues were identified as applied N-acetylglyphosate and more than 79% TRR was identified and characterised. A high absolute value of missing portions of 0.338 mg/kg has been estimated for kidney. However, when expressed in TRR, it corresponds to 6.9% TRR (<10% TRR). Kidney had a high number of extractable residues (4.708 mg/kg eq) and 90% of TRR was identified and characterised. On the other hand, it has also been reported that the concentrated extract was assumed to be representative of initial extracts, it is not expected that loses are related to any particular metabolite. RMS agrees with this last conclusion and find the data acceptable.

Most of the administrated radioactivity was excreted in urine and faeces (>85%TRR). In milk and tissues low amounts of administrated recovery was measured (up to 0.03% of the administrated dose). Most significant residues (>10% TRR) were N-acetylglyphosate, glyphosate and N-acetyl AMPA. Metabolite AMPA was a minor metabolite (<10% TRR) in ruminant matrices.

All samples were extracted and analysed within 2 months, which is acceptable.

B.7.2.4. Pigs

Studies in rabbits/rats, lactating goats and laying hen demonstrated a similar pattern of toxicokinetics and metabolism pathways. Therefore, the findings in ruminants can be extrapolated to pigs.

B.7.2.5. 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, which are relevant for conventional crops, as well as its metabolites *N*-acetyl AMPA and *N*-acetyl glyphosate, which are only relevant for tolerant crops, are all 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.

B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

B.7.3.1. Post-emergence use

A post-emergence outdoor use against weeds in orchard crops (citrus, stone and pome fruits, kiwi, tree nuts, banana, and table olives) and vines (table and wine grape, leaves not intended for human consumption) is intended. The critical GAP in NEU and SEU is identical and is as follows:

2 x 1.44 kg/ha (max. 2.88 kg/ha per year), interval 28 days, PHI 7 days (ground directed, shielded spray, band application).

The following is additionally stated in the GAP: *"Applications are performed between the crop rows. The rate refers to the treated area only, which represents not more than 50% of the total area. The application rate with reference to the total surface area is not more than 50% of the stated dose rate."* This restriction, however, is not expected to be of importance for the evaluation of the residues section and is therefore not considered during the assessment.

Furthermore, the GAP states the following: *"Avoid crop contamination during treatment."*

The intended use is less critical compared to the critical use evaluated in the previous RAR which was as follows for both NEU and SEU: 1-3 x 2.88 kg/ha (max. 4.32 kg/ha per year), interval 28 d, PHI n.a.

All studies submitted by the applicant in support of the intended orchards and vines uses are summarised in the following paragraphs. It is noted that some additional studies were identified in the previous evaluation (RAR, 2015) which were not included by the applicant in the current dossier. The RMS shortly presents the studies based on the summaries from the RAR, however, the studies are not evaluated in detail. It is not expected that the results from these studies will significantly affect the outcome of the risk assessment:

- Report MLL 30,053 (RIP9501235): Two residue trials in apples in NEU; one trial within 25% of the intended single use rate and one trial within 25% of the maximum yearly use rate. Residue levels of glyphosate and AMPA were <LOQ at an PHI of 6-8 days.
- Report EA000182 (RIP2001-557): Two residue trials in peaches in SEU; both trials are overdosed compared to the intended use rate. Residue levels of glyphosate and AMPA were <LOQ at an PHI of 7 days in fruits without stones and whole fruits. It is not unknown whether residue levels were also determined in stones.
- Report MLL30.319 (RIP9501290): Multiple residue trials in olives in SEU of which two trials were conducted at the intended single application rate and with olives sampled at a PHI of 6-7 days. Samples taken from the tree and from the ground were analysed. All residue levels of AMPA were <LOQ. Residue levels of glyphosate were <LOQ in one trial and at 0.4 mg/kg (ground-sampled olives) in the other trial. The latter value is in line with ground-sampled olives from the trials presented by the applicant.
- Report 90-Gly-02 (RIP9501289): Multiple residue trials in olives in SEU; in two trials residues were determined at a PHI of 7 days. Trials significantly underdosed (0.36 kg/ha) but residue levels of glyphosate were at 1.1 and 1.2 mg/kg at a PHI of 7 days in ground-sampled olives. Levels of AMPA were not determined.

B.7.3.1.1. Study 1

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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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

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.

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.

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 B.7.3.1.1-1: 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

Table B.7.3.1.1-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
		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 B.7.3.1.1-2: 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)
				Glyphosate	AMPA	
S13-02531-01 / [redacted] Valencia, Spain / SEU / 2013	Mandarin / Okitsu	83	Peel	<0.05	<0.05	0
			Pulp	<0.05	<0.05	0
			Whole fruit[4]	<0.05	<0.05	0
S13-02531-01 / [redacted] Valencia, Spain / SEU / 2013	Mandarin / Okitsu	83	Peel	<0.05	<0.05	0
			Pulp	<0.05	<0.05	0
			Whole fruit[4]	<0.05	<0.05	0

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 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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at a target rate of 2.88 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted. Furthermore samples were collected at a PHI of 0 days (it is not mentioned in the study report how many hours after application sampling took place) instead of the intended 7 days, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs. The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency and derivatisation efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability of glyphosate since storage stability of glyphosate was demonstrated for 18 months (covering the storage periods of specimens in this study) in commodities of five different categories for 18 months, i.e. the storage stability results can be extrapolated to all plant matrices.
In contrast to this and strictly speaking, specimens were not stored in accordance with the demonstrated period of storage stability of AMPA. Storage stability of AMPA was only determined in four commodity categories and no overall conclusion for all plant matrices can be made based on the available data. Storage stability of AMPA, however, was demonstrated for 24 months in oranges. Considering the similarity between oranges and mandarins, the available data are considered sufficient to conclude that no significant decline of residues of AMPA in mandarins occur either. In addition to this, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the uses on orchard crops and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Mandarin pulp (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Mandarin peel (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Mandarin whole fruit (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.2. Study 2

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 7029/VI/95 rev. 5 SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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 European 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 quantification (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
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

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.

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.

Application information				
Trial no.	**Application code**	**Timing**	**Application rate kg a.s./ha**	**Water volume L/ha**
S15-00018-01	2	BBCH 87	3.60	300
S15-00018-02	2	BBCH 87	3.89	324

Regions, varieties and cultivation were typical for the cultivation of tree nuts. Care was taken that the spray solution was properly homogenized by mixing before application. Application was performed with motorized 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.

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. Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA.
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 B.7.3.1.2-1: 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 LOQ (0.05 mg/kg) were found in any treated or untreated specimens of tree nuts (nutmeat). Detailed residue levels are shown in the table below.

Table B.7.3.1.2-1: 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[2,3,4] (mg/kg)		DALA[5] (days)
				Glypho-sate	AMPA	
S15-00018-01 / [redacted] Tarragona, Spain / SEU / 2015	Tree nuts / Hazelnut / Pavelet	89	Nutmeat	<0.05 (n.d.)	<0.05 (n.d.)	7
S15-00018-02 / [redacted], Sicily, Italy / SEU / 2015	Tree nuts / Pistachio / Napoletana	89	Nutmeat	<0.05 (n.d.)	<0.05 (n.d.)	7

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg
3 n.d. (not detected): <0.015 mg/kg
4 Residues of glyphosate were expressed as glyphosate while the residues of AMPA were expressed as AMPA. Results are the mean of duplicate analyses.
5 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 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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.60-3.89 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted. As already stated in the study summary, the weight of the hazelnut nutmeat sample in one trial was less than required by OECD guideline 509 (0.191 kg cf. 1 kg). Nevertheless, it is agreed with the applicant that the sample is representative considering that the number of individual nuts exceeded 50 units and nuts were sampled in a representative manner across the trial site.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices (at least 12 months)).
In contrast, AMPA was shown to be stable in soybean seeds only and data are not sufficient to allow an extrapolation to all high oil content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the uses on orchard crops and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Hazelnut (nutmeat) (SEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg
Pastachio (nutmeat) (SEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg

B.7.3.1.3. Study 3

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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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

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 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.

Application information				
Trial no.	**Application code**	**Timing**	**Application rate kg a.s./ha**	**Water volume L/ha**
S13-03425-01	A1	BBCH 87-89	2.88	300
S13-03425-02	A1	BBCH 87-89	2.87	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.

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, i.e. not constantly below -18°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.

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 fruits 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. For glyphosate and AMPA in apple (fruit), the limit of quantitation (LOQ) was 0.05 mg/kg each.
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.
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 B.7.3.1.3-1: 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 B.7.3.1.3-2: 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)
				Glypho-sate	AMPA	
S13-03425-01 / [redacted] Essex, United Kingdom / NEU / 2013	Apple / Jonagold	87-89	Fruit	<0.05	<0.05	0
S13-03425-02 / [redacted] Germany / NEU / 2013	Apple / Golden Delicious	87-89	Fruit	<0.05	<0.05	0

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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 2.88 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted. Furthermore samples were collected at a PHI of 0 days (it is not mentioned in the study report how many hours after application sampling took place) instead of the intended 7 days, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs. Lastly, it is agreed that the storage of specimens from trial S13-03425-01 at -17 °C is indeed unlikely to affect the residue level determined after analysis. It is noted that samples were only stored at -17 °C during the field phase of the study; in the analytical laboratory, samples were stored ≤ -18 °C.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency and derivatisation efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. No information is available in the study report whether residue levels are also below LOD, or only below LOQ. The following residues are selected for evaluation:

Apple (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.4. Study 4

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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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

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). Due to an application error, trial S13-03426-01 was cancelled and replaced with trial S13-03426-03; therefore no information on trial S13-03426-01 is available in the study report. 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 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.

Application schedule				
Trial no.	**Application code**	**Timing**	**Application rate kg a.s./ha**	**Water volume L/ha**
S13-03426-02	A1	89 BBCH	2.86	397
S13-03426-03	A1	89 BBCH	3.02	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.

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.

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 fruits 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. For glyphosate and AMPA in apple (fruit), the limit of quantitation (LOQ) was 0.05 mg/kg each. 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.
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 B.7.3.1.4-1: 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 B.7.3.1.4-2: 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)
				Glypho-sate	AMPA	
S13-03426-02 / [redacted] Bologna, Italy / SEU / 2013	Apple / Golden	89	Fruit	<0.05	<0.05	0
S13-03426-03 / [redacted] Aragon, Spain / SEU / 2013	Apple / Golden Delicious	89	Fruit	<0.05	<0.05	0

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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 2.86-3.02 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted. Furthermore samples were collected at a PHI of 0 days (it is not mentioned in the study report how many hours after application sampling took place) instead of the intended 7 days, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs. Lastly, the deviation in storage temperature for specimens from trial S13-03426-02 (-15.2 °C for 97.5 hours during transport) are indeed not expected to affect the study outcome considering that samples remained frozen all the time.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency and derivatisation efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Apple (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.5. Study 5

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 study design and observations:	Thirty-five trials were conducted on apples between 1973 and 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, derivatization 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.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion applicant:** 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 3b) **Conclusion RMS:** The study was not performed according to GLP, although this was not a requirement at the timepoint of the conduct of the study. The trials were performed according to a less critical GAP than intended in terms of PHI and the performance of the analytical method was not adequate. The study is not considered acceptable for evaluation.

B.7.3.1.6. Study 6

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 EC guidance working document 7029/VI/95 rev. 5 SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered acceptable.

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
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

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

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.

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 homogenized by mixing before application. Application was performed with motorized 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 (normal commercial harvest). 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. After recording the weight of fruits with and without stones, the stones were 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 6.5 hours of sampling in the field).

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 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 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 B.7.3.1.6-1: 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 B.7.3.1.6-2: 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)**
				Glypho-sate	**AMPA**	
S15-00019-01 / [redacted] Pyrenées-Orientales, France / SEU / 2015	Apricot / Royal Roussillon	87-89	Fruit without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[5]	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	
S15-00019-02 / [redacted] Pyrenées-Orientales, France / SEU / 2015	Apricot / Foubaly	87	Fruit without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[5]	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	

Table B.7.3.1.6-2: 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)**
				Glypho-sate	**AMPA**	
S15-00019-03 / [redacted], Emilia Romagna, Italy / SEU / 2015	Apricot / Precoce D'Imola	87	Fruit without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[5]	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	
S15-00019-03 / [redacted] Emilia Romagna, Italy / SEU / 2015	Apricot / Reale D'Imola	89	Fruit without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[5]	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Values represent the mean from two sampling replicates.
4 Days after last application
5 Residues in whole fruit based on residue levels in the flesh and correction for the weight ratio of flesh and stones. Residue levels in stone, however, were not determined.

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

<u>Assessment and conclusion by applicant</u>:
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.40-3.87 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted.
According to Annex I of Reg. (EC) 396/2005, stone fruits are defined as the whole product after removal of the stem, i.e. including the stone. In this study, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. However, residue levels in whole fruits were calculated based on the residue level in flesh and a correction for the weight ratio of flesh and stone; therefore, the results are considered acceptable.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. It is noted that duplicate field specimens were sampled and both specimens were analysed (sampling replicates), i.e. mean values are selected for evaluation. The following values are selected for evaluation:

Apricot, whole fruit (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.1.7. Study 7

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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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) 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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S13-03427-01	Sweet cherry	*Prunus avium*	Sweet Heart	Fruit	≥ 1.2 kg / 12 units
S13-03427-02	Sweet cherry	*Prunus avium*	Lapins	Fruit	≥ 1 kg / > 50 units

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.

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.

Crop sampling information						
Trial	**Crop**	**Commodity**	**Timing[1]**	**Growth stage (BBCH)**	**Quantity**	**Date of sampling**
S13-03427-01	Sweet cherry	Fruit	NCH	89	≥ 1.2 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 7.2.1.7-1: 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				

Table 7.2.1.7-1: Recovery results						
Matrix	**Analyte**	**Fortification level (mg/kg)**	**Recovery[1]**			
			Range (%)	**Mean (%)**	**Relative standard deviation (%)**	**Number analyses (n)**
		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 B.7.3.1.7-2: 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)**
				Glypho-sate	**AMPA**	
S13-03427-01 / [redacted] Spain / SEU / 2013	Sweet cherry / Sweet Heart	89	Whole fruit[5]	<0.05	<0.05	0
S13-03427-02 / [redacted] Italy / SEU / 2013	Sweet cherry / Lapins	87-89	Whole fruit[5]	<0.05	<0.05	0

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).
4 Days after last application
5 Residues in whole fruit based on residue levels in the flesh and correction for the weight ratio of flesh and stones. Residue levels in stone, however, were not determined.

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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 2.88 kg/ha (± 25%) was performed instead of two applications at 1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted. Furthermore samples were collected at a PHI of 0 days (it is not mentioned in the study report how many hours after application sampling took place) instead of the intended 7 days, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs. As already stated in the study summary, samples were stored above -18 °C, but never above -10 °C, for maximally 45 hours. Since samples remained frozen all the time, it is agreed with the study author that a significant impact on the study outcome is not expected. It is also noted that in none of the trials, the minimum weight of specimen as required by OECD guideline 509 (i.e. 2 kg) was achieved. Considering that 12 and 50 units from different areas in the field were sampled for trial S13-03427-01 and S13-03427-02, respectively, the specimens are considered sufficiently representative for the determination of residue levels following treatment with A12798QA.
According to Annex I of Reg. (EC) 396/2005, stone fruits are defined as the whole product after removal of the stem, i.e. including the stone. In this study, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. However, residue levels in whole fruits were calculated based on the residue level in flesh and a correction for the weight ratio of flesh and stone; therefore, the results are considered acceptable.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency and derivatisation efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Cherry, whole fruit (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.8. Study 8

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 that would impact the outcome of the study.

Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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) 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
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

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

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.

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	2.747	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.

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 B.7.3.1.8-1: 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
		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 B.7.3.1.8-2: 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)
				Glypho-sate	AMPA	
S13-03233-01 / [redacted] Emilia Romagna, Italy / SEU / 2013	Plum / Ersinger	87-89	Whole fruit[5]	<0.05	<0.05	0
S13-03233-02 / [redacted], Emilia Romagna, Italy / SEU / 2013	Plum / Angeleno	87-89	Whole fruit[5]	<0.05	<0.05	0

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
5 Residues in whole fruit based on residue levels in the flesh and correction for the weight ratio of flesh and stones. Residue levels in stone, however, were not determined.

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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 2.88 kg/ha (± 25%) was performed instead of two applications at 1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted. Furthermore samples were collected at a PHI of 0 days (it is not mentioned in the study report how many hours after application sampling took place) instead of the intended 7 days, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs. According to Annex I of Reg. (EC) 396/2005, stone fruits are defined as the whole product after removal of the stem, i.e. including the stone. In this study, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. However, residue levels in whole fruits were calculated based on the residue level in flesh and a correction for the weight ratio of flesh and stone; therefore, the results are considered acceptable.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency and derivatisation efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Plum, whole fruit (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.9. Study 9

1. Information on the study

Data point:	CA 6.3.1/009
Report author	[redacted]
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 SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
Active ingredient(s):	Glyphosate (as the isopropylammonium salt)
CAS number:	38641-94-0
Content of a.s. nominal:	360 g/L (glyphosate acid equivalents)
Content of a.s. analysed:	356.3 g/L (glyphosate acid equivalents)
Formulation type:	SL

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

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 additional trial was conducted (S12-03071-04), however, this trial was cancelled due to a premature harvest. Further details on this trial are therefore not included in this study summary. 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 2.88 kg a.s./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.

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 homogenized by mixing before application. Application was performed

with motorized 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).

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 B.7.3.1.9-1: 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
		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 B.7.3.1.9-2: Residue levels of glyphosate and AMPA in stone fruits after one application of CA2705 (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)**
				Glypho-sate	**AMPA**	
S12-03071-01 / [redacted] Bas-Rhin, France / NEU / 2012	Plum / Elena	81	Fruit	<0.05	<0.05	0
		81	Fruit	<u>≤0.05</u>	<u>≤0.05</u>	7
		85-87	Fruit	<0.05	<0.05	14
		87	Fruit	<0.05	<0.05	21
S12-03071-02 / [redacted] Alsace, France / NEU / 2012	Plum / Mirabelle	87-89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	21

Table B.7.3.1.9-2: Residue levels of glyphosate and AMPA in stone fruits after one application of CA2705 (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)**
				Glypho-sate	**AMPA**	
S12-03071-03 / [redacted], Loir et Cher, France / NEU / 2012	Plum / Mirabelle	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	21
S12-03071-05 / [redacted] Pyrenees-Orientales, France / SEU / 2012	Peach / Maillardiva	81	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	0
		81-83	Fruit	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	7
		87-89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	14
		87-89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	20
S12-03071-06 / [redacted], Tarn et Garonne, France / SEU / 2012	Peach / Brareg	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	21
S12-03071-07 / [redacted] Pyrenees-Orientales, France / SEU / 2012	Apricot / Farbaly	87-89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	21
S12-03071-08 / [redacted], Tarn et Garonne, France / SEU / 2012	Plum / Mirabelle	87-89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	21

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue in RAC, whole fruit (fruit + stone). If residue in fruits without stone are <LOQ, then residues in whole fruits are also reported as <LOQ.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 2.88 kg/ha (± 25%) was performed instead of two applications at 1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted. Only trial S12-03071-06 is an exception, in which application rates were > 25% higher than the maximum yearly use rate, however, this trial is not considered for evaluation anyway for the following reason: except for trials S12-03071-01 and S12-03071-05, no specimens were taken at the intended PHI of 7 days. Therefore, no residue values are selected from these trials. It is noted that the study report states that fruit specimens were taken at normal commercial harvest 20-21 days after application. Nevertheless, the RMS is of the opinion that specimens taken at a PHI of 7 days are also acceptable for evaluation since fruits were sampled at growth stages BBCH > 81, i.e. the final fruit size was reached. Residue levels are therefore not influenced anymore due to a potential increase in fruit weight. Lastly it is noted that in some trials, the minimum weight of specimen as required by OECD guideline 509 (i.e. 2 kg) was not achieved. Considering that ≥ 24 units from different areas in the field were sampled in each trial, the specimens are considered sufficiently representative for the determination of residue levels following treatment with CA2705.
According to Annex I of Reg. (EC) 396/2005, stone fruits are defined as the whole product after removal of the stem, i.e. including the stone. In this study, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. Furthermore, weights of the stones were not reported, i.e. it is not possible to calculate residue levels on a whole fruit basis. Since residue levels of glyphosate and AMPA were below the LOQ in the flesh, however, this deviation is considered acceptable.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Plum, whole fruit (NEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg
Peach, whole fruit (SEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg

B.7.3.1.10. Study 10

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 EC guidance working document 7029/VI/95 rev. 5 SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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

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 3.60 kg a.s./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.

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 homogenized by mixing before application. Application was performed with motorized knapsack sprayer equipped with flat fan nozzles. 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).

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 B.7.3.1.10-1: 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 B.7.3.1.10-2: 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)**
				Glypho-sate	**AMPA**	
S15-00491-01 / 74382 [redacted] Baden-Württemberg, Germany / NEU / 2015	Grape / Trollinger (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00491-02 / [redacted] Baden-Württemberg, Germany / NEU / 2015	Grape / Kerner (white)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Values represent the mean from two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.83-3.84 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted. Per trial, specimens from the upper and lower plant level were sampled. These specimens are not considered independent; residue levels from the lower plant level are selected for evaluation since these are considered more critical (as they are closer to the ground to which the product is applied). Since all residue levels were below the LOQ, this has no further implications though. It is also noted that duplicate field specimens were sampled and both specimens were analysed (sampling replicates), i.e. mean values are selected for evaluation.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices (at least 12 months)).
In contrast and with regard to acidic matrices, AMPA was shown to be stable in oranges only and data are not sufficient to allow an extrapolation to all high acid content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the use on vineyards, and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following values are selected for evaluation:

Grapes (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.11. Study 11

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 SANCO 3029/99 rev. 4

Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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	Grape	*Vitis vinifera*	Cabernet (red)	Bunches	≥ 1 kg / 12 units

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 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 3.6 kg a.s./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.

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	3.360	280

Regions, varieties and cultivation were typical for the cultivation of grapes. Care was taken that the spray solution was properly homogenized by mixing before application. Application was performed with motorized 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).

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
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 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 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 B.7.3.1.11-1: 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).

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 B.7.3.1.11-2: 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)
				Glypho-sate	AMPA	
S14-04157-01 / [redacted] Sanoe et Loire, France / NEU / 2014	Grape / Pinot noir (red)	85	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04157-02 / [redacted], Saone et Loire, France / NEU / 2014	Grape / Chardonnay (white)	85	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04157-03 / [redacted] Maine et Loire, France / NEU / 2014	Grape / Cabernet Franc (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04157-04 / [redacted] Maine et Loire, France / NEU / 2014	Grape / Chenin (white)	89	Bunches / upper plant level	<0.05	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	

Table B.7.3.1.11-2: 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)**
				Glypho-sate	**AMPA**	
S14-04157-05 / [redacted] Pyreneés-Orientales, France / SEU / 2014	Grape / Viognier (white)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	8
			Bunches / lower plant level	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	
S14-04157-06 / [redacted] Pyreneés-Orientales, France / SEU / 2014	Grape / Cabernet (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Values represent the mean from two sampling replicates.
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

<u>Assessment and conclusion by applicant</u>:
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.37-3.94 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted. Per trial, specimens from the upper and lower plant level were sampled. These specimens are not considered independent; residue levels from the lower plant level are selected for evaluation since these are considered more critical (as they are closer to the ground to which the product is applied). Since all residue levels were below the LOQ, this has no further implications though. It is also noted that duplicate field specimens were sampled and both specimens were analysed (sampling replicates), i.e. mean values are selected for evaluation. As already stated in the study summary, some specimens were transported at maximally -13 °C for maximally 32 hours during shipment. It is agreed with the study author that this does not have a significant impact on the study outcome since specimens remained frozen all the time.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices (at least 12 months)).
In contrast and with regard to acidic matrices, AMPA was shown to be stable in oranges only and data are not sufficient to allow an extrapolation to all high acid content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the use on vineyards, and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following values are selected for evaluation:

Grapes (NEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg
Grapes (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.12. Study 12

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 EC guidance working document 7029/VI/95 rev. 5 SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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

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). The RMS notes that a further trial in Germany was initially planned (S14-04158-04) which was cancelled due to pesticide restriction demands. Therefore, no results are available for this trial number. 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 3.6 kg a.s./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.

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 homogenized by mixing before application. Application was performed with motorized 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).

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

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 B.7.3.1.12-1: 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 B.7.3.1.12-2: 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)**
				Glypho-sate	**AMPA**	
S14-04158-01 / [redacted] Baden-Württemberg, Germany / NEU / 2014	Grape / Portugieser (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	
S14-04158-02 / [redacted] [redacted] Rheinland-Pfalz, Germany / NEU / 2014	Grape / Riesling (white)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	
[redacted] Baden-Württemberg, Germany / NEU / 2014	Grape / Trollinger (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<u>≤0.05</u>	<u>≤0.05</u> (n.d.)	

Table B.7.3.1.12-2: 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)**
				Glypho-sate	**AMPA**	
S14-04158-05 / [redacted] Aragon, Spain / SEU / 2014	Grape / Cabernet Sauvignon (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04158-06 / [redacted] Aragon, Spain / SEU / 2014	Grape / Garnacha (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Values represent the mean from two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.39-10.83 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted.
Per trial, specimens from the upper and lower plant level were sampled. These specimens are not considered independent; residue levels from the lower plant level are selected for evaluation since these are considered more critical (as they are closer to the ground to which the product is applied). Since all residue levels were below the LOQ, this has no further implications though. It is also noted that duplicate field specimens were sampled and both specimens were analysed (sampling replicates), i.e. mean values are selected for evaluation. As already stated in the study summary, some specimens were transported at maximally -13 °C for maximally 15.5 hours during shipment. It is agreed with the study author that this does not have a significant impact on the study outcome since specimens remained frozen all the time.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices (at least 12 months)).
In contrast and with regard to acidic matrices, AMPA was shown to be stable in oranges only and data are not sufficient to allow an extrapolation to all high acid content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the use on vineyards, and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following values are selected for evaluation:

Grapes (NEU)
Glyphosate: 3x <0.05 mg/kg
AMPA: 3x <0.05 mg/kg
Grapes (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.13. Study 13

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 EC guidance working document 7029/VI/95 rev. 5 SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS**: The study is considered to be acceptable.

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
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

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

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 3.6 kg a.s./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.

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 homogenized by mixing before application. Application was performed with motorized 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 87-89), 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).

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 maximum 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 B.7.3.1.13-1: 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

Table B.7.3.1.13-1: Recovery results							
Matrix	**Analyte**	**Fortification level (mg/kg)**	**Recovery[1]**				
			Range (%)	**Mean (%)**	**Standard deviation (%)**	**Relative standard deviation (%)**	**Number analyses (n)**
	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 B.7.3.1.13-2: 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)**
				Glypho-sate	**AMPA**	
S14-04226-01 / [redacted] Kavala, Greece / SEU / 2014	Grape / Merlot (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04226-02 / [redacted] Kavala, Greece / SEU / 2014	Grape / Sauvignon blanc (white)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04226-03 / [redacted] Aragon, Spain / SEU / 2014	Grape / Garnacha (red)	89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04226-04 / [redacted] Aragon, Spain / SEU / 2014	Grape / Garnacha (red)	87-89	Bunches / upper plant level	<0.05 (n.d.)	<0.05 (n.d.)	7
			Bunches / lower plant level	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Values represent the mean from two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.60-3.92 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted.
Per trial, specimens from the upper and lower plant level were sampled. These specimens are not considered independent; residue levels from the lower plant level are selected for evaluation since these are considered more critical (as they are closer to the ground to which the product is applied). Since all residue levels were below the LOQ, this has no further implications though. It is also noted that duplicate field specimens were sampled and both specimens were analysed (sampling replicates), i.e. mean values are selected for evaluation. As already stated in the study summary, some specimens were transported at maximally -13 °C for maximally 32 hours during shipment. It is agreed with the study author that this does not have a significant impact on the study outcome since specimens remained frozen all the time.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices (at least 12 months)).
In contrast and with regard to acidic matrices, AMPA was shown to be stable in oranges only and data are not sufficient to allow an extrapolation to all high acid content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the use on vineyards, and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following values are selected for evaluation:

Grapes (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.1.14. Study 14

Data point:	CA 6.3.1/014
Report author	[redacted]
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. Applications were performed in spring and summer, i.e. application intervals exceeded 28 days. Only at trial location Weinsberg, one application was performed. 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 (method XA001). 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. In some untreated specimens, residues of glyphosate were detected (see column 10 in the table below for further specifications).

Reasons for why the study is not considered relevant/reliable or not considered as key study:	**Conclusion applicant** (Category 2b): • 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) **Conclusion RMS:** The RMS notes that the study report is of bad quality; therefore, not all information is retrievable anymore. The evaluation is based on the readable information only. Since the applicant did not consider the study to be acceptable for evaluation, no extensive summary was presented by the applicant. To facilitate the evaluation, the RMS copied the summary table of the supervised residue trials as presented in the previous evaluation (RAR, 2015) to this document. It is noted that the table was amended since some mistakes were noted; for instance, in the previous evaluation corrected residue levels were reported. Furthermore, the RMS added recoveries to the table. The trials were performed according to a less critical GAP than intended in term of application rate. Furthermore, application intervals were higher than 28 days; therefore it is not expected that the first application contributes significantly to the residue level at harvest. Based on the available information provided in the study report, it is not clear what kind of application equipment was used, i.e. whether applications were performed with a ground-directed, shielded spray as stated in the GAP. The performance of the analytical method was not acceptable. Several procedural recoveries were below the acceptable value of 70%. Besides, the method is not validated for the determination of residue of AMPA in grapes (see Vol. 3, B.5). Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices). In contrast and with regard to acidic matrices, AMPA was shown to be stable in oranges only and data are not sufficient to allow an extrapolation to all high acid content matrices or to all plant commodities. Although AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops, a <LOQ residue situation cannot anticipated for this study as glyphosate was detected above the LOQ in the available trials. Therefore, it cannot be ascertained whether residue levels of AMPA were indeed below the LOQ following application with glyphosate, or whether residue levels declined during storage. Overall and in case this study would have been considered for evaluation (see below), additional storage stability data on AMPA would be required in this case. Taking all points stated together, the study is not considered reliable for evaluation. No residue levels are selected for evaluation. **Grapes (NEU)** Glyphosate: not selected AMPA: not selected

Table B.7.3.1.14-1: Recovery results

Matrix	Trial	Glyphosate			AMPA		
		Fortification level [mg/kg]	Individual recoveries [%]	Average ± RSD [%]	Fortification level [mg/kg]	Individual recoveries [%]	Average ± RSD [%]
Grapes	GGE 8901	0.05	65, 61, 78, 81, 83, 95	77 ± 16	0.1	50, 80, 60, 68, 74, 76	68 ± 17
		0.1	80, 70, 78, 75	76 ± 5.7	0.05	52, 56, 69, 70	62 ± 15
	GGE 8902	0.05	84, 82, 68, 82	79 ± 9.4	0.1	92, 79, 60, 72	76 ± 18
	GGE 8903	0.05	63, 69	66 ± 6.4	0.1	82, 66, 86, 65	75 ± 15
		0.2	93, 74	84 ± 16.1			
	GGE 8904	0.05	65, 58	62 ± 8.0	0.1	66, 58, 76, 58, 81	68 ± 15
		0.1	68	/			
		0.5	73, 89	81 ± 14			
	GGE 8905	0.05	75, 75, 76	75 ± 0.8	0.1	77, 75, 84	79 ± 6.0
	GGE 8906	0.05	64	/	0.1	96, 86	91 ± 7.8
		0.2	81	/			

Table B.7.3.1.14-2 Residues data summary from supervised trials (summary)

Content of a.i. (g/kg or g/l) : 180 g/l
Formulation (e.g. WP) : SL
Commercial product (name) : MON 8755 (Arcade)

Active ingredient : Glyphosate
Crop / crop group : Grape Vine
Indoors / outdoors : Outdoors (European North)
Other a.i. in formulation (content and common name) : /
Residues calculated as : 8.1 Glyphosate
8.2 AMPA

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed (grapes)	Residues, glyphosate (mg/kg)	Residues, AMPA (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
GGE 8901 Germany [redacted]	Riesling (white variety)	1) 1977 (planting) 2) 1988-06-14 - 1988-06-19 3) 1988-09-01	0.72 0.72	400 400	0.18 0.18	1988-05-18 1988-09-01[4)]	BBCH 85	middle low middle low middle low ground middle low ground middle low	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 0 4 4 8 8 14 14 14 21 21 21	4) spraying max. sample storage: 7 months Procedural recoveries: 61-65% (glyphosate); 50-80% (AMPA)
GGE 8902 Germany [redacted]	Müller-Thurgau (white variety)	1) 1976 (planting) 2) 1988-06-14 - 1988-06-18 3) 1988-08-25	0.72 0.72	400 400	0.18 0.18	1988-05-19 1988-08-25[4)]	BBCH 85	middle low middle low middle low middle low ground middle low	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 0 4 4 8 8 14 14 21 21 21	4) spraying max. sample storage: 7 months Procedural recoveries: 68-84% (glyphosate); 60-92% (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed (grapes)	Residues, glyphosate (mg/kg)	Residues, AMPA (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
GGE 8903 Germany [redacted]	Müller-Thurgau (white variety)	1) 1983 (planting) 2) 1988-06-17 - 1988-06-25 3) 1988-10-05	0.72 0.72	400 400	0.18 0.18	1988-06-14 1988-08-01[4)]	BBCH 77-79	ground middle low ground middle low ground middle low ground middle low ground middle ground middle	<0.05 <0.05 0.1 <0.05 <0.05 0.1 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 0 0 4 4 4 9 9 9 15 15 15 21 21 65 65	4) spraying max. sample storage: 8 months Procedural recoveries: 61-93% (glyphosate); 65-96% (AMPA) Control (low), 4 DAT: 0.1 mg glyphosate/kg
GGE 8904 Germany [redacted]	Riesling (white variety)	1) 1976 (planting) 2) 1988-06-14 - 1988-06-23 3) 1988-10-20	0.72	400	0.18	1988-09-27[4)]	BBCH 85	ground middle low ground middle low ground middle low ground middle low ground middle low	0.3 <0.05 0.2 0.1 <0.05 <0.05 <0.05 0.2 <0.05 0.2 <0.05 <0.05 0.1 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 0 0 4 4 4 8 8 8 14 14 14 21 21 21	4) spraying max. sample storage: 5 months Procedural recoveries: 58-89% (glyphosate); 58-78% (AMPA)

1	2	3	4			5	6	7	8.1	8.2	9	10
Report-No. Location incl. Postal code	Commodity/ Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Application rate per treatment			Dates of treatments or no. of treatments and last date	Growth stage at last treatment or date	Portion analysed (grapes)	Residues, glyphosate (mg/kg)	Residues, AMPA (mg/kg)	PHI (days)	Remarks
			kg a.i./ha	Water l/ha	kg a.i./hl							
	(a)	(b)				(c)		(a)			(d)	(e)
GGE 8905 Germany [redacted]	Spätburgunder (red variety)	1) 1981 (planting) 2) 1988-06-15 - 1988-06-20 3) 1988-10-18	0.72 0.72	400 400	0.18 0.18	1988-04-26 1988-07-29[4)]	BBCH 77-79	middle low middle low middle low middle low middle low	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	0 0 3 3 10 10 14 14 21 21	4) spraying max. sample storage: 7 months Procedural recoveries: 75-76% (glyphosate); 75-85% (AMPA)
GGE 8906 Germany [redacted]	Muscat (white variety)	1) 1971 (planting) 2) 1988-06-21 - 1988-06-28 3) 1988-09-29	0.72 0.72	400 400	0.18 0.18	1988-05-25 1988-08-16[4)]	BBCH 79-81	Grapes (not further specified where it was samples)	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	0 3 7 14 21	4) spraying max. sample storage: 8 months Procedural recoveries: 64-81% (glyphosate); 86-96% (AMPA)

B.7.3.1.15. Study 15

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: • Number of trees sampled were not provided. • Report is unclear if residue results are reported for whole fruit, including weight of stones, or if results are for flesh only.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 2a) **Conclusion RMS:** The study is considered to be acceptable.

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. Per trial site, three plots were treated and specimens were sampled 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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
AP/3065/ME/1	Olive	*Olea europaea*	Hojiblanca	Fruit	≥ 0.72 kg
AP/3065/ME/2	Olive	*Olea europaea*	Picual	Fruit	≥ 0.97 kg
AP/3065/ME/3	Olive	*Olea europaea*	Picual	Fruit	≥ 0.95 kg
AP/3065/ME/4	Olive	*Olea europaea*	Picual	Fruit	≥ 0.94 kg

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). Per trial site, three plots were treated and specimens were sampled either 28, 14, or 7 days before commercial 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.

Application information				
Trial no.	**Application code**	**Timing[1]**	**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

[1] Growth stages in terms of BBCH codes are not given in the study report. Treatments were performed when fruits were reaching maturity though.

Regions, varieties and cultivation were typical for the cultivation of olives. Care was taken that the spray solution was properly homogenized 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 tree 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.

Crop sampling information					
Trial	**Crop**	**Commodity**	**Days before harvest**	**Quantity**	**Date of sampling**
AP/3065/ME/1	Olive	Fruit, from trees Fruit, from ground	28	0.97 kg 0.92 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]	≥ 0.73 kg ≥ 0.72 kg	05.12.1995
AP/3065/ME/2	Olive	Fruit, from trees Fruit, from ground	28	≥ 1.0 kg 0.98 kg	09.11.1995
	Olive	Fruit, from trees Fruit, from ground	14	≥ 1.0 kg 0.98 kg	23.11.1995
	Olive	Fruit, from trees Fruit, from ground	7	≥ 1.0 kg 0.99 kg	01.12.1995
	Olive	Fruit, from trees Fruit, from ground	NCH[1]	≥ 1.0 kg ≥ 0.97 kg	07.12.1995 (08.12.1995, treatment 2)
AP/3065/ME/3	Olive	Fruit, from trees Fruit, from ground	28	0.96 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]	≥ 1.0 kg ≥ 0.99 kg	04.12.1995
AP/3065/ME/4	Olive	Fruit, from trees Fruit, from ground	28	0.95 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]	≥ 1.0 kg ≥ 0.94 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 B.7.3.1.15-1: 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	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 B.7.3.1.15-2: 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[1,2,3] (mg/kg)		DALA[4] (days)
				Glypho-sate	AMPA	
AP/3065/ME/1 / [redacted], Malaga, Spain / SEU / 1995	Olive / Hojiblanca	2	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	7
			Fruit from ground	9.15	<0.05 (n.d.)	0
				0.14	<0.05 (n.d.)	7
		3	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	14
			Fruit from ground	12.6	<0.05	0
				0.12	<0.05 (n.d.)	14
		4	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	28
			Fruit from ground	9.81	<0.05	0
				<0.05 (n.d.)	<0.05 (n.d.)	28
AP/3065/ME/2 / [redacted], Cordoba, Spain / SEU / 1995	Olive / Picual	2	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	7
			Fruit from ground	4.26	<0.05 (n.d.)	0
				0.11	<0.05 (n.d.)	7
		3	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	14
			Fruit from ground	5.81	<0.05 (n.d.)	0
				0.11	<0.05 (n.d.)	14
		4	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	28
			Fruit from ground	12.60	<0.05 (n.d.)	0

Table B.7.3.1.15-2: 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[1,2,3] (mg/kg)		DALA[4] (days)
				Glypho-sate	AMPA	
				<0.05 (n.d.)	<0.05 (n.d.)	28
AP/3065/ME/3 / [redacted] Cordoba, Spain / SEU / 1995	Olive / Picual	2	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	7
			Fruit from ground	5.89	<0.05 (n.d.)	0
				0.53	<0.05 (n.d.)	7
		3	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	14
			Fruit from ground	11.8	0.05	0
				0.13	<0.05 (n.d.)	14
		4	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	28
			Fruit from ground	9.31	<0.05 (n.d.)	0
				<0.05	<0.05 (n.d.)	28
AP/3065/ME/4 / [redacted], Jaen, Spain / SEU / 1995	Olive / Picual	2	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	7
			Fruit from ground	6.05	<0.05	0
				0.93	<0.05 (n.d.)	7
		3	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	14
			Fruit from ground	6.75	<0.05 (n.d.)	0
				0.93	<0.05 (n.d.)	14
		4	Fruit, from tree	<0.05 (n.d.)	<0.05 (n.d.)	0
				<0.05 (n.d.)	<0.05 (n.d.)	28

Table B.7.3.1.15-2: 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[1,2,3] (mg/kg)**		**DALA[4] (days)**
				Glypho-sate	**AMPA**	
			Fruit from ground	6.41	<0.05 (n.d.)	0
				<0.05	<0.05 (n.d.)	28

1 LOQ (limit of quantification): 0.05 mg/kg
2 n.d. (not detected): < 0.02 mg/kg
3 Residues were determined in olives without stone.
4 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:
The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 2.16 kg/ha (± 25%) was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is still within 25% of the intended maximal yearly use rate. In addition, it is not expected that a second application after the intended 28-day interval would have an impact on the residue level on table olives at harvest. Residue levels at a PHI of 7 days are selected for evaluation.
The RMS wants to note that, in contrast to what the applicant is stating, sample weights of treated olives were indeed recorded in the study report. It is agreed, however, that the number of sampled trees is unknown. In contrast to the applicant's assessment, some sample weights were slightly below the required amount of 1 kg, however, this deviation is not expected to impact the study outcome significantly.
According to Annex I of Reg. (EC) 396/2005, table olives are defined as the whole product after removal of the stem, i.e. including the stone. In this study, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. Since the weight ratio of flesh and stone was recorded in the study report, a re-calculation of residue levels would be possible. However, as residue levels of glyphosate and AMPA were below the LOQ in the flesh in tree-picked olives, a re-calculation is not required.
The analytical method was not fully validated for glyphosate and AMPA in olive fruits since recoveries were below 70% (see Vol. 3, B.5). In contrast to this, the performance of the analytical method was adequately demonstrated via the determination of concurrent recoveries and the method is therefore considered fit for purpose. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation. The only exception regarding procedural recoveries is the relative standard deviation determined for olive fruits spiked with glyphosate at 0.05 mg/kg (24%), i.e. the relative standard deviation is above the acceptable limit of 20%. Consequently, residue levels in olives might be less precise in case levels are around 0.05 mg/kg. Considering the results obtained in all supervised residue trials and that these are well in line with the levels determined in this study, the reliability of this study is not questioned.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices).
In contrast, AMPA was shown to be stable in soybean seeds only and data are not sufficient to allow an extrapolation to all high oil content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the use on olives harvested from the tree, and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens.
In line with the applicant, the RMS does only consider tree-picked olives for evaluation. Specific risk mitigation measures are discussed in Volume 1, 2.7.4.1 in detail. The following residues are selected for evaluation:

Table olives (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.1.16. Study 16

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	Study not retrievable from the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Supportive (Category 2a) **Conclusion RMS:** The study is considered acceptable for evaluation (see 'assessment and conclusion by RMS'). It is noted that the applicant stated that the study was not accepted in the previous evaluation (RAR, 2015). It is more correct to say, however, that the study was not included in the previous evaluation. It can therefore not be assessed whether the study was submitted and what would have been the reasons for non-acceptance.

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 (Greece). Per trial location, 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 of glyphosate were 0.17 mg/kg.
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
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

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

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.

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 g/mol, 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 homogenized by mixing before application. Application was performed with a CO_2-pressurized 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.

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

Crop sampling information						
Trial	**Crop**	**Commodity**	**DALA[2]**	**Growth stage (BBCH)[3]**	**Quantity**	**Date of sampling**
			7	Mature fruit	≥ 1.0 kg	12.12.1995
			13	Mature fruit	≥ 1.0 kg	18.12.1995

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 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 B.7.3.1.16-1: 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 of glyphosate 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 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 B.7.3.1.16-2: 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[3,4] (mg/kg) Glypho-sate	DALA[5] (days)
GR-95-H201 / [redacted] 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
					<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
[redacted] 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
					<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
					<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 Residues assumed to be determined in olives without stone.
5 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:
It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 0.99 or 3.3 kg glyphosate equivalents/ha was performed instead of two applications at 1.44 kg/ha, however, the higher application rate is still within 25% of the intended maximal yearly use rate. Trials with the lower application rate are not considered acceptable for evaluation. Residue levels at a PHI of 7 days are selected for evaluation. Although only one tree was sampled, a sufficient amount of olives was used for analysis which is considered representative for a treated field.
It is indeed agreed with the applicant that it is unclear if residue results are reported for whole fruit (including stones), i.e. according to Annex I of Reg. (EC) 396/2005, or if results are for flesh only. As a worst-case approach, the RMS assumes that residue levels are reported for flesh only and these values are considered for the consumer risk assessment. Furthermore, as already stated by the applicant, residue levels were indeed corrected for recoveries in case these were < 100%. From the study report, it is not possible to retrieve the corresponding procedural recovery value. As already implicated by the applicant, residue values would therefore rather be overestimated. As this also represents a worst-case scenario for the consumer risk assessment, values are accepted for evaluation. Due to these deviations, however, the trials would not be considered acceptable for MRL-setting as residues are rather overestimated, i.e. MRLs would not be calculated based on the ALARA principle.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices).
In line with the applicant, the RMS only considered tree-picked olives for evaluation. Specific risk mitigation measures are discussed in Volume 1, 2.7.4.1 in detail. The fact that only glyphosate, but not AMPA, was measured, however, does not invalidate the study. Since the two trials were conducted at the same trial location and at the same date, they are not considered independent. Consequently, only the trial yielding the highest residues is selected for evaluation (which is not relevant in the case of this study).

Table olives (SEU)
Glyphosate: <0.05 mg/kg
AMPA: not determined

B.7.3.1.17. Study 17

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	RJ 2218B
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	Study not retrievable from the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Supportive (Category 2a) **Conclusion RMS:** The study is considered acceptable for evaluation (see 'assessment and conclusion by RMS'). It is noted that the applicant stated that

	the study was not accepted in the previous evaluation (RAR, 2015). It is more correct to say, however, that the study was not included in the previous evaluation. It can therefore not be assessed whether the study was submitted and what would have been the reasons for non-acceptance.

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 (Italy). Per trial location, 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
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

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

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.

Application information				
Trial no.	**Application code**	**Timing [1]**	**Application rate kg a.s./ha [2]**	**Water volume L/ha [3]**
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 g/mol, 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 homogenized by mixing before application, and all applications were made within one hour of preparation. Applications were performed with a motorized 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 to 13-14 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.

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 B.7.3.1.17-1: 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, i.e. corrected for recovery. Detailed residue levels are shown in the table below.

Table B.7.3.1.17-2: 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[3,4] (mg/kg) Glypho-sate	DALA[5] (days)
IT10-95-H343 / [redacted]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
					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
					<u>≤0.05</u>	7
					<0.05	14
IT10-95-H344 / [redacted] 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
					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
					<u>≤0.05</u>	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 g/mol, respectively.
3 LOQ (limit of quantification): 0.05 mg/kg
4 Residues assumed to be determined in olives without stone.
5 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:
It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 0.99 or 3.3 kg glyphosate equivalents/ha was performed instead of two applications at 1.44 kg/ha, however, the higher application rate is still within 25% of the intended maximal yearly use rate. Trials with the lower application rate are not considered acceptable for evaluation. Residue levels at a PHI of 7 days are selected for evaluation.
It is indeed agreed with the applicant that it is unclear if residue results are reported for whole fruit (including stones), i.e. according to Annex I of Reg. (EC) 396/2005, or if results are for flesh only. As a worst-case approach, the RMS assumes that residue levels are reported for flesh only and these values are considered for the consumer risk assessment. Furthermore, as already stated by the applicant, residue levels were indeed corrected for recoveries in case these were < 100%. From the study report, it is not possible to retrieve the corresponding procedural recovery value. As already implicated by the applicant, residue values would therefore rather be overestimated. As this also represents a worst-case scenario for the consumer risk assessment, values are accepted for evaluation. Due to these deviations, however, the trials would not be considered acceptable for MRL-setting as residues are rather overestimated, i.e. MRLs would not be calculated based on the ALARA principle.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices).
In line with the applicant, the RMS only considered tree-picked olives for evaluation. Specific risk mitigation measures are discussed in Volume 1, 2.7.4.1 in detail. The fact that only glyphosate, but not AMPA, was measured, however, does not invalidate the study.

Table olives (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: not determined

B.7.3.1.18. Study 18

1. Information on the study

Data point:	CA 6.3.1/018
Report author	[redacted]
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. • Detailed results on the recovery experiments are missing.
Previous evaluation	Study not retrievable from the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Supportive (Category 3a) **Conclusion RMS:** The study is not considered for evaluation (see 'assessment and conclusion by RMS'). It is noted that the applicant stated that the study was not accepted in the previous evaluation (RAR, 2015). It is more correct to say, however, that the study was not included in the previous evaluation. It can therefore not be assessed whether the study was submitted and what would have been the reasons for non-acceptance.

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 per plot (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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
IT1089E001	Olive	*Olea europaea*	Perananzana	Fruit	Not specified

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.

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 g/mol, 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 motorized 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.

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 kg 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 B.7.3.1.18-1: 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[3,4] (mg/kg)	DALA[5] (days)
					Glypho-sate	
IT1089E001 / [redacted] / Italy / SEU / 1988	Olive / Perananzana	Ripening fruit	0.734	Fruit	<0.05	1
					<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 Residues assumed to be determined in olives without stone.
5 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:
It is not fully agreed with the applicant's assessment and conclusion. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 0.51, 2.03, and 4.06 kg glyphosate equivalents/ha was performed instead of two applications at 1.44 kg/ha. Therefore, none of the trials were conducted within 25% of the intended use rate. Since residues above the LOQ were determined in the trial with the highest application rate, the RMS does not consider it acceptable to select this value either.
It is indeed agreed with the applicant that it is unclear if residue results are reported for whole fruit (including stones), i.e. according to Annex I of Reg. (EC) 396/2005, or if results are for flesh only. This, however, is also true for other submitted residue studies which were accepted by the applicant for evaluation. Therefore, this deviation cannot be considered the only reason to disregard the trial from assessment.
As already stated by the applicant, residue levels were indeed corrected for recoveries in case these were < 100%. This, however, is also true for other submitted residue studies which were accepted for risk assessment purposes (but which would not be acceptable for MRL setting). Therefore, this deviation cannot be considered the only reason to disregard the trial from assessment.
In the study report, the mean procedural recovery is 46%. Since residue levels in the current trials were below the LOQ, the calculation of uncorrected values is not possible. Furthermore, no validation of the analytical method is available in Vol. 3, B.5.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices).
The RMS notes that it is not explicitly stated whether olives were picked from the tree or from the ground, although it may be assumed that olives were picked from the tree, considering that residue concentrations are in line with those from tree-picked olives from other studies.
In conclusion, due to the noted deviations, this study is not considered for evaluation. No residue levels are selected.

Table olives (SEU)
Glyphosate: not selected
AMPA: not determined

B.7.3.1.19. Study 19

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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered reliable.

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
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

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

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.

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 homogenized by mixing before application. Application was performed with motorized 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).

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 B.7.3.1.19-1: 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

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 B.7.3.1.19-2: 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)**
				Glypho-sate	**AMPA**	
S15-00469-01 / [redacted] Emilia Romagna, Italy / SEU / 2015	Kiwi fruit / Hayward	87	Peel	<0.05	<0.05 (n.d.)	7
			Pulp	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	
S15-00469-02 / [redacted] Pyreneés-Orientales, France / SEU / 2015	Kiwi fruit / Hayward	87	Peel	<0.05 (n.d.)	<0.05 (n.d.)	7
			Pulp	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): <0.015 mg/kg
3 Values represent the mean from two sampling replicates.
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

<u>Assessment and conclusion by applicant</u>:
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.51-3.88 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted.
It is noted that duplicate field specimens were sampled and both specimens were analysed (sampling replicates), i.e. mean values are selected for evaluation. Furthermore it is noted that specimens were separated in peel and pulp after harvest and both matrices were analysed separately. According to Annex I of Regulation (EC) 396/2005 and OECD Guideline 509, whole fruit specimens should be analysed for residues and for MRL setting. Since residue levels in peel and pulp were below the LOQ, the RMS does not consider that this deviation has an impact on the reliability of the study. Additionally, the weight of the specimens was below 2 kg as required by the OECD guideline. Since at least 16 individual units were sampled though, this deviations is considered acceptable. The specimens are considered representative for estimating residue levels at harvest.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability for glyphosate (18 months in all plant commodities except dry matrices (at least 12 months)).
In contrast to this and with regard to acidic matrices, AMPA was shown to be stable in oranges only and data are not sufficient to allow an extrapolation to all high acid content matrices or to all plant commodities. However, AMPA levels were much lower than glyphosate levels in the metabolism studies on fruit crops. Since a <LOQ residue situation is anticipated for the uses on orchard crops and no glyphosate is detected in the available trials, no significant levels of AMPA are expected either. Therefore, the lack of additional storage stability data on AMPA is not required in this case.
No residues above the LOQ were detected in control specimens. The following values are selected for evaluation:

Kiwi, whole fruit (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

Kiwi, peel (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

Kiwi, pulp (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.1.20. Study 20

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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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
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

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

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.

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 homogenized by mixing before application. Application was performed with motorized 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).

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 various plant commodities with a high water content (EAS Chem study S14-05172). The limit of quantitation (LOQ) for glyphosate and AMPA 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 B.7.3.1.20-1: 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
		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 B.7.3.1.20-2: 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)**
				Glyphosate	**AMPA**	
S14-04159-01 / [redacted] La Palma, Spain / SEU / 2014	Banana / Gruesa Palmera	78	Whole fruit	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	1
			Pulp	<0.05 (n.d.)	<0.05 (n.d.)	
			Peel	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04159-02 / [redacted] La Palma, Spain / SEU / 2014	Banana / Del Pais	78	Whole fruit	<0.05 (n.d.)	<0.05 (n.d.)	1
			Pulp	<0.05 (n.d.)	<0.05 (n.d.)	
			Peel	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04159-03 / [redacted], La Palma, Spain / SEU / 2014	Banana / Del Pais	78	Whole fruit	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	1
			Pulp	<0.05 (n.d.)	<0.05 (n.d.)	
			Peel	<0.05 (n.d.)	<0.05 (n.d.)	
S14-04159-04 / [redacted], La Palma, Spain / SEU / 2014	Banana / Del Pais	78	Whole fruit	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	1
			Pulp	<0.05 (n.d.)	<0.05 (n.d.)	
			Peel	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Values for whole fruit represent the mean from two sampling replicates.
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

<u>Assessment and conclusion by applicant</u>:
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application at 3.45-3.90 kg/ha was performed instead of two applications at 1.44 kg/ha, however, the trial GAP is considered more critical than the intended use. Since residues were below the LOQ at harvest, this is accepted. Furthermore samples were collected at a PHI of 1 day instead of the intended 7 days, however, it is not expected that this has a significant influence on the residue level at harvest considering that metabolism studies showed limited uptake of residues from the soil into the tree, i.e. residue levels in fruits are not expected to significantly increase with longer PHIs.
Trials S14-04159-01 and S14-04159-02 were performed at the same location and at the same date. Although crop varieties were different, the trials are considered replicates; therefore only one trial is selected for evaluation. Furthermore it is noted that residue levels for whole fruit are based on the mean value of duplicate field specimens.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). As already stated in the study summary, storage temperatures exceeded -18 °C for 13 and 18 hours during shipment. It is agreed with the study authors that this unlikely affects the study results since specimens remained frozen all the time. No residues above the LOQ were detected in control specimens. The following values are selected for evaluation:

Banana, whole fruit (SEU)
Glyphosate: 3x <0.05 mg/kg
AMPA: 3x <0.05 mg/kg

Banana, peel (SEU)
Glyphosate: 3x <0.05 mg/kg
AMPA: 3x <0.05 mg/kg

Banana, pulp (SEU)
Glyphosate: 3x <0.05 mg/kg
AMPA: 3x <0.05 mg/kg

B.7.3.1.21. Study 21

1. Information on the study

Data point:	CA 6.3.1/021
Report author	[redacted]
Report year	2018
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in citrus plants (outdoor) at 2 sites in Southern Europe 2017
Report No	S17-02881
Document No	MSL0029656
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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid, Category 1

	Conclusion RMS: The study is considered to be acceptable.

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 (orange 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 two field trials in the southern zone (in Spain in 2017). The tree plantations were treated once. The application was performed as a shielded band application directed to the soil under the trees in the row and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of orange fruit were sampled at normal harvest, which was 7 days after application and 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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S17-02881-01	Oranges	*Citrus sinensis*	Navelina	Fruit	> 2 kg / 12 units
S17-02881-02	Oranges	*Citrus sinensis*	Navelina	Fruit	> 2 kg / 12 units

B. Methods

1. Field phase

Two residue trials were conducted on oranges (outdoor) during the 2017 season in Spain (S17-02881-01 and S17-02881-02). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil as a shielded band application 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 307-325 L/ha. The main application parameters are outlined in the table below.

Application information

Trial no.	**Application code**	**Timing (BBCH)**	**Application rate kg a.s./ha**	**Water volume L/ha**
S17-02881-01	2	83	3.680	307
S17-02881-02	2	87	3.900	325

Regions, varieties and cultivation were typical for the cultivation of oranges. Care was taken that the spray solution was properly homogenized by mixing before application. Application was performed with motorized 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. 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 2.5 hours of sampling in the field).

Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S17-02881-01	Orange	Fruit	7	89	≥ 2 kg / 12 units	20.12.2017
S17-02881-02	Orange	Fruit	7	89	≥ 2 kg / 12 units	20.12.2017

1 Days after last application.

3. 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.

4. 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 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 121 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 oranges (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 B.7.3.1.21-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Orange, peel	Glyphosate	Quantification transition 168 > 63 m/z				
		0.05	81, 81, 78	80	2.2	3
		0.5	82, 80, 77	80	3.2	3
		Overall	77-82	80	2.4	6
		Confirmation transition 168 > 79 m/z				
		0.05	77, 90, 79	82	8.5	3
		0.5	77, 79, 76	77	2.0	3
		Overall	76-90	80	6.5	6
	AMPA	Quantification transition 110 > 63 m/z				
		0.05	90, 83, 82	85	4.7	3
		0.5	84, 83, 82	83	1.2	3
		Overall	82-90	84	3.5	6
		Confirmation transition 110 > 79 m/z				
		0.05	84, 83, 82	83	1.2	3
		0.5	84, 84, 83	84	0.7	3
		Overall	82-84	83	1.0	6
Orange, pulp	Glyphosate	Quantification transition 168 > 63 m/z				
		0.05	75, 82, 82	80	5.1	3
		0.5	79, 79, 79	79	0.0	3
		Overall	75-82	79	3.3	6
		Confirmation transition 168 > 79 m/z				
		0.05	89, 80, 99	89	11	3
		0.5	84, 82, 80	82	2.4	3
		Overall	80-99	86	8.6	6
	AMPA	Quantification transition 110 > 63 m/z				
		0.05	83, 86, 85	85	1.8	3
		0.5	86, 85, 85	84	1.4	3
		Overall	83-86	84	1.4	6
		Confirmation transition 110 > 79 m/z				
		0.05	79, 85, 85	83	4.2	3
		0.5	85, 86, 84	85	1.2	3
		Overall	79-86	84	3.0	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 LOQ (0.05 mg/kg) were found in any treated or untreated specimens of oranges (peel and pulp). Detailed residue levels are shown in the table below.

Table B.7.3.1.21-2: Residue levels of glyphosate and AMPA in orange 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)
				Glypho-sate	AMPA	
S17-02881-01 / [redacted] Andalucia, Spain/ SEU / 2017	Oranges/ Navelina	89	Peel	<0.05 (n.d.)	<0.05 (n.d.)	7
			Pulp	<0.05 (n.d.)	<0.05 (n.d.)	
S17-02881-02 / [redacted] Andalucia, Spain/ SEU / 2017	Oranges/ Navelina	89	Peel	<0.05 (n.d.)	<0.05 (n.d.)	7
			Pulp	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): <0.015 mg/kg
3 Since residue in both peel and pulp were <LOQ (<0.05 mg/kg), residue in whole fruit are also expected to be <LOQ (<0.05 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 oranges (peel and pulp) sampled at BBCH 89 (commercial maturity), 7 days after band application of glyphosate in the tree row at the rate of 3.68-3.90 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 at a rate of 3.7-3.9 kg a.s./ha. This application rate is 28% 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 fruit trees (and especially citrus fruit trees) in Southern Europe.

Assessment and conclusion by RMS:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is neither exactly reflecting the intended use, nor the maximum yearly application rate, i.e. one application at a target rate of 3.60 kg/ha was performed instead of two applications at 1.44 kg/ha. Since residues were below the LOQ at harvest, the overdosed trials are accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability of glyphosate and AMPA (18 months for glyphosate in all plant commodities and 24 months for AMPA in oranges).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Orange pulp (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Orange peel (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Orange whole fruit (SEU)[#]
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

Since residue in both peel and pulp were <LOQ (<0.05 mg/kg), residue in whole fruit are also expected to be <LOQ (<0.05 mg/kg).

B.7.3.1.22. Study 22

1. Information on the study

Data point:	CA 6.3.1/022
Report author	[redacted]
Report year	2018
Report title	Determination of residues of glyphosate and its metabolite AMPA after one application of MON 79351 in plums (outdoor) at 4 sites in Southern Europe 2017
Report No	S17-02878
Document No	MSL0029654
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 that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion applicant:** Valid (Category 1) **Conclusion RMS:** The study is considered to be acceptable.

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 plum (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 four field trials in the southern zone (in Spain in 2017). The tree plantations were treated once. The application was performed as a shielded band application directed to the soil under the trees and the target rate was 3.6 kg glyphosate acid equivalents per hectare. Samples of plum fruit 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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S17-02878-01	Plum	*Prunus domestica*	Black Splendor	Fruit	> 2 kg / 20 units
S17-02878-02	Plum	*Prunus domestica*	Stanley	Fruit	> 2 kg / 50 units
S17-02878-03	Plum	*Prunus domestica*	Prime Time	Fruit	> 2 kg / 30 units
S17-02878-04	Plum	*Prunus domestica*	Angelino	Fruit	> 2 kg / 40 units

B. Methods

1. Field phase

Four residue trials were conducted on plums (outdoor) during the 2017 season in Spain (S17-02878-01), Italy (S17-02878-02) and Southern France (S17-02878-03 and S17-02878-04). One application of MON 79351 (480 g/L glyphosate acid equivalents) was performed to the soil under the trees performed as a shielded band application 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 283-321 L/ha. The main application parameters are outlined in the table below.

Application information

Trial no.	Application code	Timing (BBCH)	Application rate kg a.s./ha	Water volume L/ha
S17-02878-01	2	85-86	3.857	321
S17-02878-02	2	87	3.840	320
S17-02878-03	2	85	3.640	303
S17-02878-04	2	87-89	3.400	283

Regions, varieties and cultivation were typical for the cultivation of plums. Care was taken that the spray solution was properly homogenized by mixing before application. Application was performed with motorized 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 not separated from the flesh of the fruits before freezing, but stones were removed at the analytical laboratory. 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 2.2 hours of sampling in the field).

Crop sampling information

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S17-02878-01	Plum	Fruit	7	89	> 2 kg / 20 units	06.06.2017
S17-02878-02	Plum	Fruit	7	89	> 2 kg / 50 units	08.08.2017
S17-02878-03	Plum	Fruit	7	89	> 2 kg / 30 units	20.07.2017
S17-02878-04	Plum	Fruit	7	89	> 2 kg / 40 units	29.08.2017

1 Days after last application.

3. Sample preparation

The stones were removed from the whole fruit specimens and the fruits without stones thoroughly homogenised in a cutter with dry ice before taking a representative subsample for analysis. Weights of stones and fruits without stones are recorded.

4. 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 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 295 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 plum (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 B.7.3.1.22-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]			
			Range (%)	Mean (%)	Relative standard deviation (%)	Number analyses (n)
Plum, fruit without stone	Glyphosate	Quantification transition 168 > 63 m/z				
		0.05	77, 83, 82	81	4.0	3
		0.5	72, 72, 72	72	0.0	3
		Overall	72-83	76	6.8	6
		Confirmation transition 168 > 79 m/z				
		0.05	84, 80, 83	82	2.5	3
		0.5	73, 74, 71	73	2.1	3
		Overall	71-84	78	7.1	6
	AMPA	Quantification transition 110 > 63 m/z				
		0.05	82, 84, 85	84	1.8	3
		0.5	84, 87, 86	86	1.8	3
		Overall	82-87	85	2.1	6
		Confirmation transition 110 > 79 m/z				
		0.05	81, 84, 92	86	6.6	3
		0.5	77, 79, 85	80	5.2	3
		Overall	77-92	83	6.4	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 plum (whole fruit without stone). Detailed residue levels are shown in the table below.

Table B.7.3.1.22-2: Residue levels of glyphosate and AMPA in plum 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] (mg/kg)		DALA[4] (days)
				Glypho-sate	AMPA	
S17-02878-01 / [redacted] Andalucia, Spain / SEU / 2017	Plums/ Black Splendor	89	Fruits without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[3]	<0.05 (n.d.)	<0.05 (n.d.)	7
S17-02878-02 / [redacted] Bologna, Italy / SEU / 2017	Plums/ Stanley	89	Fruits without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[3]	<0.05 (n.d.)	<0.05 (n.d.)	7
S17-02878-03 / [redacted] Tarn et Garonne,	Plums/ Prime Time	89	Fruits without stone	<0.05 (n.d.)	<0.05 (n.d.)	7

Table B.7.3.1.22-2: Residue levels of glyphosate and AMPA in plum 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] (mg/kg)		DALA[4] (days)
				Glypho-sate	AMPA	
Southern France SEU / 2017			Whole fruit[3]	<0.05 (n.d.)	<0.05 (n.d.)	7
S17-02878-04 / [redacted], Tarn et Garonne, Southern France/ SEU / 2017	Plums/ Angelino	89	Fruits without stone	<0.05 (n.d.)	<0.05 (n.d.)	7
			Whole fruit[3]	<0.05 (n.d.)	<0.05 (n.d.)	7

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): <0.015 mg/kg
3 Residues in whole fruit based on residue levels in the flesh and correction for the weight ratio of flesh and stones. Residue levels in stone, however, were not determined.
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 plums (fruit without stone) sampled at BBCH 89 (commercial maturity), 7 days after shielded band application of glyphosate in the tree row at the rate of 3.40-3.86 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 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 fruit trees (and especially stone fruit trees) in Southern Europe.

Assessment and conclusion by RMS:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. It is noted that the use pattern is neither exactly reflecting the intended use, nor the maximum yearly application rate, i.e. one application at a target rate of 3.60 kg/ha was performed instead of two applications at 1.44 kg/ha. Since residues were below the LOQ at harvest, the overdosed trials are accepted.
The distance between trial S17-02878-03 and S17-02878-04 was 3.6 km, but according to the study author this has no impact on the study outcome since different varieties, soil types and application timings had been used. The RMS agrees with this; the trials are sufficiently independent.
According to Annex I of Reg. (EC) 396/2005, stone fruits are defined as the whole product after removal of the stem, i.e. including the stone. In this study, residue levels were only determined in the fruit without stone (flesh) and not in the stone, i.e. not according to the definition set in Annex I of Reg. (EC) 396/2005. However, residue levels in whole fruits were calculated based on the residue level in flesh and a correction for the weight ratio of flesh and stone; therefore, the results are considered acceptable.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Plum, whole fruit (NEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.2. Post-harvest, pre-sowing, pre-planting, pre-emergence use

A post-harvest, pre-sowing, pre-planting, pre-emergence outdoor use against weeds is intended in the following crops or crop groups: root and tuber vegetables, bulb vegetables, fruiting vegetables, brassica, leafy vegetables, stem vegetables, and sugar beets. The critical GAP in NEU and SEU is identical and is as follows:

2 x 1.08-1.44 kg/ha (max. 2.16 kg/ha per year), interval 28 days, PHI n.a.

The intended use is less critical compared to the critical NEU and SEU use evaluated in the previous RAR which was a pre-planting, post-planting, and/or pre-emergence use in all seeded or transplanted crops according to the GAP: 1-2 x 1.08-2.16 kg/ha (max. 4.32 kg/ha per year), interval 21 d, PHI n.a.

All studies submitted by the applicant, including the relevant studies from the previous evaluations (DAR, 1998; RAR, 2015), in support of the intended post-harvest, pre-sowing, pre-planting use are summarised in the following paragraphs.

B.7.3.2.1. Study 1

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 that would impact the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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

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

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

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.

Trial no.	Application code	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00258-01	2	BBCH 00 (17 days after planting)	2.173	175
S11-00258-02	2	BBCH n r. (3 days after planting)	2.276	184
S11-00258-03	2	BBCH 00 (0 days after planting)[1]	2.218	187
S11-00258-04	2	BBCH 00 (3 days after planting)	2.374	200

1 In contrast to what is stated in the body text and in the study plan, the application in this trial was not *"at least three days after planting and before crop emergence"*, but on the same day as planting. Considering that the application was performed before emergence, i e. according to the intended use, this deviation is accepted.

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).

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 various crop matrices (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 B.7.3.2.1-1: 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 B.7.3.2.1-2: Residue levels of glyphosate and AMPA in potato 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)
				Glypho-sate	AMPA	
S11-00258-01 / [redacted], Bas-Rhin, France / NEU / 2011	Potato / Charlotte	49	Tuber	<0.05 (n.d.)	<0.05 (n.d.)	119
S11-00258-02 / [redacted], Lower Saxony, Germany / NEU / 2011	Potato / Milva	49	Tuber	<0.05 (n.d.)	<0.05 (n.d.)	138
S11-00258-03 / [redacted], Midi Pyrenees, France / SEU / 2011	Potato / Noisette	49	Tuber	<0.05 (n.d.)	<0.05 (n.d.)	114
S11-00258-04 / [redacted], Bologna, Italy / SEU / 2011	Potato / Primura	49	Tuber	<0.05 (n.d.)	<0.05 (n.d.)	98

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 10-12 months in high starch content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Potato, tuber (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Potato, tuber (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.2. Study 2

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 that would impact the outcome of the study.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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
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

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

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.

Trial no.	**Application code**	**Timing**	**Application rate kg a.s./ha**	**Water volume L/ha**
S11-00259-01	2	BBCH 00 (5 days after seeding)	2.311	187
S11-00259-02	2	BBCH 00 (1 day after seeding)[1]	2.295	193
S11-00259-04	2	BBCH 00-03 (4 days after seeding)	2.492	210
S11-00259-05	2	BBCH 05 (7 days after seeding)	2.080	169

1 In contrast to what is stated in the body text and in the study plan, the application in this trial was not *"at least three days after planting and before crop emergence"*, but one day after planting. Considering that the application was performed before emergence, i.e. according to the intended use, this deviation is accepted.

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).

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 various crop matrices (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in carrots (roots without leaves) 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 days. Samples were stored frozen at ≤ - 18 °C at the analytical facility prior to analysis.
During analysis of carrot (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 B.7.3.2.2-1: 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	-	-	-	1
		0.5	91	-	-	-	1
		Overall	91-97	94	-	-	2
	AMPA	0.05	93	-	-	-	1
		0.5	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 B.7.3.2.2-2: 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)
				Glypho-sate	AMPA	
S11-00259-01 / [redacted], Maine et Loire, France / NEU / 2011	Carrot / Montdibell	49	Roots without leaves	<0.05 (n.d.)	<0.05 (n.d.)	93
S11-00259-02 / [redacted] Wielkopolska, Poland / NEU / 2011	Carrot / Laguna	49	Roots without leaves	<0.05 (n.d.)	<0.05 (n.d.)	176
S11-00259-04 / [redacted], Haute-Garonne, France / SEU / 2011	Carrot / Maestro	49	Roots without leaves	<0.05 (n.d.)	<0.05 (n.d.)	137
S11-00259-05 / [redacted] Alicante, Spain / SEU / 2011	Carrot / Maestro	49	Roots without leaves	<0.05 (n.d.)	<0.05 (n.d.)	154

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 10-12 months in high starch content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Carrot, root without leaves (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Carrot, root without leaves (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.3. Study 3

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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 129-154 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
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

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

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.

Trial no.	**Application code**	**Timing**	**Application rate kg a.s./ha**	**Water volume L/ha**
S11-00260-01	2	BBCH 01 (13 days after seeding)	2.311	187
S11-00260-02	2	BBCH 03 (3 days after seeding)	2.413	203
S11-00260-03	2	BBCH 03 (15 days after seeding)	2.433	205
S11-00260-04	2	BBCH 00 (4 days after seeding)	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).

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 various crop matrices (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in onion (bulbs) 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 B.7.3.2.3-1: 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	-	-	-	1
		0.5	91	-	-	-	1
		Overall	91-92	92	-	-	2
	AMPA	0.05	89	-	-	-	1
		0.5	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 B.7.3.2.3-2: 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[1] (BBCH)	Commodity	Residue found[2,3] (mg/kg)		DALA[4] (days)
				Glypho-sate	AMPA	
S11-00260-01 / [redacted], Bas-Rhin, France / NEU / 2011	Bulb onion / Takmark Fl	49	Bulb	<0.05 (n.d.)	<0.05 (n.d.)	129
S11-00260-02 / [redacted] Wielkopolska, Poland / NEU / 2011	Bulb onion / Kristine	49	Bulb	<0.05 (n.d.)	<0.05 (n.d.)	143
S11-00260-03 / [redacted], Albacete, Spain / SEU / 2011	Bulb onion / Eso	49	Bulb	<0.05 (n.d.)	<0.05 (n.d.)	154
S11-00260-04 / [redacted], Bulgaria / SEU / 2011	Bulb onion / Stutgarten rijsen	49	Bulb	<0.05 (n.d.)	<0.05 (n.d.)	149

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Onion, bulb (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Onion, bulb (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.4. Study 4

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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 (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
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

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

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.

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

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).

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 various crop matrices (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 tomato (fruit) 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 B.7.3.2.4-1: 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	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-90	89	-	-	2
	AMPA	0.05	90	-	-	-	1
		0.5	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 B.7.3.2.4-2: 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)
				Glypho-sate	AMPA	
S11-00267-01 / [redacted] Baden-Württemberg, Germany / NEU / 2011	Tomato / Vanessa	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	93
S11-00267-02 / [redacted] Fejér, Hungary / NEU / 2011	Tomato / Claudius F1	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	94

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Tomato, fruit (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.5. Study 5

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 guideline	None that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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
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

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

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.

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 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).

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 various crop matrices (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.
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 200 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 cucumber and zucchini (fruit) 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 B.7.3.2.5-1: 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	-	-	-	1
		0.5	88	-	-	-	1
		Overall	88-92	90	-	-	2
	AMPA	0.05	90	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90	90	-	-	2
Cucumber, fruit	Glyphosate	0.05	90	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87-90	89	-	-	2
	AMPA	0.05	87	-	-	-	1
		0.5	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 B.7.3.2.5-2: 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)
				Glypho-sate	AMPA	
S11-00261-01 / [redacted], Baden-Württemberg, Germany / NEU / 2011	Zucchini / Monitor	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	55
S11-00261-03 / [redacted] Pyrénées-Orientales, France / SEU / 2011	Zucchini / Cigal F1	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	52
S11-00261-04 / [redacted] Latina, Italy / SEU / 2011	Cucumber / Ekron	89	Fruit	<0.05 (n.d.)	<0.05 (n.d.)	42

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Zucchini, fruit (NEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg
Zucchini, fruit (SEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg
Cucumber, fruit (SEU)
Glyphosate: <0.05 mg/kg
AMPA: <0.05 mg/kg

B.7.3.2.6. Study 6

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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 (2 trials in the northern zone and 2 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
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

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 of each head (see section 'sampling' for more details).

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.

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

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.

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 of each head.

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 crop matrices (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in cauliflower (inflorescence) 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 cauliflower (inflorescence) 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 B.7.3.2.6-1: 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	-	-	-	1
		0.5	90	-	-	-	1
		Overall	90-95	93	-	-	2
	AMPA	0.05	84	-	-	-	1
		0.5	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 B.7.3.2.6-2: 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)
				Glypho-sate	AMPA	
[redacted], Essonne, France / NEU / 2011	Cauliflower / Aviso	49	Inflorescence	<0.05 (n.d.)	<0.05 (n.d.)	75
S11-00263-02 / [redacted], Fejër, Hungary / NEU / 2011	Cauliflower / Cortes	49	Inflorescence	<0.05 (n.d.)	<0.05 (n.d.)	125
S11-00263-03 / [redacted], Emilia, Romagna, Italy / SEU / 2011	Cauliflower / Castellum	49	Inflorescence	<0.05 (n.d.)	<0.05 (n.d.)	80
S11-00263-04 / [redacted], Bulgaria / SEU / 2011	Cauliflower / Snowball	49	Inflorescence	<0.05 (n.d.)	<0.05 (n.d.)	120

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Cauliflower, inflorescence (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Cauliflower, inflorescence (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.7. Study 7

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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
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

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
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 of each head (see section 'sampling' for details).

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.

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	2.460	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

Application code	Treatment number	Timing	Application rate kg a.s./ha	Water volume L/ha
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. Roots, decayed 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 head into eight equal segments and collecting two opposite segments of each head.

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 of each head.

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 crop matrices (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in head cabbage (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 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 B.7.3.2.7-1: 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	-	-	-	1
		0.5	87	-	-	-	1
		Overall	87	87	-	-	2
	AMPA	0.05	91	-	-	-	1
		0.5	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 B.7.3.2.7-2: 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)
				Glypho-sate	AMPA	
S11-00262-01 / [redacted], Côte d'Or, France / NEU / 2011	Head cabbage / Padoc	49	Heads	<0.05 (n.d.)	<0.05 (n.d.)	67
[redacted] / [redacted] Csongrád, Hungary / NEU / 2011	Head cabbage / Pandion	49	Heads	<0.05 (n.d.)	<0.05 (n.d.)	97
S11-00262-03 / [redacted], Zaragoza, Spain / SEU / 2011	Head cabbage / Melissa	49	Heads	<0.05 (n.d.)	<0.05 (n.d.)	98
S11-00262-04 / [redacted], Bulgaria / SEU / 2011	Head cabbage / Kyose	49	Heads	<0.05 (n.d.)	<0.05 (n.d.)	99

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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Head cabbage, head (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Head cabbage, head (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.8. Study 8

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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
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

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
S11-00264-04	Head lettuce	*Lactuca sativa* var. capitata	Cervantes	Heads	≥ 1 kg / ≥ 12 units

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.

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.

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	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 various crop matrices (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 leaf lettuce (leaves) and head 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 B.7.3.2.8-1: 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 B.7.3.2.8-1: 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 B.7.3.2.8-2: 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)
				Glypho-sate	AMPA	
S11-00264-01 / [redacted], Baden-Württemberg, Germany / NEU / 2011	Leaf lettuce / Kirinia	49	Leaves	<0.05	<0.05 (n.d.)	42
S11-00264-02 / [redacted], Essex, UK / NEU / 2011	Leaf lettuce / Oak Leaf - Red	49	Leaves	<0.05	<0.05 (n.d.)	56
S11-00264-03 / [redacted] Pyrénés-Orientales, France / SEU / 2011	Head lettuce / Sucrine	49	Heads	<0.05 (n.d.)	<0.05 (n.d.)	38
S11-00264-04 / [redacted] Valencia, Spain / SEU / 2011	Head lettuce / Cervantes	49	Heads	<0.05	<0.05 (n.d.)	48

1 Growth stage at last harvest
2 <0.05 mg/kg (< LOQ)
3 n.d. (not detected): <0.015 mg/kg (< LOD)
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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Leafy lettuce, leaves (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Head lettuce, heads (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.9. Study 9

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S11-00265-01	Leek	*Allium porrum*	Kenton	Whole plant without roots	≥ 2 kg / ≥ 12 units
S11-00265-02	Leek	*Allium porrum*	Parvella	Whole plant without roots	≥ 2 kg / ≥ 12 units
S11-00265-03	Leek	*Allium porrum*	Maxim	Whole plant without roots	≥ 2 kg / ≥ 12 units
S11-00265-04	Leek	*Allium porrum*	Starozagorski 72	Whole plant without roots	≥ 2 kg / ≥ 12 units

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.

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).

Trial	Crop	Commodity	DALA[1]	Growth stage (BBCH)	Quantity	Date of sampling
S11-00265-01	Leek	Whole plant without roots	77	49	≥ 2 kg / ≥ 12 units	13.09.2011
S11-00265-02	Leek	Whole plant without roots	183	47-49	≥ 2 kg / ≥ 12 units	22.09.2011
S11-00265-03	Leek	Whole plant without roots	65	49	≥ 2 kg / ≥ 12 units	05.08.2011
S11-00265-04	Leek	Whole plant without roots	125	49	≥ 2 kg / ≥ 12 units	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 various crop matrices (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 0 days. 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 B.7.3.2.9-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
Leek / whole plant without 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

1 Residues of glyphosate and AMPA in blank / untreated matrix were below the limit of detection.

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 without roots). Detailed residue results are shown in the table below.

Table B.7.3.2.9-2: 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)
				Glypho-sate	AMPA	
S11-00265-01 / [redacted] Cote-d'Or, France / NEU / 2011	Leek / Kenton	49	Whole plant without roots	<0.05 (n.d.)	<0.05 (n.d.)	77
S11-00265-02 / [redacted] Lancashire, UK / NEU / 2011	Leek / Parvella	47-49	Whole plant without roots	<0.05	<0.05 (n.d.)	183
S11-00265-03 / [redacted], Rovigo, Italy / SEU / 2011	Leek / Maxim	49	Whole plant without roots	<0.05 (n.d.)	<0.05 (n.d.)	65
S11-00265-04 / [redacted], Bulgaria / SEU / 2011	Leek / Starozagorski 72	49	Whole plant without roots	<0.05 (n.d.)	<0.05 (n.d.)	125

1 Growth stage at harvest
2 <0.05 mg/kg (< LOQ)
3 n.d. (not detected): <0.015 mg/kg (< LOD)
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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Leek, whole plant without roots (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Leek, whole plant without roots (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.10. Study 10

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 that would impact the outcome of the study.
Previous evaluation	Yes, evaluated and accepted in the Addendum to the RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 2a **Conclusion AGG:** The study is considered to be acceptable.

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 and 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
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

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

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.

Application code	Treatment number	Timing	Application rate kg a.s./ha	Water volume L/ha
S11-00266-01	2	BBCH 01 (17 days after seeding)	2.222	180
S11-00266-02	2	BBCH 00 (6 days after seeding)	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).

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 matrices (EAS Chem study S11-03331). The limit of quantitation (LOQ) for glyphosate and AMPA in sugar beet (leaves with top and 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 and 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 B.7.3.2.10-1: 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 B.7.3.2.10-2: 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)
				Glypho-sate	AMPA	
S11-00266-01 / [redacted], Soria, Spain / SEU / 2011	Sugar beet / Sabdrina	49	Leaves with top	<0.05 (n.d.)	<0.05 (n.d.)	165
			Root	<0.05 (n.d.)	<0.05 (n.d.)	165
S11-00266-02 / [redacted], Bologna, ltaly / SEU / 2011	Sugar beet / Gea	49	Leaves with top	<0.05 (n.d.)	<0.05 (n.d.)	144
			Root	<0.05 (n.d.)	<0.05 (n.d.)	144

1 Growth stage at harvest
2 <0.05 mg/kg (< LOQ)
3 n.d. (not detected): <0.015 mg/kg (< LOD)
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:
It is agreed with the applicants conclusion; the study is considered acceptable for evaluation. It is noted that the use pattern is not exactly reflecting the intended use, i.e. one application was performed at a target rate of 2.16 kg/ha instead of two applications at 1.08-1.44 kg/ha, however, the trial GAP reflects the intended maximal yearly use rate. Since residues were below the LOQ at harvest, this is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively; 24 and 10-12 months in high starch content commodities for glyphosate and AMPA, respectively). No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Sugar beet, leaves with top (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Sugar beet, roots (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.2.11. Study 11

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 (variety Combridge Favorite) 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. Applications were performed two weeks or more before plantation of the crops. Samples of strawberry fruit were collected between 233 and 305 days (8-10 months) 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:	**Conclusion GRG:** The study was not conducted to GLP. Furthermore the average recoveries for glyphosate and AMPA are very low with 68% and 59%, respectively. Category 3b **Conclusion AGG:** The study report is rather short, and therefore, all conclusions are made based on limited information only. The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 1.8 kg/ha or 3.6 kg/ha instead of two applications at 1.08-1.44 kg/ha. The lower application rate, however, is within 25% of the intended maximal yearly use rate. The higher application rate, in contrast, is not within the acceptable 25% range of the intended single or yearly application rate. Since residues were below the LOQ at harvest, however, this deviation is considered acceptable. The RMS, however, is questioning the relatively long PHI of 8-10 months. Under normal agricultural practices, growth cycles of strawberries are expected to be significantly shorter and therefore it is questioned whether the trials can be considered representative for the intended use. The analytical method is described in the study report in more details for the analysis of glyphosate and AMPA in apples, pears, and peaches (i.e. high water content matrices), but not in strawberries (i.e. a high acid content matrix). Since the applicant did not consider the current residue study as relevant for the evaluation, the analytical method is not evaluated in more detail in Section B.5 either. Considering the low mean procedural recoveries (individual data not available), the reliability of the analytical method is questioned. As a consequence, the residue levels determined in the treated samples may also be less reliable, and could be underestimated. As a last point, no information about the storage conditions (temperature and period) is given in the study report. Taking all points together, the reliability of the study is not sufficient and the study is not used for evaluation, also noting that no data were obtained by this study that are more critical than data from available and fully reliable studies. The study was not considered for, or included in, the previous evaluation either (RAR, 2015).

B.7.3.2.12. Study 12

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), RAR (2015)
Short description of study design and observations:	Six trials were conducted on salads (open or closed leaf variety unknown) during the 1977 season in France (4 trials in Northern France, 2 trials in Southern France). In all trials, three plots were installed and applications with Roundup (MON 2139) were performed at 1.44, 4.32, and 8.64 kg/ha 7-37 days before planting the crops (for one trial the number of days is unknown). 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 (1 trial in Northern France, 1 trial in Southern France) and the United Kingdom (4 trials). In the trials conducted in France, three plots were installed and applications with Roundup (MON 2139) were performed at 1.44, 4.32, and 8.64 kg/ha 11-15 days before seeding the crops. In the trials conducted in the UK, two plots were installed and applications with Roundup (MON 2139) were performed at 1.80 and 3.60 kg/ha before seeding the crops (number of days unknown) or before emergence. 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 (1 trial in Northern France, 3 trial in Southern France). In all trials, three plots were installed and applications with Roundup (MON 2139) were performed at 1.44, 4.32, and 8.64 kg/ha before seeding the crops (0 days to 1 month before seeding). 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. The trials are not considered independent since they were performed at the same location and at the same time. In both trials, two plots were installed and there was a single application of Roundup (MON 2139) at a rate of 2.16 or 4.32 kg/ha before drilling (days not further specified). 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. Two plots were installed and there was a single application of Roundup (MON 2139) at a rate of 2.16 or 4.32 kg/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. According to the study author, 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:	**Conclusion GRG:** 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 3b. **Conclusion AGG:** *General comments* No information about the storage of specimens is given in the study report, i.e. it is not known at which temperature and for what period of time the samples were stored before analysis. Furthermore, the analytical method was developed to analyse residues of glyphosate and AMPA in apples, pears, and peaches. Whereas this might be acceptable for other high water content matrices (salad, onion, pea, and bean), it is questioned whether this accounts for high starch commodities as well (carrot). Since the applicant did not consider the current residue study as relevant for the evaluation, the analytical method is not evaluated in more detail in Section B.5 either. Considering the low mean procedural recoveries, of which individual results are not included in the study report, the reliability of the analytical method is questioned. This, however, is also the case for the analysis of commodities with a high water content since mean procedural recoveries were also low for these matrices. *Salad (lettuce; open or closed leaf variety unknown)* The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 1.44, 4.32, or 8.64 kg/ha. The application rate at 1.44 kg/ha is below the acceptable 25% range when considering the intended maximal yearly use rate (2.16 kg/ha) and the application rates at 4.32 and 8.64 kg/ha are exceeding the acceptable 25% range. Since residues were below the LOQ at the higher application rates, this deviation is considered acceptable. Residue levels of glyphosate and AMPA were all below the LOQ of 0.05 mg/kg except for one trial (SEU) where residues of glyphosate were at 0.1 mg/kg at the 1.44 kg/ha application. According to the study report, this value is considered to be the result of a contamination during harvest since the other samples at higher application rates showed no residues above the LOQ. *Onion* The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 1.44, 4.32, or 8.64 kg/ha (trials in France), or 1.80 or 3.60 kg/ha (trials in the UK). The application rate at 1.44 kg/ha is below the acceptable 25% range when considering the intended maximal yearly use rate (2.16 kg/ha) and the application rates at 3.60, 4.32 and 8.64 kg/ha are exceeding the acceptable 25% range. Higher application rates are considered acceptable in case all residues are below the LOQ at harvest, however, in one trial conducted in France (SEU), residues of glyphosate were at the LOQ (0.05 mg/kg) following an application at 8.64 kg/ha. *Carrot* The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 1.44, 4.32, or 8.64 kg/ha. The application rate at 1.44 kg/ha is below the acceptable 25% range when considering the intended maximal yearly use rate (2.16 kg/ha) and the application rates at 4.32 and 8.64 kg/ha are exceeding the acceptable 25% range. Higher application rates are considered acceptable in case all residues are below the LOQ at harvest, however, in two plots from one trial conducted in France (SEU), residues of glyphosate were above the LOQ following an application at 4.32 kg/ha (0.07 mg/kg) and 8.64 kg/ha (0.08 kg/ha). *Pea* As already stated, the two trials are not considered independent. The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 2.16 or 4.32 kg/ha. The application rate at 2.16 kg/ha, however, is at the intended maximal yearly use rate and residues of glyphosate and AMPA were below the LOQ at this GAP. At the overdosed trial, residues were below the LOQ as well. *Bean*

	The use pattern is not exactly reflecting the intended use, i.e. one application was performed at 2.16 or 4.32 kg/ha. The application rate at 2.16 kg/ha, however, is at the intended maximal yearly use rate and residues of glyphosate and AMPA were below the LOQ at this GAP. The higher application rate would be considered acceptable in case all residues are below the LOQ at harvest, however, in the available trial, residues of glyphosate were above the LOQ (0.1 mg/kg) following an application at 4.32 kg/ha. *Overall conclusion* Taking all points together, the RMS does not consider the study to be acceptable for evaluation for several reasons. Multiple trials were not performed according to the intended GAP, especially with regard to the intended (yearly) application rate. Furthermore, no information about the time between application and sowing/seeding is available in multiple trials. Trials which were performed according to the intended yearly application rate (or within the acceptable 25% range) showed residues below the LOQ. The analytical method, however, is not validated in Section B.5 and the concurrent recoveries are not acceptable either. Lastly, no information about the storage conditions is available. No data were obtained by this study that are more critical than data from available and fully reliable studies, therefore the RMS does not consider the study for evaluation. It is noted, however, that a zero-residue situation seems unlikely considering that residues above the LOQ were detected in multiple trials. The study was not considered for, or included in, the previous evaluation either (RAR, 2015).

B.7.3.2.13. Study 13

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	-
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. In all trials, two plots were installed and applications with Roundup (MON 2139) were performed at 1.80 and 3.60 kg/ha (2 trials) or 1.44 and 3.06 kg/ha (2 trials) prior to planting (pre-drilling) of the crop. In the study report it is not further specified on what date the crops were drilled, i.e. it is not possible to tell how many days were in between application and drilling. 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. In all trials, two plots were installed and applications with Roundup (MON 2139) were performed at 1.80 and 3.60 kg/ha (1 trial) or 1.44 and 3.06 kg/ha (1 trials) prior to planting (pre-drilling) of the crop. In the study report it is not further specified on what date the crops were drilled, i.e. it is not possible to tell how many days were in between application and drilling. 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:	**Conclusion GRG:** 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 3b **Conclusion AGG:** The study report is rather short, and therefore, all conclusions are made based on limited information only. The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 1.44, 1.80, 3.06, or 3.60 kg/ha instead of two applications at 1.08-1.44 kg/ha. Application rates at 1.80 kg/ha, however, are within 25% of the intended maximal yearly use rate. Higher application rates, in contrast, are not within the acceptable 25% range of the intended single or yearly application rate. Since residues were below the LOQ at harvest, however, this deviation is considered acceptable. The analytical method was designed for the determination of residues in sugar beet tops and roots and therefore the method might be adequate for kale and swede commodities (tops and roots) as well. Since the applicant did not consider the current residue study as relevant for the evaluation, the analytical method is not evaluated in more detail in Section B.5 though. Mean procedural recoveries (individual data not available) were acceptable for glyphosate in kale roots, kale leaves, and swede roots, and for AMPA in swede roots, but recoveries of AMPA were below 70% for kale roots and leaves. Lastly, no information about the storage conditions (temperature and period) is given in the study report. Taking all points together, the RMS recognises that the study is more reliable than studies A25 (CA 6.3.2/011) or A16 (CA 6.3.2/012), especially because procedural recoveries were in acceptable ranges. However, since (i) no information about the storage conditions is available, (ii) no information about the time between application and planting is available, (iii) the analytical method is not validated in Section B.5, and (iv) no data were obtained by this study that are more critical than data from available and fully reliable studies, the RMS does not consider the study for evaluation. The study was not considered for, or included in, the previous evaluation either (RAR, 2015).

B.7.3.2.14. Study 14

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	-
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-sowing at a rate of 2.10-2.16 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/green manure crop) during the 1975 season in Germany. All of the trials were conducted with a single application of Roundup (MON 2139) applied pre-sowing 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-sowing 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. Applications took place 'pre-sowing' in all trials, however, the exact date of sowing is not reported and therefore it is not possible to tell how many days past after application before plants were sown. 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:	**Conclusion GRG:** The study was not conducted according to GLP. Category 3b **Conclusion AGG:** The study report is rather short, and therefore, all conclusions are made based on limited information only. The trials were conducted according to the intended yearly use pattern of the intended use. The major information that is missing with regard to the GAP is information regarding the time period between application and sowing. The analytical method designed for the determination of residues in sugar beet tops and roots was used for analysing glyphosate and AMPA in kale and turnip commodities (tops and roots), and the analytical method designed for the determination of residues in alfalfa was used for analysing glyphosate and AMPA in serradella. Since the respective crop matrices are comparable, this might be acceptable. The adequacy of the analytical method is further substantiated by acceptable mean procedural recoveries (individual data not available) for all analytes in the respective matrices. It is noted, however, that the analytical method is not evaluated in more detail in Section B.5 since the applicant does not consider the current residue study as relevant for evaluation. Lastly, no information about the storage conditions (temperature and period) is given in the study report. Taking all points together, the RMS recognises that the study is more reliable than studies A25 (CA 6.3.2/011) or A16 (CA 6.3.2/012), especially because procedural recoveries were in acceptable ranges. However, since (i) no information about the storage conditions is available, (ii) no information about the time between application and sowing is available, (iii) the analytical method is not validated in Section B.5, and (iv) no data were obtained by this study that are more critical than data from available and fully reliable studies, the RMS does not consider the study for evaluation. The study was not considered for, or included in, the previous evaluation either (RAR, 2015).

B.7.3.2.15. Study 15

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. In the UK, two plots were installed per trial and a single application of Roundup (MON 2139) was applied at a rate of 1.44 and 2.88 kg/ha (1 trial; pre-seedbed, stubble application) or 1.8 and 2.8 kg/ha (2 trials; pre-drilling/pre-emergence and pre-emergence/post-drilling). In Belgium, a single plot was treated by a single pre-plant application of Roundup (MON 2139) at 1.44 kg/ha. The exact date of planting/drilling is not given in the study report for any trial, i.e. it is not possible to tell how many days were between application and planting. Samples of sugar beet 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:	**Conclusion GRG:** 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 3b **Conclusion AGG:** The study report is rather short, and therefore, all conclusions are made based on limited information only. The use pattern is not exactly reflecting the intended use, i.e. one application was performed at either 1.44, 1.8, 2.8, or 2.88 kg/ha instead of two applications at 1.08-1.44 kg/ha. Application rates at 1.80 kg/ha, however, are within 25% of the intended maximal yearly use rate. Higher application rates, in contrast, are not within the acceptable 25% range of the intended single or yearly application rate. Since residues were below the LOQ at harvest, however, this deviation is considered acceptable. Information regarding the time period between application and planting is missing which would have been desirable to assess whether the trials reflect the intended GAP. The analytical method designed for the determination of residues in sugar beet tops and roots was used for analysing glyphosate, however, the reliability of the analytical method is questioned since none of the mean procedural recoveries was determined above 70% (individual data not available). The analytical method is not evaluated in more detail in Section B.5 either since the applicant does not consider the current residue study as relevant for evaluation. Lastly, no information about the storage conditions (temperature and period) is given in the study report. Taking all points together, the reliability of the study is not sufficient and the study is not used for evaluation also noting that no data were obtained by this study that are more critical than data from available and fully reliable studies. In the previous evaluation (RAR, 2015), two of the four trials were considered for evaluation, however, it is not possible to retrieve why two out of four trials were acceptable, whereas the other two trials were not accepted.

B.7.3.3. Inter-row use

An outdoor inter-row use is intended on various vegetables (root and tuber vegetables, bulb vegetables, fruiting vegetables, legume vegetables, and leafy vegetables). The critical GAP in NEU and SEU is identical and is as follows:

1 x 1.08 kg/ha (max. 1.08 kg/ha per year), BBCH < 20, PHI 60 days (ground-directed, shielded spray application)

The following is additionally stated in the GAP: *"Applications are performed between the crop rows. The rate refers to the treated area only, which represents not more than 50% of the total area. The application rate with reference to the total surface area is not more than 50% of the stated dose rate."* This restriction, however, is not expected to be of importance for the evaluation of the residues section and is therefore not considered during the assessment.

Furthermore, the GAP states the following: *"Avoid crop contamination during treatment."*

In the previous evaluation for renewal of approval of glyphosate, an inter-row use was not part of the defended uses (RAR, 2015).

All studies submitted by the applicant are summarised in the following paragraphs.

B.7.3.3.1. Study 1

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	MSL0027502
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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
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

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

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.

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00482-01	2	BBCH 14	1.109	308
S15-00482-02	2	BBCH 16	1.127	313
S15-00482-03	2	BBCH 16	1.126	313
S15-00482-04	2	BBCH 19	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 and far-off the application band. 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).

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 S14-05172). 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 B.7.3.3.1-1: 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 B.7.3.3.1-2: 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)
				Glypho-sate	AMPA	
S15-00482-01 / [redacted] Ferrara, Italy / SEU / 2015	Carrot / Dordonia	49	Roots / from plants next to the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	60
			Roots / from plants far-off the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	
S15-00482-02 / [redacted] Lovech, Bulgaria / SEU / 2015	Carrot / Samson	49	Roots / from plants next to the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	60
			Roots / from plants far-off the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	
S15-00482-03 / [redacted] Pazardjik, Bulgaria / SEU / 2015	Carrot / Primo	49	Roots / from plants next to the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	61
			Roots / from plants far-off the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	
S15-00482-04 / [redacted] Pyrénées-Orientales, France / SEU / 2015	Carrot / Chambord	49	Roots / from plants next to the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	61
			Roots / from plants far-off the application band(s)	< 0.05 (n.d.)	< 0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 10-12 months in high starch content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Carrot, roots (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.3.2. Study 2

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	MSL0027500
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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
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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
S15-00467-01	Radish	*Raphanus sativus var. niger*	Candela di Ghiaccio	Roots Tops (leaves)	≥ 2 kg / > 50 units ≥ 1 kg / > 50 units
S15-00467-02	Radish	*Raphanus sativus var. niger*	Celesta	Roots Tops (leaves)	≥ 2 kg / > 50 units ≥ 1 kg / > 50 units

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.

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00467-01	2	BBCH 14	1.104	307
S15-00467-02	2	BBCH 11	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 and far-off the application band. 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).

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 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 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 B.7.3.3.2-1: 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

Table B.7.3.3.2-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
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 B.7.3.3.2-2: 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)
				Glyphosate	AMPA	
S15-00467-01 / [redacted] Bologna, Italy / SEU / 2015	Radish / Candela di Ghiaccio	49	Roots / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	31
			Roots / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
			Tops (leaves) / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
			Tops (leaves) / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00467-02 / [redacted], Lovech, Bulgaria / SEU / 2015	Radish / Celesta	49	Roots / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	30
			Roots / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
			Tops (leaves) / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
			Tops (leaves) / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

Table B.7.3.3.2-2: 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)
				Glyphosate	AMPA	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except for the fact that crops were harvested at a PHI of 30 days instead of the intended 60 days. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively; 24 and 10-12 months in high starch content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. It is noted that OECD guideline 509 does not give indications on the sample size of radish leaves, however, the RMS considers the specimens sufficiently representative since ≥ 1 kg and > 50 units were sampled. The following residues are selected for evaluation:

Radish, roots (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Radish, tops/leaves (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.3.3. Study 3

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	MSL0027499
Guidelines followed in study	OECD Test Guideline 509: Crop field trials EU Commission Working Document 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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 quantification (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
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

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

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.

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00466-01	2	BBCH 12-13	1.065	296
S15-00466-02	2	BBCH 19	1.157	321
S15-00466-03	2	BBCH 41	1.236	343
S15-00466-04	2	BBCH 41	1.008	280
S15-00466-05	2	BBCH 15	1.124	312
S15-00466-06	2	BBCH 17	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 and far-off the application band. 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).

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 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 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 B.7.3.3.3-1: 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 LOQ (0.05 mg/kg) were found in any treated or untreated specimens of onion (bulbs). Detailed residue levels are shown in the table below.

Table B.7.3.3.3-2: 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)
				Glypho-sate	AMPA	
S15-00466-01 / [redacted] Pyrénées-Orientales, France / SEU / 2015	Bulb onion / Rose de Figueres	49	Bulbs from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Bulbs from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00466-02 / [redacted] Lovech, Bulgaria / SEU / 2015	Bulb onion / Cassiopea	49	Bulbs from plants next to the application band(s)	<0.05	<0.05 (n.d.)	60
			Bulbs from plants far-off the application band(s)	<0.05	<0.05 (n.d.)	

Table B.7.3.3.3-2: 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)
				Glypho-sate	AMPA	
S15-00466-03 / [redacted] Emilia Romagna, Italy / SEU / 2015	Bulb onion / Derek	49	Bulbs from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	59
			Bulbs from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00466-04 / [redacted] [redacted] Aragon, Spain / SEU / 2015	Bulb onion / Dulce Fuentes	49	Bulbs from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Bulbs from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00466-05 / [redacted] Lower Saxony, Germany / NEU / 2015	Bulb onion / Sturon	49	Bulbs from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	61
			Bulbs from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00466-06 / [redacted] Styria, Austria / NEU / 2015	Bulb onion / Rawhide	49	Bulbs from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	61
			Bulbs from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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 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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except for the fact that the growth stage at application in trials S15-00466-03 and S15-00466-04 was at BBCH 41 instead of BBCH < 20. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Onion, bulb (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Onion, bulb (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.3.4. Study 4

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 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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
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

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

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.

Trial no.	**Application code**	**Timing**	**Application rate[1] kg a.s./ha**	**Water volume L/ha**
S15-00465-01	2	BBCH 51	1.216	338
S15-00465-02	2	BBCH 62	1.097	305
S15-00465-03	2	BBCH 29	1.075	298
S15-00465-04	2	BBCH 25	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 and far-off the application band. 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).

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 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 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 B.7.3.3.4-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			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 B.7.3.3.4-2: 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)
				Glypho-sate	AMPA	
S15-00465-01 / [redacted] Aragon, Spain / SEU / 2015	Tomato / H9036	87-89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	57
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00465-02 / [redacted], Pyrénées-Orientales, France / SEU / 2015	Tomato / Hector	87-89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	59
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00465-03 / [redacted], Emilia Romagna, Italy / SEU / 2015	Tomato / Gamlex	89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	59
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00465-04 / [redacted] Pazardjik, Bulgaria / SEU / 2015	Tomato / Rugby F1	89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	59
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except for the fact that the growth stages at application were at BBCH 25-62 instead of BBCH < 20. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Tomato, fruit (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.3.5. Study 5

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 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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

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

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

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.

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00459-01	2	BBCH 14	1.091	303
S15-00459-02	2	BBCH 24	1.101	306
S15-00459-03	2	BBCH 15	1.056	293
S15-00459-04	2	BBCH 61	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 and far-off the application. 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).

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

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
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 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 158 days, and the maximum interval from extraction to analysis was one day. 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 B.7.3.3.5-1: 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 B.7.3.3.5-2: 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)
				Glypho-sate	AMPA	
S15-00464-01 / [redacted] Pazardjik, Bulgaria / SEU / 2015	Cucumber / Timor F1	79	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00464-02 / [redacted] Pyrénées-Orientales, France / SEU / 2015	Cucumber / Raider	89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00464-03 / [redacted] Baden-Württemberg, Germany / NEU / 2015	Cucumber / Tanja	89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00464-04 / [redacted], Pays de la Loire, France / NEU / 2015	Cucumber / Raider	89	Fruit / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except for the fact that the growth stages at application in trials S15-00459-02 and S15-00459-04 were at BBCH 24 and 61, respectively, instead of BBCH < 20. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens.
It is noted that crop specimens were taken at BBCH 79 in trial S15-00459-01, whereas BBCH 89 represents normal commercial harvest. It is not expected that this will have an impact on the residue level determined in the RAC since a too early sampling time point would rather result in an overestimation of residue levels due to a potential increase in fruit weight. Besides it is noted that the RAC was indeed harvested at the intended PHI. In conclusion, the following residues are selected for evaluation:

Cucumber, fruit (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Cucumber, fruit (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.3.6. Study 6

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 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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
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

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

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.

Trial no.	**Application code**	**Timing**	**Application rate[1] kg a.s./ha**	**Water volume L/ha**
S15-00463-01	2	BBCH 10	1.179	328
S15-00463-02	2	BBCH 29	1.077	299
S15-00463-03	2	BBCH 10	1.098	305
S15-00463-04	2	BBCH 00	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 and far-off the application band. 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 in duplicate. 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).

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 in duplicate (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 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 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 B.7.3.3.6-1: 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

Table B.7.3.3.6-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		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 B.7.3.3.6-2: 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)
				Glypho-sate	AMPA	
S15-00463-01 / [redacted] Lovech, Bulgaria / SEU / 2015	Courgette / Beara	85	Fruit / from plants next to the application band(s)	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	60
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00463-02 / [redacted] Pyrénées-Orientales, France / SEU / 2015	Courgette / Lipan	89	Fruit / from plants next to the application band(s)	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	60
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00463-03 / [redacted] Baden-Württemberg, Germany / NEU / 2015	Courgette / Opera	75	Fruit / from plants next to the application band(s)	<u>≤0.05</u> (n.d.)	<u>≤0.05</u> (n.d.)	59
			Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
[redacted] Loiret, France / NEU / 2015	Courgette / Super Jedida F1	84	Fruit / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60

Table B.7.3.3.6-2: 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)
				Glypho-sate	AMPA	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates, except for "Fruit / from plants far-off the application band(s)" and "Fruit / from plants next to the application band(s)" of trials S15-00463-02 and S15-00463-03, respectively, which are values based on a single sample only.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except trials S15-00463-02 and S15-00463-04. In trial S15-00463-02, application took place at BBCH 29 instead of BBCH < 20. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted. In trial S15-00463-04, application took place at BBCH 00, i.e. before crop emergence. As already indicated by the applicant, the timing is not considered acceptable in support of an inter-row treatment. Therefore, the trial is not considered for evaluation.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens.
It is noted that crop specimens were taken before BBCH 89 in trials S15-00463-01 (BBCH 85), S15-00463-03 (BBCH 75), and S15-00463-04 (BBCH 84), whereas BBCH 89 represents normal commercial harvest. It is not expected that this will have an impact on the residue levels determined in the RACs since a too early sampling time point would rather result in an overestimation of residue levels due to a potential increase in fruit weight. Besides it is noted that the RACs were indeed harvested at the intended PHI. In conclusion, the following residues are selected for evaluation:

Courgette, fruit (NEU)
Glyphosate: <0.05 mg/kg
AMPA: 0.05 mg/kg
Courgette, fruit (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.3.7. Study 7

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 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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
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

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

Test commodities					
Trial:	Crop:	Botanical name:	Variety:	Crop parts:	Sample size:
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

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.

Trial no.	**Application code**	**Timing**	**Application rate[1] kg a.s./ha**	**Water volume L/ha**
S15-00460-01	2	BBCH 15	1.138	316
S15-00460-02	2	BBCH 15	1.022	284
S15-00460-03	2	BBCH 15	1.206	335
S15-00460-04	2	BBCH 15	0.984	273
S15-00460-05	2	BBCH 15	1.059	294
S15-00460-06	2	BBCH 15	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 and far-off the application band. 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).

Trial	**Crop**	**Commodity[1]**	**DALA[2]**	**Growth stage (BBCH)**	**Quantity**	**Date of sampling**
S15-00460-01	Head lettuce	Heads	22	49	≥ 1.1 kg / 12 units	17.06.2015[3]
S15-00460-02	Head lettuce	Heads	19	49	≥ 3.7 kg / 12 units	04.05.2015
S15-00460-03	Head lettuce	Heads	45	49	≥ 1.2 kg / 12 units	16.07.2015
S15-00460-04	Head lettuce	Heads	28	49	≥ 3.7 kg / 12 units	24.07.2015

Trial	Crop	Commodity[1]	DALA[2]	Growth stage (BBCH)	Quantity	Date of sampling
S15-00460-05	Head lettuce	Heads	31	49	≥ 2.7 kg / 12 units	15.06.2015
S15-00460-06	Head lettuce	Heads	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 (26.05.2015) and the PHI 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 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 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 B.7.3.3.7-1: 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 B.7.3.3.7-2: 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)
				Glypho-sate	AMPA	
S15-00460-01/ [redacted] Lovech, Bulgaria / SEU / 2015	Head lettuce / David	49	Lettuce heads / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	22
			Lettuce heads / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00460-02 / [redacted], Pyrénées-Orientales, France / SEU / 2015	Head lettuce / Iceberg	49	Lettuce heads / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	19
			Lettuce heads / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00460-03 / [redacted] Bologna, Italy / SEU / 2015	Head lettuce / Iceberg	49	Lettuce heads / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	45
			Lettuce heads / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00460-04 / [redacted] Aragon, Spain / SEU / 2015	Head lettuce / Aitana	49	Lettuce heads / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	28
			Lettuce heads / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00460-05 / [redacted] Baden-Württemberg, Germany / NEU / 2015	Head lettuce / Laruna NAS	49	Lettuce heads / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	31
			Lettuce heads / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00460-06 / [redacted] Styria, Austria / NEU / 2015	Head lettuce / Vitalis	49	Lettuce heads / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	34
			Lettuce heads / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except for the fact that crops were harvested at a PHI of 19-45 days instead of the intended 60 days. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Lettuce, heads (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Lettuce, heads (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.3.3.8. Study 8

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 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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
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

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

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.

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00459-01	2	BBCH 14	1.239	344
S15-00459-02	2	BBCH 12	1.151	320
S15-00459-03	2	BBCH 10	1.087	302
S15-00459-04	2	BBCH 12	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 and far-off the application band. 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).

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 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 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 B.7.3.3.8-1: 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

Table B.7.3.3.8-1: Recovery results

Matrix	Analyte	Fortification level (mg/kg)	Recovery[1]				
			Results / Range (%)	Mean (%)	Standard deviation (%)	Relative standard deviation (%)	Number analyses (n)
		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 B.7.3.3.8-2: 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)
				Glypho-sate	AMPA	
S15-00459-01 / [redacted] Bologna, Italy / SEU / 2015	Parsley / Gaia	49	Leaves / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	61
			Leaves / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00459-02 / [redacted] Lovech, Bulgaria / SEU / 2015	Parsley / Italian Gigant	49	Leaves / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Leaves / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00459-03 / [redacted] Baden-Württemberg, Germany / NEU / 2015	Parsley / Gigante d'Italia	49	Leaves / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	61
			Leaves / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00459-04 / [redacted] Styria, Austria / NEU / 2015	Parsley / Gigante d'Italia	49	Leaves / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	59
			Leaves / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Parsley, leaves (NEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg
Parsley, leaves (SEU)
Glyphosate: 2x <0.05 mg/kg
AMPA: 2x <0.05 mg/kg

B.7.3.3.9. Study 9

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 7029/VI/95 rev. 5, European Commission Working Document SANCO 3029/99 rev. 4
Deviations from current test guideline	None that would impact the outcome of the study.
Previous evaluation	New study for AIR5
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability:	**Conclusion GRG:** Valid, Category 1 **Conclusion AGG:** The study is considered to be acceptable.

2. Full summary of the study according to OECD format

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 beans 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
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

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

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.

Trial no.	Application code	Timing	Application rate[1] kg a.s./ha	Water volume L/ha
S15-00461-01	2	BBCH 15	1.171	325
S15-00461-02	2	BBCH 15	1.185	329
S15-00461-03	2	BBCH 15	1.234	343
S15-00461-04	2	BBCH 15	1.080	300
S15-00461-05	2	BBCH 15	1.199	333
S15-00461-06	2	BBCH 15	1.117	310
S15-00461-07	2	BBCH 15	1.049	291
S15-00461-08	2	BBCH 15	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 (whole 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 and far-off the application band. 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).

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 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 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 B.7.3.3.9-1: 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 B.7.3.3.9-2: Residue levels of glyphosate and AMPA in green beans 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)
				Glypho-sate	AMPA	
S15-00461-01/ [redacted] Lovech, Bulgaria / SEU / 2015	Green beans / Lodi	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	30
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

Table B.7.3.3.9-2: Residue levels of glyphosate and AMPA in green beans 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)
				Glypho-sate	AMPA	
S15-00461-02 / [redacted] Gard, France / SEU / 2015	Green beans, / Venice	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	35
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00461-03 / [redacted] Emilia, Romagna, Italy / SEU / 2015	Green beans, / Schubert	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	47
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00461-04 / [redacted] Navarra, Spain / SEU / 2015	Green beans, / Cocobel	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	45
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00461-05 / [redacted] Morbihan, France / NEU / 2015	Green beans, / Flagrano	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	60
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00461-06 / [redacted] Lower Saxony, Germany / NEU / 2015	Green beans, / Maxi	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	44
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00461-07 / [redacted] Baden-Württemberg, Germany / NEU / 2015	Green beans, / Maxi	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	29
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	
S15-00461-08 / [redacted] Styria, Austria / NEU / 2015	Green beans, / Imayca	79	Whole pods / from plants next to the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	45
			Whole pods / from plants far-off the application band(s)	<0.05 (n.d.)	<0.05 (n.d.)	

1 Growth stage at harvest
2 LOQ (limit of quantification): 0.05 mg/kg; n.d. (not detected): < 0.015 mg/kg
3 Residue levels are mean values of two sampling replicates.
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:
It is agreed with the applicant's assessment and conclusion. The study is considered acceptable for evaluation. The trials were conducted according to the intended inter-row use, except for the fact that crops were harvested at a PHI of 29-47 days instead of the intended 60 days in seven out of the eight trials. Since this represents a worst-case situation and residues were below the LOQ at harvest, this deviation is accepted.
It is noted that temperatures during shipment of specimens from trials S15-00461-04 exceeded -18 °C three times (9-62.5 hours, maximally -4 °C). Since samples remained frozen all the time throughout shipment, and were received in frozen conditions at the analytical facility, this deviation is not considered to have a significant impact on the study outcome.
The performance of the analytical method was sufficiently demonstrated and the method is considered acceptable. It is noted, however, that additional information regarding the extraction efficiency is needed for confirmation.
Specimens were stored in accordance with the demonstrated period of storage stability (24 and 18 months in high water content commodities for glyphosate and AMPA, respectively).
No residues above the LOQ were detected in control specimens. The following residues are selected for evaluation:

Green beans, whole pods (NEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg
Green beans, whole pods (SEU)
Glyphosate: 4x <0.05 mg/kg
AMPA: 4x <0.05 mg/kg

B.7.4. FEEDING STUDIES

B.7.4.1. Poultry

B.7.4.1.1. Study 1

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:	Applicant's conclusion: Valid (Category 2a) Conclusion RMS: Acceptable

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. There was also a control group (group 6), which was administrated with water.

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:

Analytical phase:

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
[redacted] 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:	Purchased from [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, [redacted] and then shipped to the Analytical Phase facility [redacted]) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table B.7.4.1-1: 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, [redacted]

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 [redacted] 20009 for *N*-acetyl glyphosate and relevant degradates (see Volume 3, B-5). 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 B.7.4.1-2: 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 B.7.4.1-2: 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 B.7.4.1-2: 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 B.7.4.1-3: 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 B.7.4.1-4: 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[2,3]	
					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*	*84.17*	49.98	*743.95*	*39.98*	*595.16*
	2	*1.66*	*0.102*	*82.78*	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*	*0.112*	*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 B.7.4.1-5: 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[4]
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 B.7.4.1-6: 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[4]
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.
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

Table B.7.4.1-7: 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[4]
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.
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

Table B.7.4.1-8: 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[4]
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.
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

Table B.7.4.1-9: 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[4]
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.
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

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 B.7.4.1-10: 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[4]
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.
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples.

Table B.7.4.1-11: 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[4]
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
4 Mean total residue values were determined as the sum of replicate subgroup values divided the number of replicate samples..

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 7.4.1-12: 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[4]	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.
4 Sum of N-acetyl glyphosate, glyphosate, AMPA and N-acetyl AMPA, expressed as glyphosate.

Table B.7.4.1-13: 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[4]	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.
4 Sum of N-acetyl glyphosate, glyphosate, AMPA and N-acetyl AMPA, expressed as glyphosate

Table B.7.4.1-14: 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[4]	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.
4 Sum of N-acetyl glyphosate, glyphosate, AMPA and N-acetyl AMPA, expressed as glyphosate

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: The study is considered acceptable to conclude on magnitude of residues in poultry tissues after administration of N-acetyl-glyphosate. It is noted that in the evaluation residues which were measured above the LOD, but below the LOQ were reported as <0.025 mg/kg and value of "0.025 mg/kg" was taken into account when total residues were determined. This means that total values reported in this RAR document are different than total residues reported in the study report, where it was calculated with actual

measured residues and ND residues were not taken into consideration. The values calculated and reported in this evaluation are considered more conservative.
Eggs samples were analysed within 30 days of extraction. The maximum storage interval for liver, fat and muscle was max. 80 days, which is covered by the available stability data for glyphosate and AMPA.
No separate storage stability study is available for N-acetyl-glyphosate and N-acetyl-AMPA in animal matrices. However, concurrently within this feeding study, storage was investigated for the maximum storage days (80 days) in liver, fat and muscle for all investigated compounds. All storage recoveries were within acceptable ranges and it is concluded that all analytes were stable for 80 days.

Further, it has been concluded that the performance of the analytical method was sufficiently demonstrated (Volume 3, B-5).

B.7.4.1.2. Study 2

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]37-43
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	No. It is stated that although not GLP, study was performed following appropriate EPA GD by using accepted laboratory methods and standard data management.
Acceptability/Reliability:	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Not valid. Analytical method used in the studies is considered not acceptable (See Volume 3, B5)

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: [redacted]

In-Life phase:

Analytical phase:

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:	Purchased from [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, [redacted] and then shipped to the Analytical Phase facility ([redacted]) where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table B.7.4.1-15: 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, [redacted]

An analytical method 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 [redacted] 87-43, RAR Volume 3, B-5) 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, liver 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 B.7.4.1-16: 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
	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

Table B.7.4.1-16: 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)
		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 B.7.4.1-17: 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 B.7.4.1-18: 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 B.7.4.1-19: 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 B.7.4.1-20: 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:
In general RMS agree with the study evaluation.
It has been reported that since TMS (trimethylsulfonium cation) is not relavant for the dossier, data of this analyte have not been reported in the summary. RMS agrees with this conclusion.

All samples were analyzed within 69 days of collection, which is covered by the available stability data for glyphosate and AMPA.

In the study summary Limit of detection (LOD) is reported and defined as minimum concentration that could be reliably quantitated. RMS wants to point out that this is definition of LOQ and therefore, LOQ is as follow: in eggs 0.01 mg/kg for PMG and 0.02 mg/kg for AMPA. For fat, liver, kidney and muscle LOQ was 0.05 mg/kg.
It is noted that no procedural recoveries were analysed at the LOQ level of investigated tissues (0.05 mg/kg). This is considered as a relevant deviation, since performance of the method cannot be determined at the LOQ level. Moreover, it has been observed that in the lowest investigated fortification level recoveries are rather low: 66 – 73 % for PMG and 58-66% for AMPA (except fat). Since it is reported that in most of the samples in all feeding levels no residues were detected, performance of the method at the LOQ level is desirable.
Taking into account lack of data on method performance at the LOQ in tissues, the study is considered as supportive only for those matrices.
From the residue perspective results of the study are acceptable for eggs, however analytical method used in the study is considered not acceptable (Volume 3, B-5). Therefore results of this study are not taken into account for further evaluation.

B.7.4.1.3. Study 3

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:	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Not valid. The analytical method is considered not acceptable (Volume 3, B-5)

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. Total nominal dose was 40, 120 and 400 mg/kg of glyphosate and AMPA in the 9:1 ratio.
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 AMPA 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 14–28 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:

In-Life phase:

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:	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:	Purchased from [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, [redacted] and then shipped to the Analytical Phase facility [redacted] where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table 7.4.1-21: 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, [redacted]

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 B.7.4.1-22: 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 (for details of method evaluation see Volume 3, B-5). 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 (HPLC) 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 B.7.4.1-23: 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

Table B.7.4.1-23: 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)
		Overall	82.4–89.5	85.1	2.6	3.1	6
	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 B.7.4.1-24: 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 B.7.4.1-25: 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*	*0.12*	*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 B.7.4.1-26: 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*

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.

4 Study Day 28 is at the end of the 28-day dosing period

Table B.7.4.1-27: 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 B.7.4.1-28: 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 B.7.4.1-29: 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 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	<0.050	<0.050	<0.050	<0.050
			2	<0.050		<0.050	
	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*
	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	0.139	0.138	0.069	0.070
			2	0.137		0.070	
	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*
	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 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	0.621	0.614	0.331	0.329
			2	0.607		0.327	
	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*
	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 B.7.4.1-30: 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 finalization 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: RMS agrees with the study evaluation.

It is noted that in the study report and evaluation of the study in previous RAR (Germany, 2015) reported results were corrected for the recovery. Data reported within this evaluation is not corrected for the recovery (raw data). It is noted that in the Volume 3, B-5 analytical method by the study ML-6676 is considered at the moment as not validated.

No exact storage period of the analysed samples has been reported. Based on the analytical dates, period of max. 25 months of storage has been estimated, which is covered by the available storage data for poultry fat, muscle and liver. The data for those matrices is considered acceptable.

For kidney max. 13 months storage has been demonstrated. Glyphosate and AMPA are considered stable for max. 14 months in eggs, since in one of the provided study decline was observed at later timepoints. Therefore, the data in kidney and eggs cannot be considered acceptable.

It should be noted, however, that analytical method used in the study is considered not acceptable (Volume 3, B-5). Therefore results of this study are not taken into account for further evaluation.

B.7.4.1.4. Study 4 : Relevant published article from Literature Search Report

Data point	CA 6.4.1/004
Report author	Shehata A., 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:	Conclusion of the applicant: Yes/ reliable with restrictions Conclusion RMS: Supportive information. Article has been reviewed, no consequences for conclusion.

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.

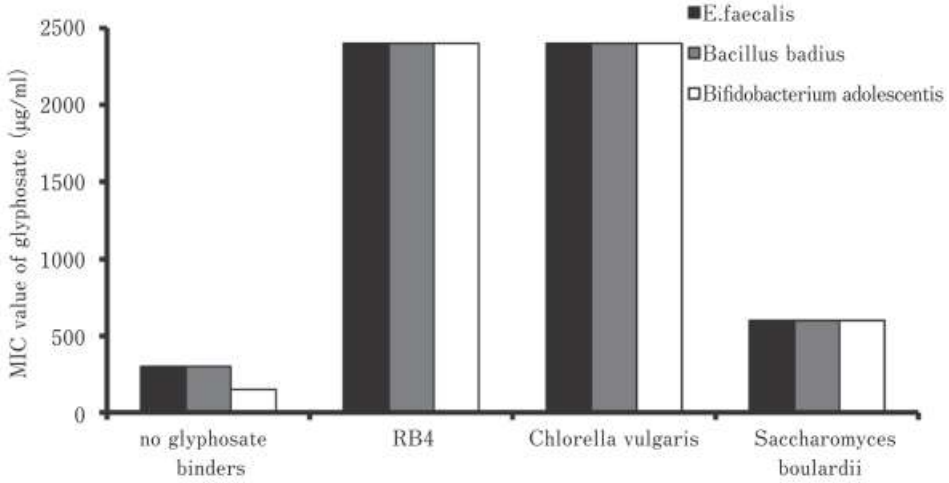
Sample N=30	Glyphosate (ng/gm)		
	Minimum	Maximum	Mean±SD
Feed	190.0	400.0	370.0±92.0
Liver	6.0	13.6	9.8±3.0
Spleen	11.8	25.0	21.1±17.0
Lung	12.0	25.0	24.2±9.0
Intestine	20.0	120.0	98.3±42.0
Heart	17.0	20.0	20.4±0.6
Muscles	3.6	4.9	5.0±0.3
Kidney	0.4	17.6	16.0±13.0

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 (* $P=0.05$, ** $=P<0.001$).

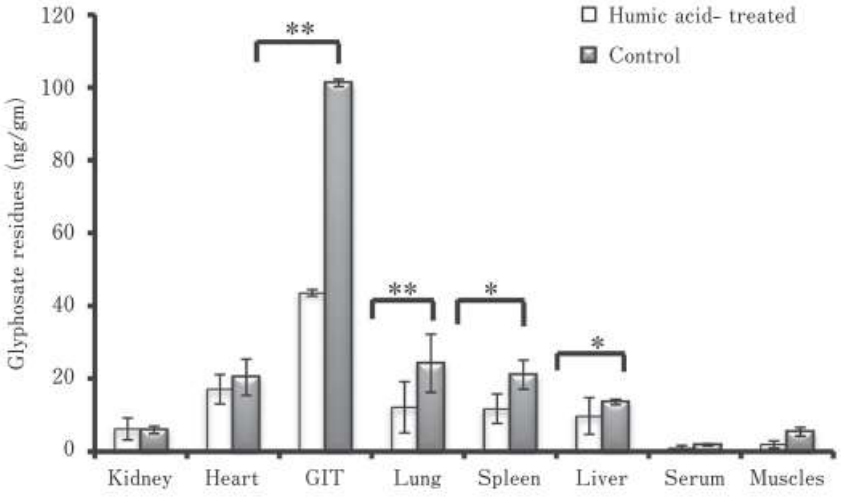


Table 2: Effect of humic acid supplementation on the production parameters.

Parameter	Humic acid-treated chickens	Non-treated chickens
Total number	22500	23100
Slaughtered number at 30-day-old	6509	6509
Slaughtered number at 39-day-old	14581	15573
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.

Assessment and conclusion by RMS:
The article investigates glyphosate residue in feed and different organs of poultry (chicken) and impact of humic acid, *Chlorella vulgaris* and *Saccharomyces boulardi* of glyphosate neutralisation. It has been demonstrated that glyphosate could be detected in poultry tissues and different organs. Additionally, humic acid, *Chlorella vulgaris* and *Saccharomyces boulardi* showed neutralisation of the antimicrobial effect of glyphosate *in-vitro*. No information is given on source of glyphosate concentration in animal tissues, no reliable transfer factors could be estimated. Further, obtained information can be considered as supplementary. It is concluded that this publication has no further impact on the existing risk assessment parameters.

B.7.4.1.5. Study 5: Relevant published article from Literature Search Report

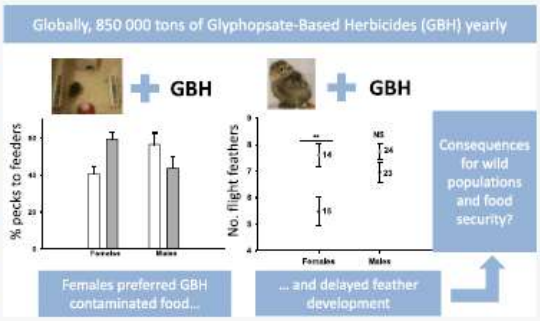
Study reported below was not submitted by the applicant in the dossier submission in the literature section for residues. It has been submitted in the dossier for ecotoxicology section, however it investigates, among other things, residues of glyphosate in edible poultry matrices, therefore RMS decided to include abstract and short assessment of this study in this residue part.

Data point	CA 6.4.2/007
Report author	Ruuskanen, S.
Report year	2020
Report title	Female Preference and Adverse Develop
Document No.	Environ. Scie Technol. 2020, 54 1128-1135
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion of the applicant: - Conclusion RMS: Supportive information. Article has been reviewed, no consequences for conclusion.

Assessment and conclusion by RMS:

Study abstract:

ABSTRACT: Controversial glyphosate-based herbicides (GBHs) are the most frequently used herbicides globally. An increasing number of studies have identified GBH residues in soil, water, and even human food that may expose nontarget organisms including wildlife, livestock, and humans to health risks. After a heated debate, the European Union allowed the use of GBHs to continue until 2022, after which their risks will be re-evaluated. Thus, decision makers urgently need scientific evidence on GBH residues and their possible effects on ecosystems. An important, yet neglected, aspect is to assess whether animals show preference or avoidance for GBH-contaminated food, as it can influence the likelihood of adverse health effects in wildlife. Here, using Japanese quails (*Coturnix japonica*) as our model, we show that females preferred GBH-contaminated food compared to control food. In females, exposure to GBHs caused delayed plumage development, and GBH residues were present in eggs, muscles, and liver. These results indicate that female preference is not adaptive, potentially exposing nontarget animals to greater risk of adverse effects of GBHs in natural and agricultural environments. Our results on tissue residues suggest that further studies are needed to understand the risks of such residues in the food chain.



Summary of the study:

The study involves a two/choice experiment, where birds (Japanese quails) were provided with feed containing glyphosate (GBH) or control feed (no glyphosate added). The concentration of glyphosate in GBG food was aimed at 160 mg/kg food (12-20 mg glyphosate/ kg body mass/day in full-grown Japanese quails.

Table 1. Glyphosate Residues (mg/kg Tissue, Average, and Standard Deviation) in Various Tissues and Excreta (Fecal and Urine Combined), Separately for Females and Males[a]

tissue type	treatment	sex	exposure (months)	average glyphosate (mg/kg)	SD
egg	GBH	female	10	0.76	0.16
liver	GBH	male	5	0.74	0.50
liver	GBH	male	12	1.33	0.21
liver	GBH	female	12	4.10	1.10
muscle	GBH	male	12	0.10	0.02
muscle	GBH	female	12	0.24	0.09
excreta	GBH	male	12	209.05	8.40
excreta	GBH	female	12	179.50	NA

[a]Samples from several, randomly chosen animals have been pooled (see the text for details on sample sizes).

Eggs, liver, muscle and excreta were analyzed (see table above). The highest concentration of glyphosate has been measured in excreta, followed by liver, eggs and muscle. Those findings are in general in agreement with the OECD complied, feeding studies in poultry, where also significant highest residue level was found in excreta, compared to other tissues.

It should be noted that concentration of glyphosate in feed was aimed at 12-20 mg glyphosate/ kg body mass/day in full-grown Japanese quails, which is extremely higher than calculated dietary burden for representative uses: 0.006 mg/kg bw/d (but also for uses in the Article 12 MRL review, EFSA 2019, where max. dietary burden for poultry was 2.15 mg/kg glyphosate bw/d).

Results from this study can be considered as supplementary. This publication has no further impact on the existing risk assessment parameters.

B.7.4.2. Ruminants

B.7.4.2.1. Study 1

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:	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Acceptable

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, 17, 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 [redacted] 20009 analytical method (See Volume 3, B-5)

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 summarized 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 0.10 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:
Analytical phase:

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:	Supplied by [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 (at least 8 days), 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 B.7.4.2-1: 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 B.7.4.2-2: 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, [redacted]

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 [redacted] 20009 analytical method for *N*-acetyl glyphosate and relevant degradates (See Volume 3, B-5). 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 B.7.4.2-3: 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
		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

Table B.7.4.2-3: 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)
		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
		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

Table B.7.4.2-3: 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	77–103	87	9	10	12
N-acetyl-AMPA	Whole Milk	0.025	82, 90, 117, 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
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
	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

Table B.7.4.2-3: 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)
		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 B.7.4.2-4: 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 B.7.4.2-5: 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

Table B.7.4.2-5: 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
	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*
	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

Table B.7.4.2-5: 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
	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
		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

Table B.7.4.2-5: 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	<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
		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

Table B.7.4.2-5: 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

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 B.7.4.2-6: 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

Table B.7.4.2-6: 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
		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 0.10 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 7.4.2-7: 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 7.4.2-8: 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

Table 7.4.2-8: 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
	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
	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 7.4.2-9: 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*

Table 7.4.2-9: Residue level results in fat

	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

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 B.7.4.2-10: 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 0.10 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:
RMS agreed with the study assessment. The study is considered acceptable to conclude on magnitude of residues in ruminants after administration of N-acetyl-glyphosate.
It is noted that in the evaluation residues which were measured above the LOD, but below the LOQ were reported as <0.025 or <0.05 mg/kg and value of "0.025 mg/kg / 0.05 mg/kg" was taken into account when mean residues were determined. This means that mean values reported in this RAR document are different than mean values reported in the study report, where it was calculated with actual measured residues and ND residues were not taken into consideration. The values calculated and reported in this evaluation are considered more conservative. In the study tissues and milk were analysed for N-acetyl-glyphosate, glyphosate, AMPA and N-acetyl AMPA.
Milk samples were analysed within 30 days of collection. The storage interval for kidney, liver, fat and muscle was 71, 74, 83 and 77 days, respectively, which is covered by the available storage stability data for glyphosate and AMPA.

No separate storage stability study is available for N-acetyl-glyphosate and N-acetyl-AMPA in animal matrices. However, concurrently within this feeding study, storage was investigated in liver, fat and muscle for all investigated compounds during duration of the study. All storage recoveries were within acceptable ranges and it is concluded that all analytes were stable for 90-91 days.

Further, the performance of the analytical method was sufficiently demonstrated and the method is considered acceptable (Volume 3 – B.5)

B.7.4.2.2. Study 2

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 testing facilities	Yes
Acceptability/Reliability:	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Not acceptable. The analytical method is considered not acceptable (Volume 3, Part B-5)

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. Total nominal concentration was 40, 120 and 400 mg/kg feed.
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 AMPA 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, 0.78 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: [redacted]

In-Life phase:

Analytical phase:

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:	Purchased in [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 (Total 40 mg/kg feed) 3X: nominal at 108 mg/kg glyphosate + 12 mg/kg AMPA in total diet (120 mg/kg feed) 10X: nominal at 360 mg/kg glyphosate + 40 mg/kg AMPA in total diet (Total 400 mg/kg feed)

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, [redacted] and then shipped to the Analytical Phase facility [redacted] where they continued to be stored frozen (<-20 °C) until analysed.

A summary of the sampling information is shown in the table below.

Table B.7.4.2-11: 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, [redacted]

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 B.7.4.2-12: 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

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. 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 B.7.4.2-13: 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

Table B.7.4.2-13: 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)
	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
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 deviations for individual standard fortification levels which were not included in the study report were calculated separately for this study summary and are presented here in italics.
*Recovery is corrected for background in controls.

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 B.7.4.2-14: 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	47.1
	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

*Reported results were corrected for recoveries

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 7.4.2-15: 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	059	*5.5*	*571*	*21.1*	*2365*	*4.1*	*112.0*	*263*	*0.46*	*12.4*

Table 7.4.2-15: 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
[108 mg/kg glyphosate + 12 mg/kg AMPA in dry feed (total diet)]	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*
	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*	*353.1*	*660*	*1.22*	*39.0*
	Average:	*5.3*	*598*	*21.9*	*7625*	***12.7***	***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 measurable 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 B.7.4.2-16: 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): 108.8 mg/kg in feed; 4.1 mg/kg bw AMPA (average): 12.0 mg/kg in feed; 0.46 mg/kg bw	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	
	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	

Table B.7.4.2-16: 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
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 B.7.4.2-17: 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; 4.1 mg/kg bw AMPA (average): 12.0 mg/kg in feed; 0.46 mg/kg bw	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	
	105	28	1	~~0.69~~ / **0.88**	~~*0.69*~~ / ***0.78***	0.19	*0.20*
			2	0.69		0.20	
	Study Day 28, 3X treatment group average:				~~*0.75*~~ ***0.78***		*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	

Table B.7.4.2-17: 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
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: RMS agrees with the assessment.
It is noted that in the study report and evaluation of the study in previous RAR (Germany, 2015) reported results from residue levels in the tissues were corrected for the recovery. Data reported within this evaluation are not corrected for the recovery (raw data). One value from the 28 days 3X treatment from animal number 105 was corrected by RMS according with the study report.
Reported recoveries are considered acceptable and therefore performance of the analytical method has been sufficiently addressed, including LOQ level.

No exact storage period of the analysed samples has been reported. Based on the analytical dates, period of max. 22 months of storage has been estimated, which is covered by the available storage data for glyphosate and AMPA in ruminant fat, muscle, kidney and liver. Also stability of glyphosate in milk is sufficiently demonstrated to covered the estimated storage period. The data for those matrices is considered acceptable.

AMPA is considered stable for 16 months in milk. Therefore, stability for this matrix is not demonstrated sufficiently. On the other hand, based on available data from metabolism study and other feeding studies, residues of AMPA in milk are not expected above the LOQ level. Therefore, this is consider as a minor deficiency of the study.

It should be noted that analytical method used in the study is considered not acceptable (Volume 3, B-5). Therefore results of this study are not taken into account for further evaluation.

B.7.4.2.3. Study 3

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 guideline	A review of this study indicates the following deviations from OECD 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:	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Not acceptable, since analytical method is considered not acceptable (Volume 3, B-5).

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:

Analytical phase:

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:	Purchased in [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 (l,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 B.7.4.2-18: 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.

[redacted]y and were shipped by air to the Analytical test facility [redacted] 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, [redacted]

Residues of CMP and AMPA in milk and tissues were determined using analytical Method [redacted]87-41 (See Volume 3, Part B-5). 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 B.7.4.2-19: 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

[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 B.7.4.2-20: 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 /	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

Table B.7.4.2-20: 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]
50 mg/kg glyphosate-trimesium in dry feed (total diet)	**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 B.7.4.2-21: 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 tables 4 and 5 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 B.7.4.2-22: : 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
	L00001	28	<0.05	*	*	*
	L00018	28	*	<0.2	<0.05	<0.05

Table B.7.4.2-22: : Residues of CMP in tissues

T1, Glyphosate-trimesium *0.51 mg/kg in feed; 0.018 mg/kg bw*	*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 B.7.4.2-23: : 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

Assessment and conclusion by applicant:

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:

In general RMS agree with the study evaluation.

It has been reported that since TMS (trimethylsulfonium cation) is not relevant for the dossier, data of this analyte have not been reported in the summary. RMS agrees with this conclusion.

All the samples were analysed within 69 days of collection, which is covered by the available storage stability data for glyphosate and AMPA .

It is noted that in the study a lower limit of detection was reported (LOD) which is, however, defined as lowest quantification level (LOQ). Therefore, RMS concludes that LOQ for glyphosate and AMPA in milk was 0.02 mg/kg, in liver 0.2 mg/kg and in muscle, fat and kidney 0.05 mg/kg.
It is noted that no procedural recoveries were analysed at the LOQ level of investigated tissues (0.05 mg/kg and 0.2 mg/kg in liver). This is considered as a relevant deviation, since performance of the method cannot be determined at the LOQ level. Since it is reported that in most of the lower fortification levels no residues were detected, performance of the method at the LOQ level is desirable. It is also observed that recoveries for AMPA in liver are not within acceptable ranges.
Taking into account lack of data on method performance at the LOQ in tissues, and not acceptable performance for AMPA in liver the study is considered not acceptable.

It should be noted that analytical method used in the study is considered not acceptable (Volume 3, B-5). Therefore results of this study are not taken into account for further evaluation.

B.7.4.2.4. Study 4: Relevant published article from Literature Search Report

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, pasteurized 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:	Conclusion of the applicant: Yes/ reliable Conclusion RMS: Supportive information. Article has been reviewed, no consequences for conclusion.

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.

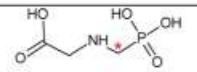
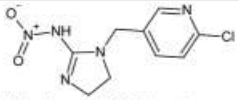
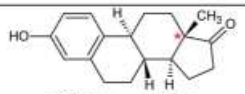
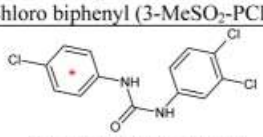
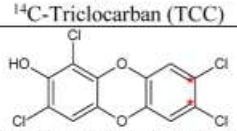
Table 1: Drug Structures and Physicochemical Properties.

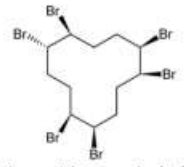
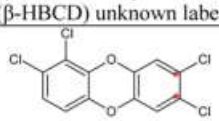
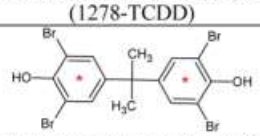
[a] Compound radioactively labeled with a directed label and specified on the structure with a red asterisk. An asterisk within a ring indicates a uniform label on the ring. Exceptions: IMI and *β*-HBCD carbon labels are unknown.

[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]
 ^{14}C-Glyphosate (GLY)	Herbicide/ pesticide	169.07 g/mol 50 (20 nM/ 200 nM) 5.0 (2000 nM)	-3.26 ± 1.53	5.89 ± 0.40	-4.24 ± 1.49
 ^{14}C-Imidacloprid (IMI) unknown label	Insecticide/ pesticide	255.66 g/mol 25.3 (20 nM/ 200 nM) 2.5 (2000 nM)	0.39 ± 0.59	5.28	-0.38 ± 0.59
 ^{14}C-Bisphenol A (BPA)	Plasticizer	228.28 g/mol 53.5 (20nM/ 200nM) 6.0 (2000nM)	3.60 ± 0.27		3.60 ± 0.27
 ^{14}C-Estrone (E1)	Hormone	270.37 g/mol 51.3 (20nM/ 200nM) 5.7 (2000nM)	3.62 ± 0.45		3.62 ± 0.45
 ^{14}C-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
 ^{14}C-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
 ^{14}C-2-hydroxy-1,3,7,8-tetrachloro dibenzo-*p*-dioxin (2-OH-1378-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
 ^{14}C-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-pentabromodi-phenyl 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	17.86	3.52
	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 (70)	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	17.85	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	1.85		
	2000 (2067)	3.21	2.11	1.52		
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	0.79	0.34	18.01	22.96
	200 (234)	3.53	9.36	0.38		
	2000 (1815)	21.63	76.47	0.28		
BDE-99	20 (20)	0.12	0.03	4.0	6.66	1.20
	200 (178)	1.05	0.38	2.76[f]		
	2000 (1890)	18.33	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 (GraphPad 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, 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.

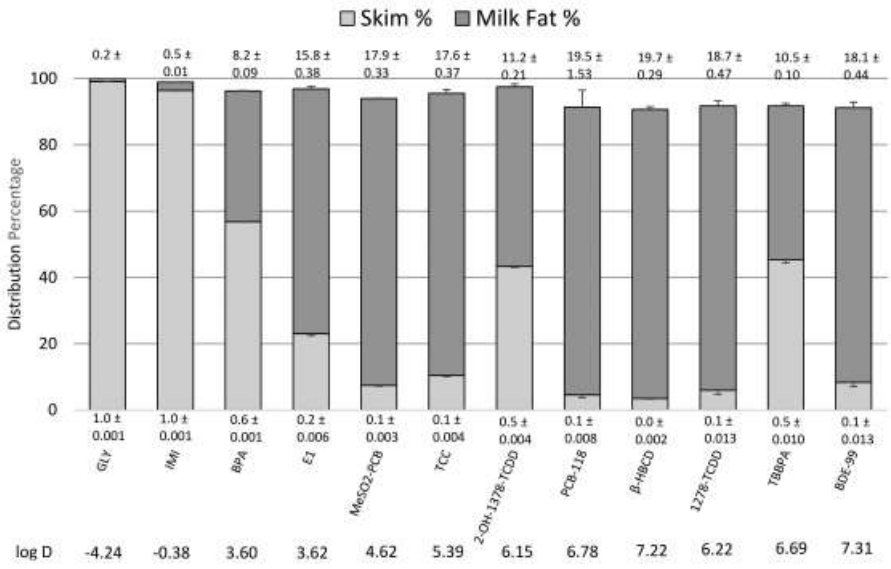


Table S5: GLY Phase 1 Average Distribution Data and Ratios.

Phase 1	Whole			Skim Milk				Milk Fat				Percent Recovery	Obs. Ratio
Mean	Spike DPM	Volume (mL)	Initial nM	Total DPM	Skim Milk 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	116,815	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	1,167,088	46.52	226.03	99.07	11,398	2.38	43.21	0.97	100.04	0.19
2000nM	1,117,710	48.90	2,059.36	1,107,061	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.

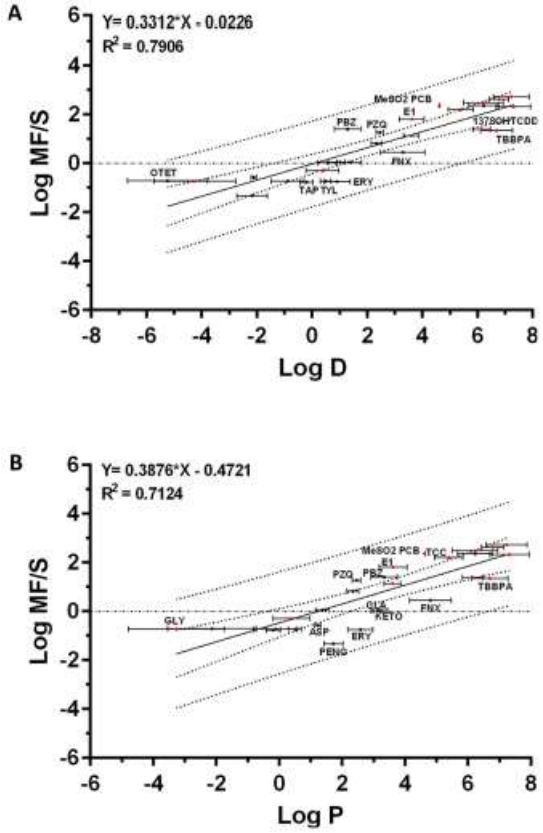
Compounds with a log *D* or *P* value of about 6 consistently concentrated in milk fat (or cream as cited in references). Concentrations of dichlorodiphenyltrichloroethane (DDT, Table S4) (log *D* 6.22 and log *P* = 5.92) in raw whole milk (5 % lipid), skim milk, and cream (70 % lipid) were reported 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.

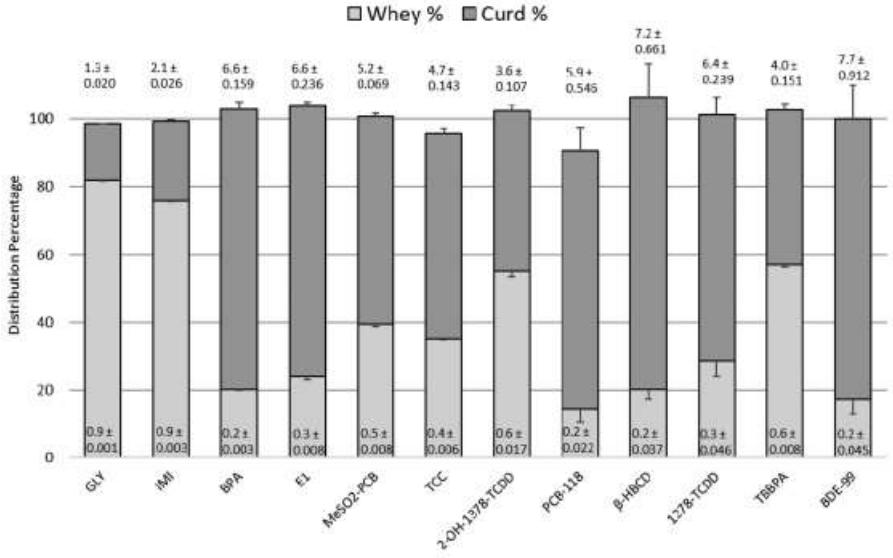


Table S17: GLY Phase 2 Average Distribution Data and Ratios.

Phase 2	Skim Milk			Whey Fraction					Curd Fraction					Percent Recovery	Obs. Ratio	Adj. Ratio
Average	Initial DPM	Volume (mL)	Initial nM	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	$[GLY]_{Curd}/[GLY]_{whey}$	$[GLY]_{0\%moisCurd}/[GLY]_{whey}$
0nM		45.33			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	1,139,999	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	1,081,531	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.

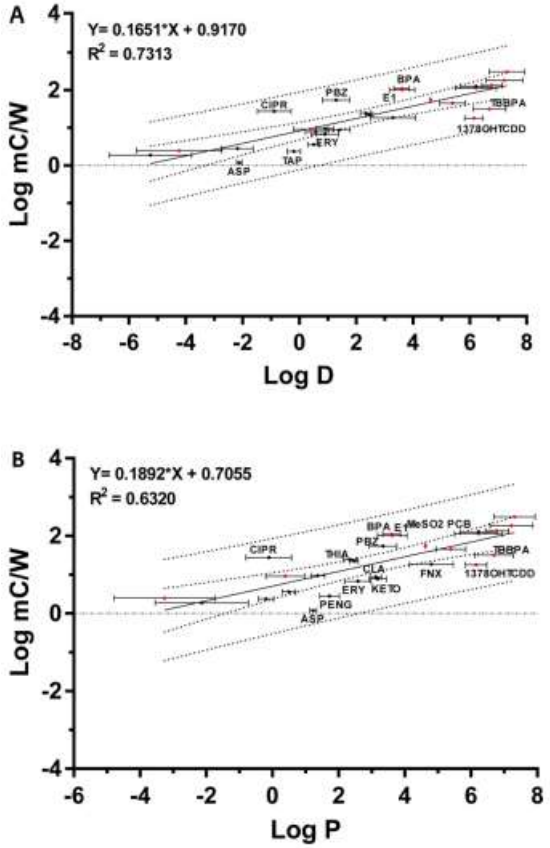
Lipophilic compounds in this study concentrated in the curd to a greater extent than whey, but the lipophilic pesticide lindane (log *P* = 3.99), a cyclo-chlorinated structure with similarities to *β*-HBCD, did not concentrate in cheese or yogurt (produced from curd) made from contaminated raw milk [20]. 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]$_{0\ \%moisture\ curd}$ /[chemical]$_{whey}$ (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).

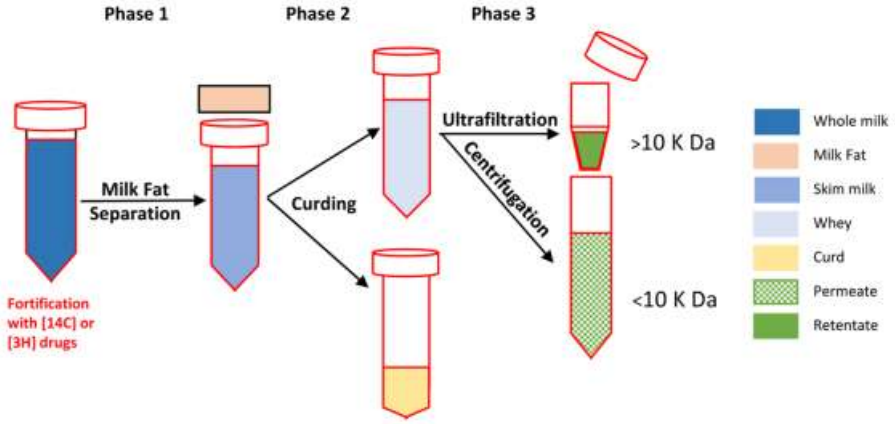


Figure 6: Drug distribution from whey into permeate, retentate, and filter fractions. Bars represent percent mean of all concentrations (n = 3 concentrations, concentration exceptions are PCB-118 and β-HBCD n = 2 concentrations; n = 3 replicates per concentration, replicate exceptions n = 2 replicates 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).

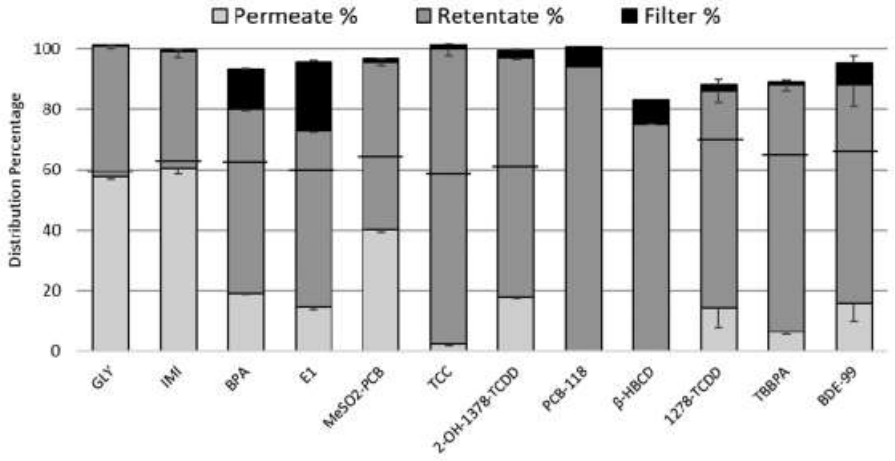


Table S29: GLY Phase 3 Average Distribution Data and Ratios.

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	4.6
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	101.91	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	0.01	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	0.01	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	1.57	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).

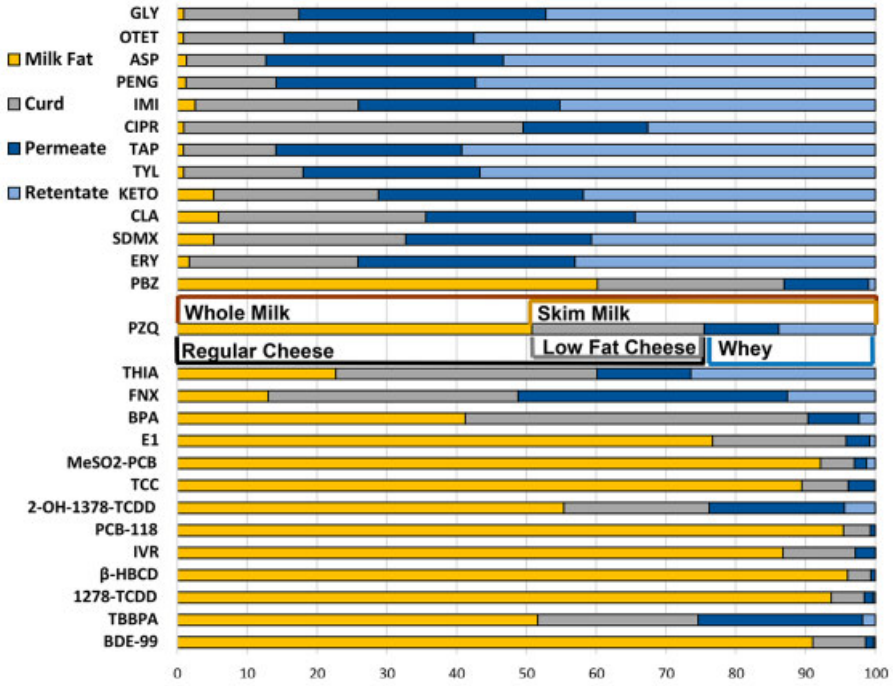
Previously studied chemicals that had higher association for whey proteins versus caseins were PENG (casein/whey ratio = 0.2), ERY (0.5), KETO (0.4), SDMX (0.8) [8]; TAP (0.5), CLA (0.4), and FNX (0.25) [9]. Although the distribution between lipid and aqueous phases was markedly dependent on the property 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.

Assessment and conclusion by RMS:
The article investigates partitioning several environmental contaminants, including glyphosate in milk fractions. Partitioning between milk fat and skim milk and between 0% moisture curd and whey was corelated with the compound's lipophilicity. Glyphosate was found in skim milk (further processed to whey and curd). Only very low amount of glyphosate portioned in milk fat. The obtained information on glyphosate behaviour in milk can be considered as supplementary. It has been concluded in the available feeding study that transfer of glyphosate to milk is very limited. It is concluded that this publication has no further impact on the existing risk assessment parameters.

B.7.4.2.5. Study 5 : Relevant published article from Literature Search Report

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:	Conclusion of the applicant: Yes/ reliable Conclusion RMS: Supportive information. Article has been reviewed, no consequences for conclusion.

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:

$$NE\ balance\ [MJ\ NE_L/d] = Energy\ intake\ [MJ\ NE_L/d] - \{NE_M\ [MJ\ NE_L/d] + NE_L\ [MJ\ NE_L/d]\}$$

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.

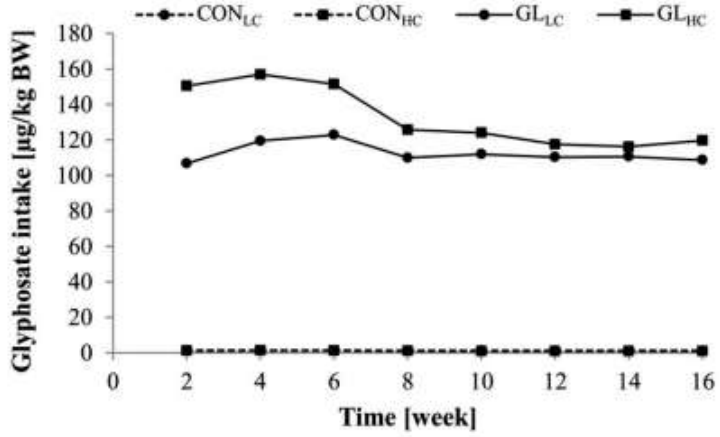
The chemical composition of the cows' ration components (Table 1) was within the normal range for the respective feedstuffs (DLG 1997). The average daily GL intake in Groups CON LC and CON HC was 0.8 and 0.8 mg/d, respectively, and in Groups GL LC and GL HC 73.8 and 84.5 mg/d, respectively. Figure 1 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[§]	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); [§]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.

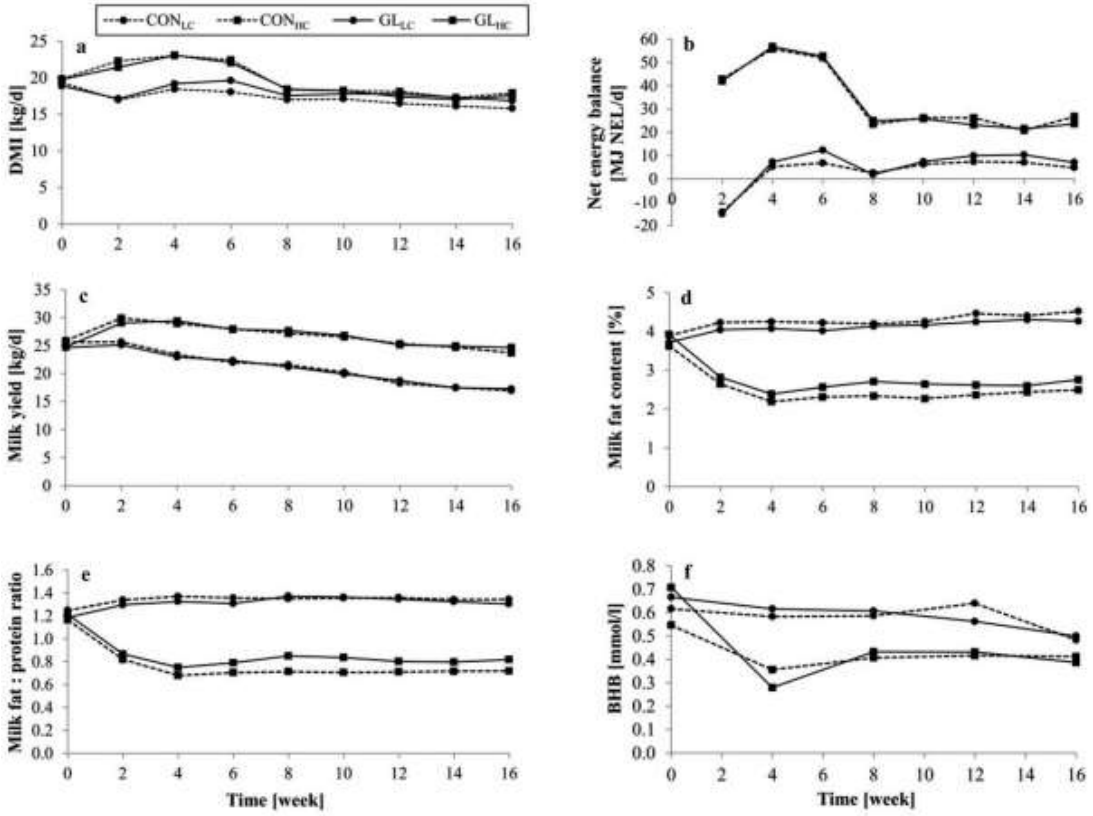
Table 2: Effects of glyphosate residues and concentrate feed proportion (CFP) in total mixed rations (TMR) on body condition score, body weight, dry matter intake (DMI), net energy intake and net energy balance.

	Control (CON)		Glyphosate (GL)		*p*-Value[7]					
	LC[4] (*n* = 16)	HC[5] (*n* = 16)	LC (*n* = 14)	HC (*n* = 15)	GL	CFP	t[6]	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	0.021	0.673	<0.001	0.689
Body weight [kg]	646 ± 5	675 ± 5	641 ± 5	669 ± 5	0.232	0.870	<0.001	0.240	<0.001	0.070
DMI [kg/d]*	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	0.707
Water intake [kg/d]	73.8 ± 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[2] [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	0.837	<0.001	0.333	0.030	0.418
NEFA[3] [mmol/l]	0.22 ± 0.02	0.20 ± 0.02	0.22 ± 0.02	0.29 ± 0.02	0.012	0.175	<0.001	0.013	0.052	0.696

[4]LC, low concentrate proportion (30% concentrate in TMR); [5]HC, high concentrate proportion (60% concentrate in TMR); [6]t, time effect of trial week; [7]GL × CFP × t ($p > 0.05$) for all variables; *analysed with DMI week 0 as covariance factor; [2]BHB, β-hydroxybutyrate; [3]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.

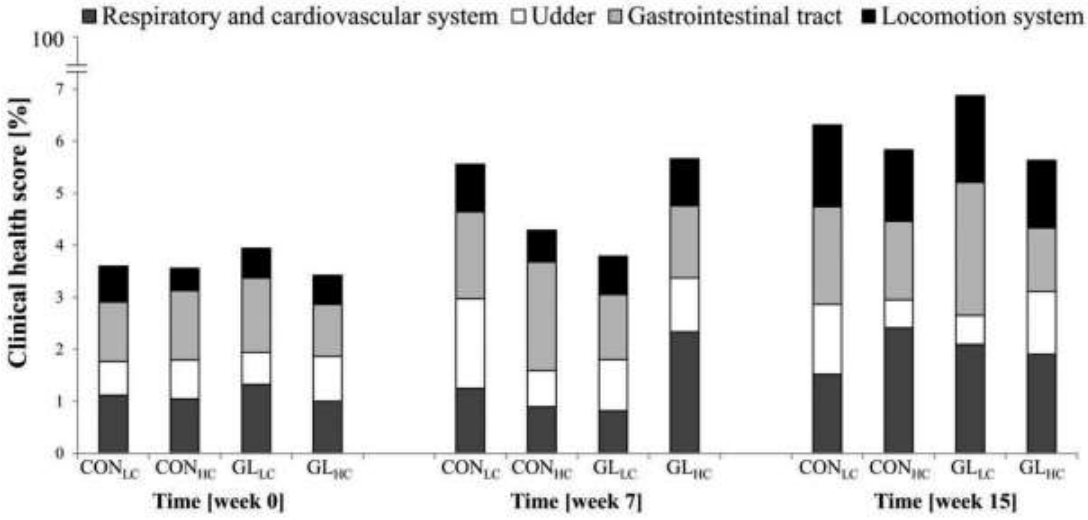
Table 3: Effects of glyphosate residues and concentrate feed proportion (CFP) in total mixed ration (TMR) on milk performance parameters.

	Control (CON)		Glyphosate (GL)		p-Value						
	LC[a] (n = 16)	HC[b] (n = 16)	LC (n = 14)	HC (n = 15)	GL	CFP	t[c]	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	0.011
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[d] [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	0.980
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	0.633	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); [b]HC, high concentrate proportion (60% concentrate in TMR); [c]t, time effect of trial week; [d]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 (Duke and Powles 2008). According to von Soosten *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.

Assessment and conclusion by RMS:
RMS agrees with the applicant's evaluation. The article investigates health, energy and characteristic of dairy cow fed with glyphosate contaminated feed (GL treated peas, wheat straw and grain). No residues were detected in milk, which is in line with GLP feeding studies. The clinical examination showed no adverse effect of glyphosate containing diet on cow's health and performance. Obtained information can be considered as supplementary. It is concluded that this publication has no further impact on the existing risk assessment parameters.

B.7.4.2.6. Study 6: Relevant published article from Literature Search Report

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
Document No.	DOI 10.3168/jds.2015-10585 E-ISSN 1525-3198
Guidelines followed in study	None stated
Deviations from current test guideline	Not applicable
GLP/Officially recognised testing facilities	Not applicable
Acceptability/Reliability:	Conclusion of the applicant: Yes/ reliable Conclusion RMS: Supportive information. Article has been reviewed, no consequences for conclusion.

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.

	Experiment					
Component (%, unless noted)	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 ± 0.01
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	0.14 ± 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)	1.59 ± 1.77	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.

Assessment and conclusion by RMS:
RMS agrees with applicant's assessment. In the submitted article excretion pathways of glyphosate and AMPA in dairy cows fed with diets containing the two residues were investigated. During the study feed, feces urine, milk and duodenal contents were analyzed. Elimination via milk was negligible and it has been concluded that gastrointestinal absorption of glyphosate is of minor importance. Fecal excretion was the major pathway. This conclusions are in line with observations in GLP feeding studies.
Obtained information can be considered as supplementary. It is concluded that this publication has no further impact on the existing risk assessment parameters.

B.7.4.3. Pigs

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:	Conclusion applicant: Valid (Category 2a) Conclusion RMS: Study is acceptable.

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. Total levels of administrated compounds were 40, 120 and 400 mg/kg.
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 AMPA (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:

Analytical phase:

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:	Purchased from [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 B.7.4.3-1: 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, [redacted]

An analytical methodology was developed and validated for the determination of glyphosate and AMPA in the feed diet (See Volume 3, Part B-5). 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 B.7.4.3-2: 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 B.7.4.3-2: 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. 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 B.7.4.3-3: 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 B.7.4.3-3: 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 B.7.4.3-4: 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*
	1	*339.4*	*36.5*
	2	*329.7*	*36.8*
	3	*346.5*	*36.8*

Table B.7.4.3-4: 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	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 B.7.4.3-5: 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*
	Average:	*92*	*3.0*	*11.13*	*1022.8*	*1.20*	*110.6*

Table B.7.4.3-5: 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)

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 B.7.4.3-6: 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 B.7.4.3-7: 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*
	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*

Table B.7.4.3-7: 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			2	0.483		0.225	
	Study Day 28, 10X treatment group average:				*0.598*		*0.337*
Glyphosate (average): *340.2 mg/kg in feed; 11.13 mg/kg bw*	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	
AMPA (average): *36.8 mg/kg in feed; 1.20 mg/kg bw*	*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 B.7.4.3-8: 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	142	25[5]	1	0.130	*0.136*	<0.050	*<0.050*
			2	0.141		<0.050	
Glyphosate (average): *33.4 mg/kg in feed; 0.98 mg/kg bw*	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	
AMPA (average): *3.6 mg/kg in feed; 0.11 mg/kg bw*	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	135	28	1	2.87	*2.91*	0.300	*0.308*
			2	2.94		0.316	
Glyphosate (average): *105.2 mg/kg in feed; 3.24 mg/kg bw*	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	
AMPA (average): *11.0 mg/kg in feed; 0.34 mg/kg bw*	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 B.7.4.3-8: 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:
RMS agreed with study evaluation.
It is noted that in the study report and evaluation of the study in previous RAR (Germany, 2015) reported results were corrected for the recovery. Data reported within this evaluation is not corrected for the recovery (raw data). Reported recoveries are considered acceptable and therefore performance of the analytical method has been sufficiently addressed, including LOQ level, except for glyphosate in liver at the LOQ (67% mean recovery).

No exact storage period of the analysed samples has been reported. Based on the analytical dates, period of max. 27 months of storage has been estimated. Storage stability for glyphosate and AMPA have been demonstrated for 26 months, except AMPA in pig fat. Estimated storage time of 27 seems an overestimation. For AMPA in fat, stability for maximum 15 months has been demonstrated. However, taking into account available data from metabolism and other feeding studies, residues of glyphosate and AMPA are not expected in (pig) fat. Therefore, the data for investigated matrices is considered acceptable.

It has been concluded that the analytical method used in this study is fit for purpouse (See Volume 3, Part B-5).

B.7.4.4. Fish

Not applicable (see B.7.2.5).

B.7.5. EFFECTS OF PROCESSING

Refer to separate Volume 3 B.7.5 – B.7.8.

B.7.6. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS

Refer to separate Volume 3 B.7.5 – B.7.8.

B.7.7. OTHER STUDIES

Refer to separate Volume 3 B.7.5 – B.7.8.

B.7.8. REFERENCES RELIED ON

Refer to separate Volume 3 B.7.5 – B.7.8.