Glyphosate & its IPA-, K-, NH4- and DMA salts

Herbicide

Application for Renewal of Approval (AIR 2) according to Commission Regulation (EC) N° 1141/2010

ANNEX II
Summary documentation, TIER II

Document M:

Point 6: Metabolism and residue data

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IIA 6 Metabolism and residue data

IIA 6.1 Stability of residues

IIA 6.1.1 Stability of residues during storage of samples

The conclusions from the 2001 EU evaluation as well as the supporting studies still apply but are supplemented with studies to fulfil the current data requirements.

<u>Conclusions from the 2001 EU evaluation : Glyphosate Monograph</u>
The stability of spiked crop samples (exogenous fortifications) has been determined over a period of 0 to 31-32 months while the stability of endogenous (plant incorporated) residues has been determined over a period of 2 to 5 years in frozen storage ([redacted] 1991 RIP95-01332).

Endogenous residues of both glyphosate and AMPA are proven to be stable in the seven crop commodities included in the study (corn grain, soy forage, sorghum stover, clover, tomatoes, alfalfa seed and potatoes) after 2-5 years in frozen storage. Although the exogenous AMPA residues show some decline over the course of this stability study, the decline is minimal. Coupled with the high stability of endogenous residues of AMPA, these results show that both glyphosate and AMPA are stable in different crop types (water, oil, protein, and starch containing and dry material) in frozen storage.

The stability of exogenous residues of glyphosate and AMPA in animal commodities has been demonstrated ([redacted] 1998, RIP95-01253). Samples of swine, cow, and chicken fat, muscle, liver and kidney along with cow milk and chicken eggs were fortified with a solution of glyphosate and AMPA and stored frozen at ≤-20 °C. Samples were stored for up to 13 to 32 months. The data indicate a slight decrease in the glyphosate and AMPA residues for most matrices over the course of the study. However, these results show that losses due to instability have a negligible effect on the results of the feeding studies on swine, dairy cow and laying hens.

<u>Conclusions from the 2001 EU evaluation : Glyphosate-Trimesium Monograph</u>
The stability of glyphosate and AMPA residues in representative raw agricultural commodities stored at -20 °C, including sorghum grain, soy bean, soy bean straw, and wheat grain, has been demonstrated ([redacted] 1989, RIP95-00028). Samples were removed for analysis at intervals up to 2 years after fortification. In addition, sorghum grain was also analysed at 4 years after fortification. Analysis showed that glyphosate and AMPA were stable in all samples taken. A further storage stability study ([redacted] 1995, RIP96-00003) on samples of wheat and oats processed products including grain, groats, glumes, flakes, bread, and flour confirms that incurred residues of glyphosate are stable over periods of up to 20 months.

Storage stability of glyphosate and AMPA has been demonstrated in muscle, liver, kidney, eggs and milk for a minimum of 689 days (1.9 years). ([redacted] 1987, RIP95-00024 and [redacted] 1987, RIP95-00025).

<u>Studies added to complete the EU-renewal Submission</u>
There are additional crop storage stability studies that were not included in the 2001 EU evaluation but are needed to fulfil the current EU data requirements (supplementary or confirmatory data). These studies are summarized below.

In these studies, samples of soybean seed and straw, pasture grass, wheat, rye and barley grain and straw, maize (corn), sugar beet root and leaves and oranges were spiked with glyphosate and AMPA and stored at a temperature of -10°C to -20°C over a period of one year and up to 3.5 years.
Glyphosate and AMPA were stable for at least 6 months in the soybean seeds, 12 months in pasture grass and at least 13 months in soybean straw. In wheat and rye grain and straw glyphosate was stable for at least 3.5 years and AMPA was stable for at least 288 days in grain and at least 190 days in straw.

Glyphosate and AMPA residues in barley (grain and straw), maize and sugar beet were stable for at least 18 months. In oranges, glyphosate and AMPA were stable for at least 2 years.

In addition, samples of beans, oilseed rape and linseed were spiked with glyphosate and stored at about -18 °C. The residues in all matrices were stable for about 18 months.

Together these studies provide new data on stability of glyphosate and AMPA in acidic crop commodities (oranges), and supplement the previous data on stability in oil seeds, cereals, root crops, forage and straw.

Annex point	Author(s)	Year	Study title
IIA 6.1.1/01	[redacted]	1993	Determination of glyphosate in soybean raw agricultural commodities (RAC) stability report [redacted] Study No.: 91210 [redacted] Doc. No.: 455 GLY (June 1993) GLP: yes unpublished

Guideline: US EPA Pesticides Assessment Guidelines (171-4)
Subdivision O of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

Deviations: None

Dates of experimental work: October 1991-June 1993

Study owner: Cheminova

Executive Summary

The storage stability of glyphosate and AMPA (aminomethylphosphonic acid) in soybean seed and straw was investigated. Samples were spiked with the test items at a concentration level of 1.0 mg/kg each and stored at <−10°C for about one year. Over the period tested, glyphosate and AMPA were stable 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 were spiked 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 for soybean straw and at four samplings over a period of 183 days 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 [redacted] method [redacted]-045-91 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.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1.1-1. The analytical results used for the stability calculation were corrected for recoveries. Glyphosate and AMPA in soybean seeds were stable for about 6 months and in soybean straw for about 13 months.

Table 6.1.1-1: Storage stability of glyphosate and AMPA in soybean seeds and straw

Matrix / Analyte	Days	Residues (mg/kg) Single values		Average	Corrected[1]	Concurrent recoveries (mg/kg)	% Change from initial analysis[2]
Seeds							
Glyphosate	5	0.76	0.74	0.75	1.15	0.654	
	14	0.74	0.70	0.72	1.02	0.704	-11
	45	0.53	0.75	0.64	1.28	0.500	+11
	183	0.86	0.80	0.83	1.31	0.636	+14
AMPA	5	0.79	0.78	0.78	1.13	0.692	-2
	14	0.74	0.81	0.77	0.89	0.865	-23
	45	0.67	0.77	0.72	1.37	0.524	+19
	183	0.73	0.70	0.72	1.00	0.721	-13
Straw							
Glyphosate	0	0.85	0.71	0.78	1.06	0.736	
	15	0.76	0.61	0.68	0.85	0.798	-20
	44	0.85	0.80	0.82	1.21	0.675	+14
	102	0.71	0.63	0.67	0.90	0.747	-15
	300	0.67	0.77	0.72	0.79	0.914	-25
	398	0.72	0.79	0.75	1.06	0.707	0
AMPA	0	0.80	0.73	0.77	1.04	0.741	-2
	15	0.63	0.70	0.66	0.87	0.757	-18
	44	0.69	0.68	0.68	1.05	0.649	-1
	102	0.52	0.55	0.53	0.76	0.695	-28
	300	0.41	0.56	0.49	0.56	0.879	-47
	398	0.61	0.52	0.56	0.86	0.648	-19

[1]Residue values corrected for concurrent recovery
[2]Percent change in corrected residue value from initial analysis (Day 5 for seeds and Day 0 for straw)

III. CONCLUSIONS

Over the period tested, glyphosate and AMPA were stable 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.

Annex point	Author(s)	Year	Study title
IIA 6.1.1/02	[redacted]	1993	Determination of glyphosate in pasture grasses stability report [redacted] Study No.: 91212 [redacted] Doc. No.: 456 GLY (June 1993) GLP: yes unpublished
Guideline:			US EPA Pesticides Assessment Guidelines (171-4) Subdivision O of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)
Deviations:			None
Dates of experimental work:			October 1991-June 1993
Study owner:			Cheminova

Executive Summary

The storage stability of glyphosate and AMPA in pasture grasses was investigated. Samples were spiked with the test items at a concentration level of 1.0 mg/kg each and stored at <−10°C for about one year. Over the period tested, glyphosate and AMPA were stable in pasture grasses (representative of high water content fodder crops) for at least 12 months when stored ≤-10°C.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

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

2. **Test Commodity:**

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

B. STUDY DESIGN

1. Test procedure

The storage stability of glyphosate and AMPA in pasture grasses was investigated.
Duplicate samples were spiked 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.
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.
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 [redacted] method [redacted]-045-91 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.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1.1-2. The analytical results used for the stability calculation were corrected for recoveries. Glyphosate and AMPA in pasture grasses were stable for about 12 months.

Table 6.1.1-2: Storage stability of glyphosate and AMPA in pasture grasses

Matrix / Analyte	Days	Residues (mg/kg) Single values		Average	Corrected[1]	Concurrent recoveries (mg/kg)	% Change in corrected residues[2]
Pasture grasses							
Glyphosate	6	0.78	0.93	0.86	0.99	0.861	
	10	1.0	1.1	1.06	1.02	1.04	+3
	19	0.92	0.91	0.92	1.01	0.906	+2
	51	0.83	0.70	0.77	0.78	0.976	-21
	95	0.76	0.64	0.70	0.84	0.833	-15
	187	0.70	0.77	0.74	0.93	0.793	-6
	362	0.85	0.76	0.81	1.04	0.722	+5
AMPA	6	0.63	0.55	0.59	0.73	0.805	-
	10	0.71	0.73	0.72	0.79	0.909	+8
	19	0.69	0.69	0.69	0.86	0.800	+18
	51	0.69	0.64	0.66	0.90	0.741	+23
	95	0.54	0.63	0.59	0.74	0.797	+1
	187	0.55	0.65	0.60	0.81	0.737	+11
	362	0.76	0.73	0.74	0.96	0.777	+32

[1]Residue values corrected for concurrent recovery
[2]Percent change in corrected residue value from Day 6 analysis

III. CONCLUSIONS

Over the period tested, glyphosate and AMPA were stable in pasture grasses (representative of high water content fodder crops) for at least 12 months when stored ≤-10°C.

Annex point	Author(s)	Year	Study title
IIA 6.1.1/03	[redacted]	1995	Storage Stability of Glyphosate and AMPA in Wheat Grain and Straw and in Rye Grain and Straw [redacted] Study No.: 303614 [redacted] Doc. No.: 325 GLY 30 November 1995 GLP: yes unpublished

Guideline:	'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:	None
Dates of experimental work:	December 1991-August 1995
Study owner:	Cheminova

Executive Summary

The storage stability of glyphosate and AMPA in wheat grain and straw and in rye grain and straw was investigated. Samples were spiked 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 until analysis for about 3.5 years. Glyphosate is stable in wheat and rye matrices (grain and straw) (representative of high starch content cereal crops) for at least 3.5 years when stored under deep freeze conditions. AMPA in cereal grain is stable for at least 288 days and in straw for at least 190 days 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 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 until analysis.
At six samplings over a period of 1349 days the samples were tested for the stability of glyphosate and AMPA.

2. Description of analytical procedures

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.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1.1-3. The analytical results used for the stability calculation were not corrected for recoveries

Table 6.1.1-3: Storage stability of glyphosate and AMPA in grain and straw of wheat and rye

Matrix	Storage time (days)	Residues (mg/kg)	Sample recoveries (%)	Concurrent recoveries (%)	% Change from initial analysis[1]
Wheat grain					
Glyphosate	0	0.76	76	-	--
	190	0.80	80	-	+5
	288	0.82	82	74	+8
	643	0.65	65	57	-14
	1349	0.69	69	72	-9
AMPA	0	0.39	79	-	--
	190	0.41	83	-	+5
	288	0.41	81	80	+5
	643	0.28	55	64	-28
	1349	0.23	46	76	-41
Wheat straw					
Glyphosate	0	0.87	87	70	--
	190	0.86	86	86	-1
	288	0.80	80	82	-8
	643	0.73	73	75	-16
	1349	1.08	108	108	+24
AMPA	0	0.36	72	79	--
	190	0.41	83	86	+14
	288	0.32	63	76	-11
	643	0.25	49	68	-31
	1349	0.29	57	89	-19
Rye grain					
Glyphosate	0	0.71	71	76	--
	190	0.88	88	107	+24
	288	0.88	88	84	+24
	643	0.75	75	73	+6
	1349	0.68	68	90	-4
AMPA	0	0.40	80	73	--
	190	0.40	79	89	0
	288	0.40	79	79	0
	643	0.33	66	66	-18
	1349	0.27	53	91	-33
Rye straw					
Glyphosate	0	0.85	85	94	--
	190	0.96	96	-	+13
	288	0.78	78	82	-8
	643	0.60	60	82	-29
	1349	0.95	95	114	+12
AMPA	0	0.43	86	101	--
	190	0.40	79	-	-7
	288	0.30	59	71	-30
	643	0.23	45	75	-47
	1349	0.20	39	92	-53

[1]Percent change in uncorrected residue value from Day 0 analysis

III. CONCLUSIONS

Glyphosate is stable in wheat and rye matrices (grain and straw) (representative of high starch content cereal crops) for at least 3.5 years when stored under deep freeze conditions. AMPA in cereal grain is stable for at least 288 days and in straw for at least 190 days under freezer conditions.

Annex point	Author(s)	Year	Study title
IIA 6.1.1/04	[redacted]	1997	Determination of the Storage Stability of Glyphosate in Beans, Oilseed Rape and Linseed [redacted] Study No.: [redacted]-94/13882-00 [redacted] Doc. No.: 394 GLY 13 February 1997 GLP: yes unpublished

Guideline: US EPA Pesticides Assessment Guidelines (171-4)
Subdivision O of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

Deviations: None

Dates of experimental work: December 1994-June 1996

Study owner: Cheminova

Executive Summary

The storage stability of glyphosate in beans, oilseed rape and linseed stored at about ≤−18°C was investigated. The samples were spiked with glyphosate at a concentration level of 2.6 mg/kg, 0.6 mg/kg and 5.6 mg/kg, respectively. In all matrices investigated (representative of high oil content oilseed crops and high water content fresh legume vegetables), glyphosate residues were stable for about 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:	Legume vegetable	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.
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 the samples were tested for the stability of glyphosate.

2. Description of analytical procedures

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.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1.1-4. The analytical results used for the stability calculation were not corrected for recoveries. Glyphosate residues were stable for at least 18 months.

Table 6.1.1-4: Storage stability of glyphosate in plant matrices

Matrix	Days	Concentration in stored samples (mg/kg)		Recovery instored samples (%)		Concurrent recoveries (mg/kg)	% Change from initial analysis[1]
		nominal values	actual value	single values	mean value		
Beans	0	2.60	2.30	89	90	81	--
		2.60	2.34	90			
	174	2.60	2.45	94	92	89	+2
		2.59	2.33	90			
	371	2.60	2.84	109	108	79	+20
		2.60	2.76	106			
	456	2.60	2.70	104	105	84	+17
		2.60	2.75	106			
	551	2.59	2.56	99	98	97	+9
		2.60	2.51	96			
Oilseed rape	0	0.608	0.584	96	87	78	--
		0.609	0.470	77			
	174	0.610	0.529	87	88	85	+1
		0.607	0.531	88			
	371	0.606	0.564	93	95	68	+9
		0.608	0.589	97			
	456	0.610	0.633	104	110	83	+26
		0.609	0.698	115			
	551	0.609	0.590	97	96	102	+10
		0.608	0.580	95			
Linseed	0	5.68	5.34	94	93	86	--
		5.67	5.18	91			
	182	5.68	5.17	91	88	96	-5
		5.69	4.82	85			
	371	5.68	4.98	88	97	74	+4
		5.67	6.03	106			
	456	5.69	6.22	109	106	87	+14
		5.65	5.82	103			
	551	5.66	5.05	89	89	87	-4
		5.69	5.06	89			

[1]Percent change in mean uncorrected percent recovery value from Day 0 analysis

III. CONCLUSIONS

Over the period tested for at least 18 months, glyphosate residues were stable in beans (representative of high water content fresh legume vegetables), oilseed rape and linseed (representative of high oil content oilseed crops) when stored ≤-18°C.

Annex point	Author(s)	Year	Study title
IIA 6.1.1/05	[redacted]	2010	Storage stability of residues of Glyphosate and AMPA in various plant materials [redacted] Study No.: [redacted]-0707 11 March 2010 GLP: yes unpublished

Guideline: EU Directive 91/414/EEC as amended by 96/46/EC 4.2.1
EU Commission Working Document 1607/VI/97, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97)
US EPA Residue Chemistry Test Guidelines, OPPTS 860.1380, Storage Stability Data

Deviations: None

Dates of experimental work: 22 May 2008- 8 December 2009

Study owner: Feinchemie Schwebda GmbH

Executive Summary

The storage stability of glyphosate and AMPA in barley (grain and straw), maize (corn) and sugar beet (root and leaves) stored at about ≤−18°C was investigated. The samples were spiked with glyphosate and AMPA at a concentration level of 1 mg/kg, respectively. In all matrices investigated, glyphosate and AMPA residues were stable for at least 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 may, Beta vulgaris*
Crop parts(s) or processed commodity:	Barley (grain and straw), maize (corn), 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 (corn) and sugar beet (root and leaves) stored at about ≤−18°C was investigated.
Samples were spiked with the test items at a concentration level of 1.0 mg/kg for both glyphosate and AMPA. The samples were stored at about −18°C until analysis.
At four samplings over a period of 18 months the samples were tested for the stability of glyphosate and AMPA,.

2. Description of analytical procedures

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.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1.1-5.

Table 6.1.1-5: Storage stability of glyphosate and AMPA in various plant matrices

Matrix	Storage time (months)	Glyphosate			AMPA		
		Mean recoveries (%)	Mean recoveries corrected (%)	Recoveries in freshly fortified samples (%)	Mean recoveries (%)	Mean recoveries corrected (%)	Recoveries in freshly fortified samples (%)
Barley grain	0	74	100	-	97	100	-
	6	74	101	73	83	111	75
	12	71	101	70	72	88	82
	18	70	99	71	68	96	71
Barley straw	0	74	100	-	75	100	-
	6	66	92	72	53	75	71
	12	68	87	78	36	42	85
	18	72	86	84	77	100	77
Maize corn	0	80	100	-	94	100	-
	6	66	87	76	82	106	77
	12	79	100	79	79	93	85
	18	73	96	76	86	108	80
Sugar beet root	0	84	100	-	90	100	-
	6	89	95	94	88	110	80
	12	75	95	79	70	89	79
	18	81	114	71	67	91	74
Sugar beet root	0	84	100	-	87	100	-
	6	72	90	80	70	86	81
	12	67	96	70	56	69	81
	18	68	85	80	74	101	73

III. CONCLUSIONS

Glyphosate and AMPA are stable in barley (grain and straw), maize (corn) and sugar beet (root and leaves) for at least 18 months when stored under deep freeze conditions.

Annex point	Author(s)	Year	Study title
IIA 6.1.1/06	[redacted]	2012	Storage stability of residues of Glyphosate and AMPA in citrus fruit [redacted] Study No.: [redacted]-09-234 1 February 2012 GLP: yes unpublished

Guideline:	EU Directive 91/414/EEC as amended by 96/46/EC 4.2.1 EU Commission Working Document 1607/VI/97, Appendix H: Storage Stability 7032/VI/95 rev. 5 (22/Jul/97)
Deviations:	None
Dates of experimental work:	18 June 2009- 21 June 2011
Study owner:	Glyphosate Task Force (GTF)

Executive Summary

The storage stability of glyphosate and AMPA in citrus fruit (oranges) stored at about ≤−18°C was investigated. The samples were spiked with glyphosate and AMPA at a concentration level of 0.5 mg/kg, respectively. In all matrices investigated, glyphosate and AMPA residues were stable for at least 24 months.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

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

2. Test Commodity:

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

B. STUDY DESIGN

1. Test procedure

The storage stability of glyphosate and AMPA in orange (whole fruit) stored at about ≤−18°C was investigated.
Samples were spiked with the test items at a concentration level of 0.5 mg/kg glyphosate and AMPA, respectively. The samples were stored at about −18°C 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.

2. Description of analytical procedures

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 labeled glyphosate and AMPA internal standards then passed through solid phase extraction media for final cleanup. 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.

II. RESULTS AND DISCUSSION

The results are presented in Table 6.1.1-6.

Table 6.1.1-6: Storage stability of glyphosate and AMPA in various plant matrices

Matrix	Storage time (months)	Glyphosate			AMPA		
		Mean recoveries (%)	Mean recoveries corrected (%)	Recoveries in freshly fortified samples (%)	Mean recoveries (%)	Mean recoveries corrected (%)	Recoveries in freshly fortified samples (%)
Orange whole fruit	0	88.2	100.0	-	86.9	100.0	-
	30	93.2	102.3	91.1	90.7	98.6	92.0
	97	91.2	102.1	89.3	89.3	100.0	89.3
	196	92.1	104.7	88.0	89.4	101.1	88.4
	273	87.2	101.3	86.1	87.2	100.5	86.8
	372	93.2	105.8	88.1	91.4	106.7	85.7
	546	89.2	100.5	88.8	87.6	102.8	85.2
	727	88.5	103.3	85.7	84.2	91.6	91.9

III. CONCLUSIONS

There was essentially no change in corrected recovery for glyphosate up to 24 months and for AMPA up to 18 months, At 24 months, AMPA corrected recovery was still >90%. Glyphosate and AMPA are stable in oranges (whole fruit) for at least 24 months when stored under deep freeze conditions.

IIA 6.1.2 Stability of residues in sample extracts

The sample extract stability was investigated as part of the method validation for the analytical method for the determination of glyphosate in plant materials and is included in the analytical methods section. It was found to be stable.

IIA 6.2 Metabolism, distribution and expression of residues

IIA 6.2.1 In plants, in at least three crops representative of the different categories of crop.

Conclusions from the 2001 EU evaluation: Glyphosate Monograph

Studies were included in the 2001 glyphosate EU evaluation covering metabolism of glyphosate in both conventional crops and glyphosate tolerant crops.

Conventional Crop Metabolism

The metabolism and distribution of ^{14}C-glyphosate in more than 20 varieties of conventional crops was reviewed in the 2001 EU glyphosate evaluation and is summarised in the glyphosate monograph. Application methods that were investigated include application to soil and hydroponic solutions, applications to stems and trunks, and foliar applications of glyphosate to conventional crops. The conclusions from the 2001 EU evaluation as well as the supporting studies still apply.

The crops studied and the types of application used are listed in the Table 6.2.1-1 below along with the reference from the 2001 glyphosate monograph.

Table 6.2.1-1: Metabolism Studies of Glyphosate in Crops

Crop	Application Method	Reference
Citrus mitis	Directed soil application, foliar	[redacted] 1975, RIP95-01194
Walnut, Almond, Pecan	Directed soil application, foliar	[redacted] 1976, RIP95-01196
Apple	Directed soil application, foliar, trunk	[redacted] 1974, RIP95-01190
Grapes	Directed soil application, foliar, trunk, hydroponics	[redacted] 1974, RIP95-01191
Potatoes	Preemergence soil, foliar	[redacted] 1975, RIP95-01193
Soybeans, Cotton, Wheat, Maize	Preemergence soil, hydroponics	[redacted], 1973, RIP96-00099
Barley, Oat, Rice, Sorghum	Preemergence soil, hydroponics	[redacted] 1974, RIP95-01189
Sugar Beets	Preemergence soil, foliar	[redacted] 1976, RIP95-01195
Sugarcane	Hydroponics, foliar	[redacted] 1975, RIP95-01198
Coffee Plants	Directed soil application, foliar, trunk, hydroponics	[redacted] 1975, RIP95-01192
Pasture crops: Fescue, Alfalfa, Clover, Grass	Preemergence soil, foliar	[redacted] 1976, RIP95-01197

Uptake and Translocation

Soybeans, cotton, wheat, maize, barley, oats, rice, sorghum, potatoes, sugar beets, and pasture crops were treated with a pre-emergence application of glyphosate at application rates equivalent to 4.48 kg/ha.

For root uptake from the soil in apple trees, grapes, coffee plants, citrus, walnut, almond, and pecan trees, glyphosate was applied to the soil surface of pots containing the emerged crops, while shielding the foliage, at glyphosate application rates of between 2.24 kg/ha and 5.07 kg/ha.
In all cases, maximum uptake of radioactivity into plants grown in soil treated with ^{14}C-glyphosate was less than 1% of the total applied radioactivity, demonstrating that very little of the applied glyphosate is taken up from the soil.
To simulate uptake of glyphosate through trunks and stems following post-emergence directed spray applications in orchard and vineyards, a formulated solution of ^{14}C-glyphosate was directly applied to trunks and stems of apple trees, grapes, and coffee plants. In all cases less than 3% of the applied radioactivity was incorporated into the plants. These results show that very little of the applied glyphosate will be present as a residue in orchard and vineyard crops as a result of inadvertent applications of glyphosate to trunks and stems following post-emergence directed spray treatments.
The distribution and metabolism of glyphosate following foliar applications has been investigated in apple trees, grapes, coffee plants, potatoes, citrus, sugar beets, walnut, almond, and pecan trees by application of subherbicidal levels of a formulated solution of ^{14}C-glyphosate to the surfaces of leaves. In all cases glyphosate was found to be rapidly and extensively translocated throughout the plant.

Metabolic Pathway
The majority of the plant-contained ^{14}C-radioactivity was released by aqueous extraction in almost all cases. Glyphosate was the major ^{14}C-component of the extract, and AMPA was the major ^{14}C-containing metabolite. Glyphosate was almost always present in higher amounts than AMPA, except in corn foliage following hydroponic application of ^{14}C-glyphosate, where glyphosate and AMPA were present at comparable levels. In addition to glyphosate and AMPA several minor metabolites that typically constituted less than 1% of the TRR were also occasionally detected. Several of these minor metabolites were identified, as N-methylaminomethylphosphonic acid (N-methyl-AMPA), methylphosphonic acid, and N-methyl-glyphosate. No significant metabolites other than AMPA were observed.

Glyphosate Tolerant Crop Metabolism
While glyphosate-tolerant crop uses are not being included in the current dossier, the original monograph included four metabolism studies in glyphosate-tolerant crops. The crops all received over-the-top, direct foliar applications of glyphosate during the growing stages of the crop. The crops studied, the tolerance mechanism, and reference from the 2001 glyphosate monograph are listed in Table 6.2.1-2.

Table 6.2.1-2: Metabolism Studies of Glyphosate in Glyphosate-Tolerant Crops

Crop	Glyphosate Tolerance Mechanism	Reference
Maize	EPSPS and GOX	[redacted] 1995, RIP97-00618
Oilseed rape	EPSPS and GOX	[redacted] 1994, RIP98-00118
Cotton	EPSPS	[redacted] 1997, RIP97-00619
Soybeans	EPSPS	[redacted] 1994, RIP98-00117

Two of the studies were in crops (soybean and cotton) that included only CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) conferring glyphosate tolerance, and two of the studies were in crops (maize and oilseed rape) that included both CP4 EPSPS and GOX (glyphosate oxidoreductase), which metabolizes glyphosate to AMPA.
The studies on metabolism of glyphosate in tolerant maize and oilseed rape plants revealed a rapid metabolism of glyphosate to AMPA caused by the presence of GOX. In contrast, cotton and soybean did not contain GOX and thus were similar to the non-tolerant plants, and metabolised glyphosate only slowly to AMPA.

Conclusions from the 2001 EU evaluation: Glyphosate-Trimesium Monograph

Studies on the metabolism of ^{14}C-glyphosate (labelled in the glyphosate anion portion of the molecule and applied as the trimesium salt) were summarised in the glyphosate trimesium monograph. The studies included: directed application to soil in citrus, directed application to soil and intentional overspray in grapes, pre-emergence application to soil in soybean, and preharvest application in wheat, and are listed in the Table 6.2.1-3.

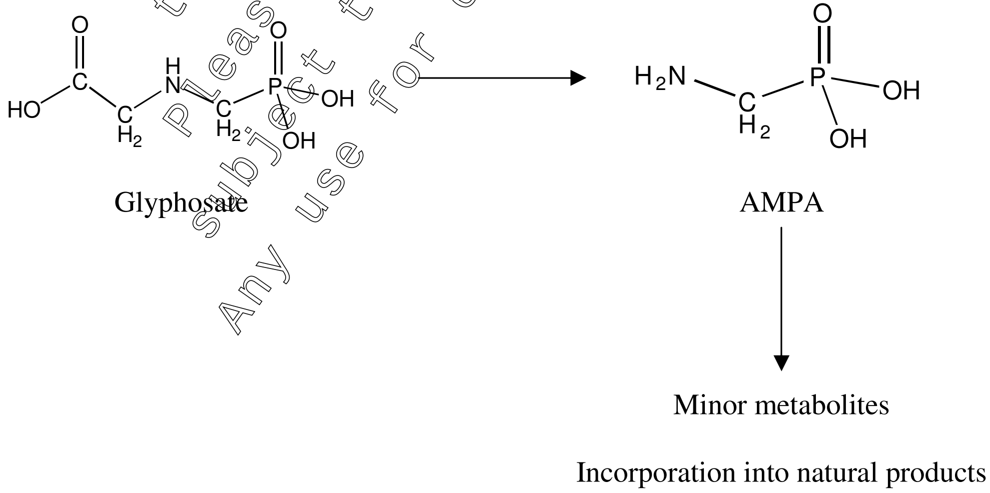
Table 6.2.1-3: Metabolism Studies of Glyphosate in Crops Following Application of Glyphosate-Trimesium

Crop	Application Method	Reference
Citrus	Directed soil application	[redacted] 1985, RIP95-00011
Grapes	Directed soil application, foliar overspray	[redacted] 1990, RIP95-00017, [redacted] 1991, RIP95-00012
Soybeans	Preemergence soil	[redacted] 1992, RIP95-00015
Wheat	Preharvest	[redacted] 1989, RIP95-00014

The studies demonstrated minimal residues of glyphosate or AMPA in plants following application to soil, either prior to emergence or as a directed application around the crop. When ^{14}C-glyphosate was applied directly to the crop, as the preharvest application in wheat or deliberate overspray in grapes, the majority of the residues remained as glyphosate. The only significant metabolite was AMPA. It was usually a minor component of the TRR, but in several of the soybean commodities, AMPA residues exceeded those of glyphosate. No other significant metabolites were identified.

Summary

The results of all the numerous plant uptake and metabolism studies demonstrate that glyphosate is slowly metabolised in plants to AMPA. With only a few exceptions (some soybean commodities and hydroponically-grown maize forage where AMPA levels were comparable to or greater than glyphosate levels), glyphosate is the major compound present in plant tissues. In all cases, AMPA accounts for less than 27% of the radioactive residues, and typically is less than 10%. With the exception of AMPA, no other metabolites of glyphosate are detected that account for greater than 5% of the total radioactive residues.



IIA 6.2.2 Poultry

The conclusions from the 2001 EU evaluation as well as the supporting studies still apply.

Conclusions from the 2001 EU evaluation : Glyphosate Monograph
Two different studies on laying hens were included in the original glyphosate monograph to determine the absorption, distribution, metabolism and excretion in livestock.
In one study ([redacted] 1988, RIP95-01205; [redacted] 1988, RIP95-01206), animals were dosed with 9:1 ratio of glyphosate and aminomethylphosphonic acid, AMPA, which is the primary plant metabolite of glyphosate. The hens were dosed at a level corresponding to a total dietary concentration of 120 and 400 mg/kg.
For the other study ([redacted] 1994, RIP95-01208), hens were dosed with glyphosate alone at a level corresponding to a total dietary concentration of 200 mg/kg.
Glyphosate and AMPA were rapidly excreted mainly in the faeces and urine, primarily as unchanged parent compound, resulting in low residue levels in edible tissues and eggs. There was minimal metabolism of glyphosate to AMPA, as clearly demonstrated in the study conducted with glyphosate alone. Metabolites resulting from the degradation of glyphosate and AMPA in tissues were either insignificant or entirely absent.

Conclusions from the 2001 EU evaluation: Glyphosate-Trimesium Monograph
An animal metabolism study in hens was included in the glyphosate trimesium monograph ([redacted] 1994, RIP95-00020). The animals were dosed with ^{14}C-glyphosate in the form of the trimesium salt at a level equivalent to 62-64 mg/kg of glyphosate acid in the diet.
Glyphosate-trimesium radiolabelled in the glyphosate portion was rapidly and nearly completely excreted by hens. The radioactive residues found in tissues and eggs consist mainly of glyphosate and the metabolite AMPA. In addition, a part of the radioactivity was incorporated into naturally occurring products.

Summary
Results from all three sets of animal metabolism studies are consistent. Both glyphosate and AMPA were rapidly and extensively excreted after dosing in hens. Tissue levels were generally low, and AMPA was the only significant metabolite present. Other metabolites resulting from degradation of glyphosate and AMPA were either insignificant or absent.

IIA 6.2.3 Lactating ruminants (goat or cow)

The conclusions from the 2001 EU evaluation as well as the supporting studies still apply.

Conclusions from the 2001 EU evaluation: Glyphosate Monograph
Two different studies on lactating goats were included in the original glyphosate monograph to determine the absorption, distribution, metabolism and excretion in livestock.
In one study ([redacted] 1988, RIP95-01203; [redacted], 1988, RIP95-01204), animals were dosed with a 9:1 ratio of glyphosate and aminomethylphosphonic acid, AMPA, which is the primary plant metabolite of glyphosate. The goats were dosed at a level corresponding to a total dietary concentration of 120 mg/kg.
For the other study ([redacted] 1994, RIP95-01207), goats were dosed with glyphosate alone at a level corresponding to a total dietary concentration of 200 mg/kg.
Glyphosate and AMPA were rapidly excreted mainly in the faeces and urine, primarily as unchanged parent compound, resulting in low residue levels in edible tissues and milk. There was minimal metabolism of glyphosate to AMPA, as clearly demonstrated in the study conducted with glyphosate alone. Metabolites resulting from the degradation of glyphosate and AMPA in tissues were either insignificant or entirely absent.

Conclusions from the 2001 EU evaluation: Glyphosate-Trimesium Monograph

An animal metabolism study in goats was included in the glyphosate trimesium monograph ([redacted], 1994, RIP95-00022). The animals were dosed with ^{14}C-glyphosate in the form of the trimesium salt at a level equivalent to 62-64 mg/kg of glyphosate acid in the diet.

In goats, the glyphosate portion of glyphosate-trimesium is rapidly excreted mainly in the faeces. Tissue residues were generally low with the highest value reached in the kidneys. The radioactive residues found in tissues consisted mainly of glyphosate itself and the metabolite AMPA. The major radioactive residues in milk were natural products in the form of lactose, triglycerides and protein. Lactose and triglycerides constituted over 45% TRR in milk, while material associated with post extraction milk solids comprised 20% TRR, which is consistent with natural incorporation of radiocarbon into proteins. Residues of glyphosate did not accumulate in fat, tissues or milk.

Summary

Results from all three sets of animal metabolism studies are consistent. Both glyphosate and AMPA were rapidly and extensively excreted after dosing in goats. Tissue levels were generally low, and AMPA was the only significant metabolite present. Other metabolites resulting from degradation of glyphosate and AMPA were either insignificant or absent.

IIA 6.2.4 Pigs

No metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly.

IIA 6.2.5 Nature of residue in fish

This OECD point is not covered by or part of an EC point according to current data requirements. Hence data / documents do not need to be submitted.

IIA 6.2.6 Chemical identity (emphasis on impurities of residual concern)

This OECD point is not covered by or part of an EC point according to current data requirements. Hence data / documents do not need to be submitted.

IIA 6.3 Residue trials for crops or plant products used as food or feed in which use is proposed or where residues from soil can be taken up

Numerous supervised residue trials have been conducted to establish MRLs for glyphosate. In cases where residues resulting from different glyphosate formulations have been compared in side by side field trials, no differences were found. Thus, it is possible to extrapolate from data obtained on the active substance in accordance with the requirements of Annex II 6.3.

Good agricultural practices for the application of glyphosate can be grouped into six categories based on the types of applications:

a. Pre-harvest broadcast applications yielding detectable glyphosate residues that require establishment of MRLs.
b. Applications prior to crop emergence that result in undetectable glyphosate residues.
c. Grassland applications.
d. Directed spray applications underneath the foliage of existing crops (post-directed applications).
e. Selective equipment applications (e.g. recirculating sprayer and wiper applicator applications).
f. Forestry applications.

In-crop, pre-harvest applications are currently approved in various European Union Member States for cereals (wheat, barley, oats, and rye), pulses (beans and peas), oil seed crops and forage grasses. Maximum glyphosate residues in grain and seed resulting from pre-harvest applications according to approved uses reached 20 mg/kg.

In-crop selective equipment or between-the-row applications of glyphosate may also result in detectable residues in crops. For example, an MRL of 1 mg/kg was set for maize that has received inter-row selective applications.

A major method of glyphosate application is as a pre-plant or pre-emergence treatment that does not result in significant residues.

EU MRLs were adopted and included in Annex II of Regulation (EC) No 396/2005, which adequately support claimed uses (**COMMISSION REGULATION (EC) No 839/2008 of 31 July 2008 and COMMISSION REGULATION (EC) No 149/2008 of 29 January 2008**).

Upon review of the database supporting the current uses, it was determined that while there were numerous residue studies of pre-plant and pre-emergence applications in a variety of crops, many were older, non-GLP studies and did not always represent the current GAP. In order to provide an up-to-date set of studies, a representative set of trials was recently conducted. The glyphosate and AMPA residues for all trials of all crops were below the LOQ (<0.05 mg/kg), and most were below the LOD (<0.015 mg/kg) and therefore support the existing MRLs of 0.1 mg/kg for pre-plant/pre-emergence uses.

The crops selected for the residue trials included:

Crop Group	**Crops Used in Residue Trial**
Root and tuber vegetables	Potatoes and carrots
Bulb vegetables	Onions
Fruiting vegetables	Tomatoes
Cucurbits	Cucumbers and zucchini
Brassica	Cauliflower and cabbage
Leafy vegetables	Head and leaf lettuce
Stem vegetables	Leeks
Sugar plants	Sugar beets

In the following sections, the new studies are presented. They show the residue behaviour of glyphosate when the application is made pre-plant or in the pre-emergence stage of the crops.

IIA 6.3.1 Potatoes

Annex point	Author(s)	Year	Study title
IIA 6.3.1/01	[redacted]	2012	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
			[redacted]
			S11-00258
			28 February 2012
			GLP: yes
			unpublished

Guidelines:	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:	None
Dates of experimental work:	02 March 2011- 07 February 2012
Study owner:	Glyphosate Task Force (GTF)

Executive Summary

Four residue trials were conducted on potatoes treated at least 3 days after planting and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Potato tuber samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on potatoes (outdoor) during 2011 in Northern France, Germany, Southern France and Italy. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at least 3 days after planting and before crop emergence, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.17-2.37 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of potato tuber were taken by hand at BBCH 49.

The samples (potato tuber) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01, which was previously validated for potato (tuber) in [redacted] Study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from potato (tuber) fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (85-88%). Details of recovery data are shown in Table 6.3.1-2.
All trials are summarised below in Table 6.3.1-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of potato (tuber).

III. CONCLUSIONS

Four residue trials were conducted with MON 52276, containing 360 g/L glyphosate on potato in pre-emergence state, two in northern Europe and two in southern Europe. The product was applied in accordance with the proposed use pattern (slight deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of the trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of potato (tuber) sampled at BBCH 49 (commercial maturity).

Table 6.3.1-1: Residues of glyphosate and AMPA in potato (tuber) following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00258 S11-00258-01 Yes 2011	potato, Charlotte	France, Europe, North	360 SL[1]	1	2.173	1.242	00	tuber	119	< 0.015	< 0.015
S11-00258 S11-00258-02 Yes 2011	potato, Milva	Germany, Europe, North	360 SL[1]	1	2.276	1.237	n/a	tuber	138	< 0.015	< 0.015
S11-00258 S11-00258-03 Yes 2011	potato, Noisette	France, Europe, South	360 SL[1]	1	2.218	1.186	00	tuber	114	< 0.015	< 0.015
S11-00258 S11-00258-04 Yes 2011	potato, Primura	Italy, Europe, South	360 SL[1]	1	2.374	1.187	00	tuber	98	< 0.015	< 0.015

FL = formulation GS = growth stage at last application DALT = days after last treatment
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.1-2: Procedural recoveries for glyphosate and AMPA in potato (tuber)

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite		Fortification level	Recovery (%)		
				n	(mg/kg)	single value	mean	RSD
S11-00258 S11-00258-01 S11-00258-02 S11-00258-03 S11-00258-04 Yes 2011	potato	potato, tuber	glyphosate	1 1	0.05 0.50	88 88	88	-
			AMPA	1 1	0.05 0.50	85 87	86	-

IIA 6.3.2 Carrots

Annex point	Author(s)	Year	Study title
IIA 6.3.2/01	[redacted]	2012	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 [redacted] Study No.: S11-00259 28 February 2012 GLP: yes unpublished
Guidelines:			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:			None
Dates of experimental work:			14 March 2011- 09 January 2012
Study Owner:			Glyphosate Task Force

Executive Summary

Four residue trials were conducted on carrots treated at least 3 days after planting and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Samples of carrot root without leaves were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on carrots (outdoor) during 2011 in Northern France, Poland, Southern France and Spain. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at least 3 days after seeding and before crop emergence, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.08-2.49 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of carrots were taken by hand at BBCH 49.

The samples (carrot roots without leaves) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01, which was previously validated for carrot (roots) in [redacted] Study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from carrots (root without leaves) fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (90-97%). Details of recovery data are shown in Table 6.3.2-2.
All trials are summarised below in Table 6.3.2-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of carrot (root without leaves).

III. CONCLUSIONS

Four residue trials were conducted with MON 52276, containing 360 g/L glyphosate on carrots in pre-emergence state, two in northern Europe and two in southern Europe. The product was applied in accordance with the proposed use patterns (slight deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of the trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of carrot (roots without leaves) sampled at BBCH 49 (commercial maturity).

Table 6.3.2-1: Residues of glyphosate and AMPA in carrots following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00259 S11-00259-01 Yes 2011	carrot, Montdibell	France Europe, North	360 SL[1]	1	2.311	1.236	00	roots without leaves	93	< 0.015	< 0.015
S11-00259 S11-00259-02 Yes 2011	carrot, Laguna	Poland, Europe, North	360 SL[1]	1	2.295	1.189	00	roots without leaves	176	< 0.015	< 0.015
S11-00259 S11-00259-04 Yes 2011	carrot, Maestro	France, Europe, South	360 SL[1]	1	2.492	1.187	00	roots without leaves	137	< 0.015	< 0.015
S11-00259 S11-00259-05 Yes 2011	carrot, Maestro	Spain, Europe, South	360 SL[1]	1	2.080	1.231	00	roots without leaves	154	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.2-2: Procedural recoveries for glyphosate and AMPA in carrots

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite		Fortification level	Recovery (%)		
				n	(mg/kg)	single value	mean	RSD
S11-00259 S11-00259-01 S11-00259-02 S11-00259-04 S11-00259-05 Yes 2011	carrot	root without leaves	glyphosate	1 1	0.05 0.50	97 91	94	-
			AMPA	1 1	0.05 0.50	93 90	92	-

IIA 6.3.3 Bulb Onions

IIA 6.3.3/01 [redacted] 2012 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
[redacted] Study No.: S11-00260
28 February 2012
GLP: yes
unpublished

Guideline: 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: None

Dates of experimental work: 14 March 2011- 10 January 2012

Study owner: Glyphosate Task Force

Executive Summary

Four residue trials were conducted on bulb onions treated at least 3 days after planting and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Onion bulb samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on bulb onion (outdoor) during 2011 in Northern France, Poland, Spain and Bulgaria. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at least 3 days after seeding and before crop emergence, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.31-2.43 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of bulb onion were taken by hand at BBCH 49.

The samples (onion bulbs) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01, which was previously validated for onion (bulbs) in [redacted] Study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from onion bulbs (bulbs) fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (88-92%). Details of recovery data are shown in Table 6.3.3-2.
All trials are summarised below in Table 6.3.3-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of bulb onion (bulb).

III. CONCLUSIONS

Four residue trials were conducted with MON 52276, containing 360 g/L glyphosate on bulb onions in pre-emergence state, one in northern Europe and three in southern Europe. The product was applied in accordance with the proposed use patterns (slight deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of bulb onions sampled at BBCH 49 (commercial maturity).

Table 6.3.3-1: Residues of glyphosate and AMPA in bulb onions following application of MON 52276

Study No.			Application					Residues			
Trial No. GLP Year	**Crop Variety**	**Country**	**FL**	**No.**	**kg/ha (a.s.)**	**kg/hL (a.s.)**	**GS**	**Portion analysed**	**DALT (days)**	**glyphosate (mg/kg)**	**AMPA (mg/kg)**
S11-00260 S11-00260-01 Yes 2011	bulb onion, Takmark F1	France, Europe, South	360 SL[1]	1	2.311	1.236	01	bulbs	129	< 0.015	< 0.015
S11-00260 S11-00260-02 Yes 2011	bulb onion, Kristine	Poland, Europe, North	360 SL[1]	1	2.413	1.189	03	bulbs	143	< 0.015	< 0.015
S11-00260 S11-00260-03 Yes 2011	bulb onion, Eso	Spain, Europe, South	360 SL[1]	1	2.433	1.187	03	bulbs	154	< 0.015	< 0.015
S11-00260 S11-00260-04 Yes 2011	bulb onion, Stutgarten rijsen	Bulgaria, Europe, South	360 SL[1]	1	2.386	1.236	00	bulbs	149	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.3-2: Procedural recoveries for glyphosate and AMPA in bulb onions

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%) single value	mean	RSD
S11-00260 S11-00260-01 S11-00260-02 S11-00260-03 S11-00260-04 Yes 2011	bulb onion	bulbs	glyphosate	1 1	0.05 0.50	92 91	92	-
			AMPA	1 1	0.05 0.50	89 88	89	-

IIA 6.3.4 Tomato

IIA 6.3.4/01 [redacted] 2012 Determination of residues of glyphosate and AMPA after one application of MON 52276 in tomato (outdoor) at 2 sites in Hungary and Germany 2011

[redacted] Study No.: S11-00267

28 February 2012

GLP: yes

unpublished

Guideline: 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: None

Dates of experimental work: 21 April 2011- 20 January 2012

Study owner: Glyphosate Task Force

Executive Summary

Two residue trials were conducted on tomatoes treated 3 days prior to transplanting seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Tomato fruit samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Two residue trials were conducted on tomato (outdoor) during 2011 in Germany and Hungary. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at 3 days before planting the seedlings, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.28-2.30 kg glyphosate acid/ha.
In both trials in northern and southern Europe samples of tomato fruit were taken by hand at BBCH 89.

The samples (tomato fruit) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01 as validated in [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from tomato fruits fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (87-90%). Details of recovery data are shown in Table 6.3.4-2.
All trials are summarised below in Table 6.3.4-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of tomato (fruit).

III. CONCLUSIONS

Two residue trials were conducted with MON 52276, containing 360 g/L glyphosate at 3 days before planting of tomato seedlings in northern Europe and southern Europe. The product was applied in accordance with the proposed use patterns (slight deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of tomato (fruit) sampled at BBCH 89 (commercial maturity).

Table 6.3.4-1: Residues of glyphosate and AMPA in tomatoes following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00267 S11-00267-01 Yes 2011	tomato, Vanessa	Germany, Europe, North	360 SL[1]	1	2.304	1.232	n/a	fruit	93	< 0.015	< 0.015
S11-00267 S11-00267-02 Yes 2011	tomato, Claudius F1	Hungary, Europe, South	360 SL[1]	1	2.283	1.234	n/a	fruit	94	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.4-2: Procedural recoveries for glyphosate and AMPA in tomatoes

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite		Fortification level	Recovery (%)		
				n	(mg/kg)	single value	mean	RSD
S11-00267 S11-00267-01 S11-00267-02 Yes 2011	tomato	fruit	glyphosate	1 1	0.05 0.50	90 87	89	-
			AMPA	1 1	0.05 0.50	90 88	89	-

IIA 6.3.5 Cucumber and Zucchini

IIA 6.3.5/01	[redacted]	2012	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 [redacted] Study No.: S11-00261 28 February 2012 GLP: yes unpublished
Guideline:			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:			None
Dates of experimental work:			21 March 2011- 10 January 2012
Study owner:			Glyphosate Task Force

Executive Summary

Three residue trials were conducted on cucumber or zucchini treated 3 days prior to transplanting seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Cucumber and zucchini fruit samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Three residue trials were conducted on cucumber or zucchini (outdoor) during 2011 in Germany, Southern France and Italy. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at 3 days before planting the seedlings, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.22-2.55 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of cucumber or zucchini fruit were taken by hand at BBCH 89.

The samples (cucumber or zucchini fruit) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01 as validated in [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from cucumber and zucchini fruits fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (87-92%). Details of recovery data are shown in Table 6.3.5-2.
All trials are summarised below in Table 6.3.5-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of cucumber or zucchini fruit.

III. CONCLUSIONS

Three residue trials were conducted on cucumber or zucchini with MON 52276, containing 360 g/L glyphosate at 3 days before planting of cucumber or zucchini seedlings in northern Europe and southern Europe. The product was applied in accordance with the proposed use patterns (deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of cucumber or zucchini fruit sampled at BBCH 89 (commercial maturity).

Table 6.3.5-1: Residues of glyphosate and AMPA in cucumber and zucchini following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00261 S11-00261-01 Yes 2011	zucchini, Monitor	Germany, Europe, North	360 SL[1]	1	2.551	1.232	n/a	fruit	55	< 0.015	< 0.015
S11-00261 S11-00261-03 Yes 2011	zucchini, Cigal F1	France, Europe, South	360 SL[1]	1	2.222	1.234	n/a	fruit	52	< 0.015	< 0.015
S11-00261 S11-00261-04 Yes 2011	cucumber, Ekron	Italy, Europe, South	360 SL[1]	1	2.239	1.237	n/a	fruit	42	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.5-2: Procedural recoveries for glyphosate and AMPA in cucumber and zucchini

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%) single value	mean	RSD
S11-00261 S11-00261-01 S11-00261-03 S11-00261-04 Yes 2011	zucchini	fruit	glyphosate	1 1	0.05 0.50	92 88	90	-
			AMPA	1 1	0.05 0.50	90 90	90	-
	cucumber	fruit	glyphosate	1 1	0.05 0.50	90 87	89	-
			AMPA	1 1	0.05 0.50	87 90	89	-

IIA 6.3.6 Cauliflower

IIA 6.3.6/01 [redacted] 2012 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
[redacted] Study No.: S11-00263
28 February 2012
GLP: yes
unpublished

Guideline: 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: None

Dates of experimental work: 14 March 2011- 10 January 2012

Study owner: Glyphosate Task Force

Executive Summary

Four residue trials were conducted on cauliflower treated 3 days prior to transplanting seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Cauliflower inflorescence samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on cauliflower (outdoor) during 2011 in Northern France, Hungary, Italy and Bulgaria. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at 3 days before planting the seedlings, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.17-2.41 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of cauliflower inflorescence were taken by hand at BBCH 49.
The samples (cauliflower inflorescence) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01as in [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from cauliflower inflorescence fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (84-95%). Details of recovery data are shown in Table 6.3.6-2.
All trials are summarised below in Table 6.3.6-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of cauliflower inflorescence.

III. CONCLUSIONS

Four residue trials were conducted on cauliflower (outdoor) with MON 52276, containing 360 g/L glyphosate at 3 days before planting of cauliflower seedlings in northern Europe and southern Europe. The product was applied in accordance with the proposed use patterns (deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of cauliflower inflorescence sampled at BBCH 49 (commercial maturity).

Table 6.3.6-1: Residues of glyphosate in cauliflower following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00263 S11-00263-01 Yes 2011	cauli-flower, Aviso	France, Europe, North	360 SL[1]	1	2.256	1.240	n/a	inflores-cence	75	< 0.015	< 0.015
S11-00263 S11-00263-02 Yes 2011	cauli-flower, Cortes	Hungary, Europe, South	360 SL[1]	1	2.172	1.234	n/a	inflores-cence	125	< 0.015	< 0.015
S11-00263 S11-00263-03 Yes 2011	cauli-flower, Castellum	Italy, Europe, South	360 SL[1]	1	2.413	1.189	n/a	inflores-cence	80	< 0.015	< 0.015
S11-00263 S11-00263-04 Yes 2011	cauli-flower, Snowball	Bulgaria, Europe, South	360 SL[1]	1	2.332	1.234	n/a	inflores-cence	120	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.6-2: Procedural recoveries for glyphosate and AMPA in cauliflower

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%) single value	mean	RSD
S11-00260 S11-00263-01 S11-00263-02 S11-00263-03 S11-00263-04 Yes 2011	cauliflower	inflorescence	glyphosate	1 1	0.05 0.50	95 90	93	-
			AMPA	1 1	0.05 0.50	84 89	87	-

IIA 6.3.7 Head Cabbage

IIA 6.3.7/01	[redacted]	2012	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 [redacted] Study No.: S11-00262 08 March 2012 GLP: yes unpublished
Guideline:			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:			None
Dates of experimental work:			10 June 2011- 07 February 2012
Study owner:			Glyphosate Task Force

Executive Summary

Four residue trials were conducted on head cabbage treated 3 days prior to transplanting seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Head cabbage (head) samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on head cabbage (outdoor) during 2011 in Northern France, Hungary, Spain and Bulgaria. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at least 3 days before planting the seedlings, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.13-2.56 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of head cabbage (head) were taken by hand at BBCH 49.

The samples (head cabbage) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01 as validated in [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from head cabbage fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (87-91%). Details of recovery data are shown in Table 6.3.7-2.
All trials are summarised below in Table 6.3.7-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of head cabbage (head).

III. CONCLUSIONS

Four residue trials were conducted on head cabbage (outdoor) with MON 52276, containing 360 g/L glyphosate at least 3 days before planting of seedlings in northern Europe and southern Europe. The product was applied in accordance with the proposed use patterns (deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of head cabbage (head) sampled at BBCH 49 (commercial maturity).

Table 6.3.7-1: Residues of glyphosate and AMPA in head cabbage following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00262 S11-00262-01 Yes 2011	head cabbage, Padoc	France, Europe, North	360 SL[1]	1	2.558	1.236	n/a	heads	67	< 0.015	< 0.015
S11-00262 S11-00262-02 Yes 2011	head cabbage, Pandion	Hungary, Europe, South	360 SL[1]	1	2.127	1.237	n/a	heads	97	< 0.015	< 0.015
S11-00262 S11-00262-03 Yes 2011	head cabbage, Melissa	Spain, Europe, South	360 SL[1]	1	2.140	1.237	00	heads	98	< 0.015	< 0.015
S11-00262 S11-00262-04 Yes 2011	head cabbage, Kyose	Bulgaria, Europe, South	360 SL[1]	1	2.345	1.234	00	heads	99	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.7-2: Procedural recoveries for glyphosate and AMPA in head cabbage (head)

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite		Fortification level	Recovery (%)		
				n	(mg/kg)	single value	mean	RSD
S11-00262-01 S11-00262-02 S11-00262-03 S11-00262-04 Yes 2011	head cabbage	head	glyphosate	1 1	0.05 0.50	87 87	87	-
			AMPA	1 1	0.05 0.50	91 90	91	-

IIA 6.3.8 Lettuce

IIA 6.3.8/01	[redacted]	2012	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 [redacted] Study No.: S11-00264 08 March 2012 GLP: yes unpublished
Guideline:			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:			None
Dates of experimental work:			21 March 2011- 07 February 2012
Study owner:			Glyphosate Task Force

Executive Summary

Four residue trials were conducted on leaf and head lettuce treated at 3 days prior to transplanting seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Lettuce leaf and head samples were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOQ (0.05 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on leaf lettuce or head lettuce (outdoor) during 2011 in Germany, UK, Southern France and Spain. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at 3 days before planting the seedlings, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.26-2.47 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of leaf lettuce (leaves) or head lettuce (head) were taken by hand at BBCH 49.

The samples (leaf lettuce (leaves) or head lettuce (head)) were analysed for glyphosate and AMPA according to [redacted] AG-ME-1294-01 as validated in [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from leaf lettuce and head lettuce fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (84-91%). Details of recovery data are shown in Table 6.3.8-2.
All trials are summarised below in Table 6.3.8-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any of the treated and untreated samples of leaf lettuce (leaves) and head lettuce (head).

III. CONCLUSIONS

Four residue trials were conducted on leaf lettuce or head lettuce (outdoor) with MON 52276, containing 360 g/L glyphosate at least 3 days before planting of seedlings in northern Europe and southern Europe. The product was applied in accordance with the proposed use patterns (deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.05 mg/kg) present in any sample of leaf lettuce (leaves) or head lettuce (head) sampled at BBCH 49 (commercial maturity).

Table 6.3.8-1: Residues of glyphosate and AMPA in lettuce following application of MON 52276

Study No.			Application					Residues			
Trial No. GLP Year	**Crop Variety**	**Country**	**FL**	**No.**	**kg/ha (a.s.)**	**kg/hL (a.s.)**	**GS**	**Portion analysed**	**DALT (days)**	**glyphosate (mg/kg)**	**AMPA (mg/kg)**
S11-00264 S11-00264-01 Yes 2011	leaf lettuce, Kirinia	Germany, Europe, North	360 SL[1]	1	2.469	1.235	n/a	leaves	42	< 0.05	< 0.015
S11-00264 S11-00264-02 Yes 2011	leaf lettuce, Oak Leaf - Red	UK, Europe, North	360 SL[1]	1	2.258	1.188	n/a	leaves	56	< 0.05	< 0.015
S11-00264 S11-00264-03 Yes 2011	head lettuce, Sucrine	France, Europe, South	360 SL[1]	1	2.334	1.185	n/a	heads	38	< 0.015	< 0.015
S11-00264 S11-00264-04 Yes 2011	head lettuce, Cervantes	Spain, Europe, South	360 SL[1]	1	2.413	1.189	n/a	heads	48	< 0.05	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.8-2: Procedural recoveries for glyphosate and AMPA in lettuce

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%) single value	mean	RSD
S11-00264 S11-00264-01 S11-00264-02 S11-00264-03 S11-00264-04 Yes 2011	leaf lettuce	leaves	glyphosate	1 1	0.05 0.50	91 86	89	-
			AMPA	1 1	0.05 0.50	86 85	86	-
	head lettuce	head	glyphosate	1 1	0.05 0.50	93 88	91	-
			AMPA	1 1	0.05 0.50	85 84	85	-

IIA 6.3.9 Leek

IIA 6.3.9/01 [redacted] 2012 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
[redacted] Study No.: S11-00265
28 February 2012
GLP: yes
unpublished

Guideline: 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: None

Dates of experimental work: 02 March 2011- 19 January 2012

Study owner: Glyphosate Task Force

Executive Summary

Four residue trials were conducted on leek treated 3 days prior to transplanting seedlings at a target rate of 2.16 kg glyphosate acid per hectare. Leek samples (whole plant without root) were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOQ (0.05 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Four residue trials were conducted on leek (outdoor) during 2011 in Northern France, the United Kingdom, Italy and Bulgaria. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at 3 days before planting the seedlings, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.14-2.54 kg glyphosate acid/ha.
In all trials in northern and southern Europe samples of leek (whole plant without root) were taken by hand at BBCH 49.

The samples (leek, whole plant without root) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-as validated in [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively.

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from leek (whole plant without root) fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (87-90%). Details of recovery data are shown in Table 6.3.9-2.
All trials are summarised below in Table 6.3.9-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOQ (0.05 mg/kg) were found in any of the treated and untreated samples of leek (whole plant without root).

III. CONCLUSIONS

Four residue trials were conducted on leek (outdoor) with MON 52276, containing 360 g/L glyphosate at 3 days before planting of leek seedlings in northern Europe and southern Europe. The product was applied in accordance with the proposed use patterns (deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.05 mg/kg) present in any sample of leek (whole plant without root) sampled at BBCH 49 (commercial maturity).

Table 6.3.9-1: Residues of glyphosate and AMPA in leek following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00265 S11-00265-01 Yes 2011	leek, Kenton	France, Europe, North	360 SL[1]	1	2.539	1.192	n/a	whole plant without root	77	< 0.015	< 0.015
S11-00265 S11-00265-02 Yes 2011	leek, Parvella	United Kingdom, Europe, North	360 SL[1]	1	2.413	1.189	n/a	whole plant without root	183	< 0.05	< 0.015
S11-00265 S11-00265-03 Yes 2011	leek, Maxim	Italy, Europe, South	360 SL[1]	1	2.255	1.187	n/a	whole plant without root	65	< 0.015	< 0.015
S11-00265 S11-00265-04 Yes 2011	leek, Staroza-gorski 72	Bulgaria, Europe, South	360 SL[1]	1	2.140	1.237	n/a	whole plant without root	125	< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.9-2: Procedural recoveries for glyphosate and AMPA in leek

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%)		
						single value	mean	RSD
S11-00265 S11-00265-01 S11-00265-02 S11-00265-03 S11-00265-04 Yes 2011	leek	whole plant without root	glyphosate	1 1	0.05 0.50	90 89	90	-
			AMPA	1 1	0.05 0.50	89 87	88	-

IIA 6.3.10 Sugar Beet

IIA 6.3.10/01	[redacted]	2012	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 [redacted] Study No.: S11-00266 08 March 2012 GLP: yes unpublished
Guideline:			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:			None
Dates of experimental work:			23 February 2011- 06 February 2012
Study owner:			Glyphosate Task Force

Executive Summary

Two residue trials were conducted on sugar beets treated at least 3 days after planting and before crop emergence at a target rate of 2.16 kg glyphosate acid per hectare. Sugar beet samples (leaves with tops and roots) were analyzed for glyphosate and AMPA. No residues of glyphosate or AMPA above the LOD (0.015 mg/kg) were found in any of the treated or untreated samples.

I. MATERIALS AND METHODS

Two residue trials were conducted on sugar beet (outdoor) during 2011 in Spain and Italy. One spray application of MON 52276 (360 g/L glyphosate) was performed to the bare soil at 6.0 L/ha at least 3 days after seeding and before crop emergence, diluted with water immediately prior to application to a spray volume of 175 L/ha. The actual used product rates correspond to 2.22-2.45 kg glyphosate acid/ha.
In both trials in southern Europe samples of sugar beet (leaves with tops and roots) were taken by hand at BBCH 49.

The samples (leaves with top and roots) were analysed for glyphosate and AMPA according to [redacted] method AG-ME-1294-01 and [redacted] study S11-03331, with a limit of quantitation of 0.05 mg/kg and limit of detection of 0.015 mg/kg for both analytes, respectively

II. RESULTS AND DISCUSSION

Recoveries of glyphosate and AMPA were obtained from sugar beet (leaves with tops and roots) fortified at levels of 0.05 mg/kg and 0.50 mg/kg. Single recoveries and mean recoveries over both levels were all within acceptable ranges (87-96% leaves with tops; 89-93% roots). Details of recovery data are shown in Table 6.3.10-2.
All trials are summarised below in Table 6.3.10-1 and in greater detail in the Tier 1 summary forms.

No residues of glyphosate and AMPA above the LOD (0.015 mg/kg) were found in any of the treated and untreated samples of sugar beet (leaves with tops and roots).

III. CONCLUSIONS

Two residue trials were conducted on sugar beet (leaves with tops and roots) with MON 52276, containing 360 g/L glyphosate at least 3 days after seeding and before crop emergence in southern Europe. The product was applied in accordance with the proposed use patterns (deviations were within EU tolerances), and the tests were carried out according to GLP principles.
The results of trials presented above demonstrate that there are no residues (< 0.015 mg/kg) present in any sample of sugar beet leaves with tops or roots sampled at BBCH 49 (commercial maturity).

Table 6.3.10-1: Residues of glyphosate and AMPA in sugar beet following application of MON 52276

Study No. Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No.	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	glyphosate (mg/kg)	AMPA (mg/kg)
S11-00266 S11-00266-01 Yes 2011	sugar beet, Sandrina	Spain, Europe, South	360 SL[1]	1	2.222	1.234	01	leaves with top	165	< 0.015	< 0.015
								roots		< 0.015	< 0.015
S11-00266 S11-00266-02 Yes 2011	sugar beet, Gea	Italy, Europe, South	360 SL[1]	1	2.453	1.185	00	leaves with top	144	< 0.015	< 0.015
								roots		< 0.015	< 0.015

FL = formulation GS = BBCH growth stage at last application DALT = days after last treatment
n/a = not applicable
Formulations used in trials: 1 = MON 52276 (360 SL), containing 360 g/L glyphosate acid

Table 6.3.10-2: Procedural recoveries for glyphosate and AMPA in sugar beet

Study No. Trial No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%) single value	mean	RSD
S11-00266 S11-00266-01 S11-00266-02 Yes 2011	sugar beet	sugar beet, leaves with tops	glyphosate	1	0.05	96	95	-
				1	0.50	93		
			AMPA	1	0.05	94	91	-
				1	0.50	87		
		sugar beet, roots	glyphosate	1	0.05	91	91	-
				1	0.50	90		
			AMPA	1	0.05	93	91	-
				1	0.50	89		

IIA 6.4 Livestock feeding studies

The conclusions from the 2001 EU evaluation as well as the supporting studies still apply. The studies included in the Glyphosate Monograph and the Glyphosate Trimesium Monograph are listed below in Table 6.4-1.

Table 6.4-1: Animal Livestock Feeding Studies

OECD Point	Animal	Dose Levels (mg/kg in diet)	Dosing Material	Monograph	Reference
IIA 6.4.1	Poultry	0, 40, 120, 400	9:1 mixture of glyphosate acid and AMPA	Glyphosate	[redacted] 1987, RIP95-01252
IIA 6.4.1	Poultry	0. 0.34, 3.4, 34	Glyphosate trimesium (dose level in glyphosate acid equivalents)	Glyphosate Trimesium	[redacted] 1987, RIP95-00025
IIA 6.4.2	Cows	0, 40, 120, 400	9:1 mixture of glyphosate acid and AMPA	Glyphosate	[redacted] 1987, RIP95-01250
IIA 6.4.2	Cows	0. 0.34, 3.4, 34, 207. 690	Glyphosate trimesium (dose level in glyphosate acid equivalents)	Glyphosate Trimesium	[redacted] 1987, RIP95-00024
IIA 6.4.3	Swine	0, 40, 120, 400	9:1 mixture of glyphosate acid and AMPA	Glyphosate	[redacted], 1987, RIP95-01251

Livestock feeding studies reflect the potential exposure of livestock through different types of feed. The residues of Glyphosate are from treated fodder, such as grass. The highest potential residues in fodder arise from crops treated before harvest, when the grower is changing the rotation into arable or horticultural crops. This use is not covered by the Representative Good Agricultural Practice that is supported in this dossier but represents the critical GAP in defining residues in animal tissues.

Supervised residue trials to determine residues in grasses after treatment of pasture pre-harvest with glyphosate formulation were conducted in North-Europe (Denmark, Germany, Finland, France and UK). These data were provided in the dossier for the first Annex I inclusion of Glyphosate and are summarized in Table 6.4-2. Glyphosate application rates ranged from 0.72 to 4.32 kg a.s./ha . The label for Northern Europe recommends a pre-harvest interval of minimum 5 days. Following treatment at rates ranging from 0.72 to 2.88 kg a.s./ha the glyphosate residues in fresh grass taken the day of application or one day later ranged between 14.6 to 252.3 mg/kg (STMR* = 76.5 mg/kg). The samples taken 3 - 8 DAT (thus within the GAP), ranged from 1 to 139 mg/kg (STMR * = 8.2 mg:kg). Only a limited number of trials (4) were conducted at the exaggerated rate of 4.32 kg a.s./ha and yielded residues within the range of values described above. Residue levels in silage did not differ significantly from the residue levels in fresh grass used for preparation of this feedingstuff. No degradation takes place during the silage process.

Table 6.4-2: Residue Studies in Grass, Hay and Silage

Commodity	Reference
Grass, hay and silage	[redacted] 1982. RIP95-01242.
Grass, hay and silage	[redacted] 1982. RIP95-01245.
Grass and silage	[redacted] 1983. RIP95-01264.
Grasss	[redacted] 1979. RIP95-01228.
Grass, hay and silage	[redacted] 1984. RIP95-01273.
Grass, hay and silage	[redacted] 1984. RIP95-01271
Grass, hay and silage	[redacted] 1988. RIP95-01281
Grass	[redacted] 1976. RIP95-01213
Grass	[redacted] 1977. RIP95-01214
Grass and hay	[redacted] 1994. RIP95-01308
Grass and silage	[redacted] 1994. RIP95-01312

Conclusions from the 2001 EU evaluation: Glyphosate Monograph

Animal feeding studies using glyphosate and AMPA have been conducted with lactating cows, poultry, and swine ([redacted] 1987, RIP95-01250; [redacted] 1987, RIP95-01252; [redacted] 1987, RIP95-01251). For these studies, test groups of animals were fed a daily ration containing a nine to one mixture of glyphosate and AMPA at total combined daily dietary levels of 40, 120, and 400 mg/kg for 28 days. The dosing levels are assumed to represent, respectively, lx, 3x, and l0x the maximum expected residue levels of glyphosate and AMPA in the diet. Animals were sacrificed either following the last day of treatment or following a 28 day depuration period. Milk samples were taken in the cow study and eggs were collected in the poultry study at various time points during treatment and depuration. At sacrifice, residue levels were determined in fat, muscle, liver and kidney.

For all three species, glyphosate and AMPA residues were less than 0.05 mg/kg (undetectable) in all fat and muscle samples from all treatment levels following the 28-day dosing period, except muscles samples from swine and fat samples from chickens dosed at the highest level, which had residues of 0.06 to 0.07 mg/kg of glyphosate.

The highest glyphosate and AMPA residues were found in kidneys. At the end of the 28-day dosing period glyphosate residues in kidney of cow, swine and chicken dosed at the 10x level were 3.0, 7.63, and 3.82 mg/kg, respectively. AMPA residue levels in the same tissues were 0.07, 0.29, and 0.96 mg/kg, respectively. Significantly lower levels of glyphosate and AMPA were found in liver tissues collected at the end of the 28-day dosing period. For the l0x dose level liver samples, glyphosate residues were 0.20, 0.60, and 0.61 mg/kg, respectively. AMPA residues in the same tissues were <0.05, 0.12, and 0.39 mg/kg, respectively.

Analysis of tissues following the 28 day depuration period demonstrate that glyphosate and AMPA are rapidly eliminated. Following a 28-day depuration period, AMPA residues were less than 0.05 mg/kg in all samples. Glyphosate residues in the 28-day depurated animal tissues were less than 0.05 mg/kg in all tissues except kidney samples at the 3x and l0x dose levels, which contained average glyphosate residues of 0.08 and 0.18 mg/kg, respectively.

Glyphosate and AMPA residues were less than 0.025 mg/kg (undetectable) in all milk samples collected from cows dosed at the l0x dose level.

Glyphosate residues were undetected in all egg samples collected from hens dosed at the 1x level, and were up to 0.131 mg/kg in eggs of hens dosed at the 10x level. AMPA residues in the same samples were less than 0.025 mg/kg in all cases. All glyphosate residues in eggs collected after a 7-day depuration period were less than 0.025 mg/kg.

Conclusions from the 2001 EU evaluation: Glyphosate-Trimesium Monograph

Animal feeding studies were conducted with glyphosate-trimesium in cattle and poultry.

Laying hens were fed with glyphosate-trimesium at dose levels of 0.5, 5 and 50 mg glyphosate-trimesium/kg in feed (equivalent to 0.34, 3.4 and 34 mg/kg of glyphosate acid) ([redacted], 1987, RIP95-00025). The hens were dosed for 28 consecutive days. Certain hens were selected for an additional withdrawal period of 7 days in which no glyphosate-trimesium was administered. No treatment-related effects on feed consumption, body weight or egg production were evident throughout the study. Glyphosate-trimesium, when fed continuously at a level equivalent to 34 mg/kg of glyphosate acid to laying hens, produced low concentrations of residues in eggs and edible tissues. Residues of glyphosate in eggs ranged from <0.01 – 0.015 mg/kg. Residues of glyphosate in kidney were 0.31 mg/kg, and were not detected (<0.05 mg/kg) in liver, fat and muscle. Residues of AMPA were below the limit of determination in all tissues and eggs. All residues were below the limit of determination by 7 days after cessation of dosing.

Lactating dairy cattle were dosed daily for 28 days with five rates of glyphosate-trimesium technical, at rates equivalent to 0.5, 5, 50, 300 and 1000 mg/kg in the diet (equivalent to 0.34, 3.4, 34, 207 and 690 mg/kg of glyphosate acid in the diet) ([redacted] 1987, RIP95-00024). Two animals from each group were sacrificed after 28 days and the remainder were sacrificed after 7 days of withdrawal. Feed consumption, milk production and body weights of dairy cows were not affected by daily administration of glyphosate-trimesium at dose levels up to 300 mg/kg in the diet. At a dose level of 1000 mg/kg treatment related effects were observed including lethargy with reduced feed consumption, milk production and bodyweight.
Glyphosate-trimesium, when fed continuously for 28 days, at a level equivalent to 207 mg/kg of glyphosate acid to dairy cattle, produced low concentrations of residues in milk and edible tissues. One milk sample had glyphosate residues at 0.02 mg/kg, all others were below the limit of determination (<0.02 mg/kg). In kidney, glyphosate residues were 1.8 – 2.6 mg/kg and AMPA residues were 0.47 – 0.58 mg/kg immediately after dosing, and declined to 0.12 mg/kg and <0.05 mg/kg, respectively, 7 days after cessation of dosing. In fat, glyphosate residues were 0.06 mg/kg and AMPA was <0.05 mg/kg. Glyphosate and AMPA levels in liver and muscle were below the limit of determination in all samples.

Summary
Results in both sets of livestock feeding studies are consistent. Glyphosate and AMPA are rapidly excreted. The highest residues are in kidney, with lower residues in the liver. Residues in milk, eggs, tissue and fat were either not detected or were very low. Residues declined quickly after dosing was stopped.

IIA 6.5 Effects of industrial processing and/or household preparation (representative processing simulations) on

IIA 6.5.1 The nature of residue

Annex point	Author(s)	Year	Study title
IIA 6.5.1/01	[redacted]	2010	Nature of [^{14}C]glyphosate residues in processed commodities – High temperature hydrolysis, [redacted] Report Number: [redacted]0023072, [redacted] Report Number: 1925[redacted]-001. GLP: yes unpublished

Guideline: OECD Guideline 507
Deviations: None
Dates of experimental work: September 2009-October 2009
Study owner: Glyphosate Task Force

Executive Summary

The degradation of [^{14}C]glyphosate was studied under hydrolytic conditions at high temperatures in sterile aqueous buffers at pH 4, 5 and 6 for periods of up to 60 minutes, simulating common processing practices as pasteurisation, baking, brewing, boiling and sterilisation.

The test solutions were analysed by high performance liquid chromatography (HPLC) with liquid scintillation counting (LSC) analysis before and after the heating. Radiocarbon recoveries ranged from 95.6 to 99.4% of the applied dose for all solutions. The experiments showed that glyphosate did not degrade at temperatures ranging from 90°C to sterilizing conditions (121 °C) in any of the buffer systems tested, indicating that glyphosate should be stable in/on processed commodities during common processing practices.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	[^{14}C]glyphosate : labelled at phosphonomethylene carbon
Description:	not reported
Lot/Batch #:	53463-3-23
Purity:	radiochemical purity: ≥ 98%
Specific act.:	10.28 MBq/mg or 6.17 x 10^5 DPM/µg

2. Test system

A stock solution of the [^{14}C]glyphosate test material was prepared in HPLC grade water at a concentration of 5.71 x 10^7 DPM/mL and radiochemical purity of 98.7%. The stock solution was mixed with sterile aqueous buffer solutions of three different pH values (pH 4, 5 and 6). All buffer solutions were prepared with potassium biphthalate. Buffer solutions were sterilized by passing through a sterile filter into previously autoclaved vials/bottles. Prior to application, nitrogen was bubbled for at least 5 minutes through each buffer via sterile bacterial air filter, to avoid the effects of oxygen on the test systems. The concentration of glyphosate in buffered solutions ranged from 1.07 mg/L to 1.15 mg/L.

The samples were prepared in duplicate for each buffer system.

B. STUDY DESIGN

1. Experimental conditions

<u>pH 4 and 90°C - pasteurisation</u>
The test solutions were placed in an oven for 20 min at 90 °C and pH 4.0±0.1 in amber glass vials (4-mL capacity) with Teflon-lined caps.

<u>pH 5 and 100°C - baking, brewing, boiling</u>
The test solutions were placed in an oven for 60 min at 100 °C and pH 5.0±0.1 in amber glass vials (4-mL capacity) with Teflon-lined caps.

<u>pH 6 and 120°C - sterilisation</u>
The test solutions were placed in an autoclave for 20 min at 121 °C and pH 6.0±0.1 in amber glass vials (4-mL capacity) with Teflon-lined caps.

Duplicate samples were analysed immediately for time zero where no heat was used. After heating duplicate samples were retrieved from the respective oven or autoclave. The pH of the solution was measured and recorded. Triplicate aliquots (3 x 0.1 mL) were taken for analysis. All solutions were analysed by HPLC within two days of sampling.

II. RESULTS AND DISCUSSION

A. pH VALUES

The pH of the samples was measured at each sampling time. The results indicate that the buffering capacity was maintained in the solution throughout the study.

B. HIGH TEMPERATURE HYDROLYSIS

The hydrolysis of glyphosate test substance was examined at pH 4, pH 5 and pH 6 at 90°C, 100°C and 121°C, respectively. The mass balance for the high temperature hydrolysis tests ranged from 95.6% to 99.4% applied radioactivity. The overall radioactivity before and after each test performance is given in Table 6.5.1-1.

Table 6.5.1-1: Material balance of radiocarbon following hydrolysis of $[^{14}C]$glyphosate at high temperatures

Test	pH 4, 90°C, 20 min	pH 5, 100°C, 60 min	pH 6, 121°C, 20 min
before test [% of applied dose]			
Rep A	96.1	96.7	98.3
Rep B	95.7	96.6	97.6
after test [% of applied dose]			
Rep A	95.6	98.9	98.3
Rep B	95.9	99.3	99.4

III. CONCLUSIONS

Glyphosate was stable to hydrolysis in all test systems and temperatures conducted, indicating that glyphosate should be stable in/on processed commodities during common processing practices.

IIA 6.5.2 Distribution of the residue in peel/pulp

Processing studies in many crops were included in the initial glyphosate and glyphosate trimesium dossiers. Glyphosate concentrates primarily in processed fractions such as hulls and bran of cereals and citrus peel due to surface residues; in meal after removal of oil fractions; and in concentrated liquid fractions such as molasses. Glyphosate does not partition into oil, and is removed from highly processed fractions such as sugar.

IIA 6.5.3 Residue levels – balance studies on a core set of representative processes

Please refer to IIA 6.5.2

IIA 6.5.4 Residue levels – follow-up studies to determine concentration or dilution factors

Please refer to IIA 6.5.2

IIA 6.6 Residues in succeeding crops

IIA 6.6.1 Theoretical consideration of the nature and level of the residue

Since the actual study gives more detailed information, no theoretical consideration of the nature and the level of residues in succeeding crops has been performed.

IIA 6.6.2 Metabolism and distribution studies on representative crops

The conclusions from the 2001 EU evaluation as well as the supporting studies still apply.

Conclusions from the 2001 EU evaluation: Glyphosate Monograph
A confined rotational crop study was included in the glyphosate monograph ([redacted] 1990, RIP95-01201; [redacted] 1990, RIP95001202) . The primary crop, soybeans, received a preplant application of 4.15 kg/ha of ^{14}C-glyphosate. Carrots, lettuce and barley were planted as rotational crops at 30, 119 and 365 days after application.

Total ^{14}C-radioactivity expressed as glyphosate equivalents, was less than 0.2 mg/kg in all rotational crop samples and decreased with time. Release of ^{14}C-radioactivity upon aqueous extraction of rotational crop samples was less than 60% of the radioactivity in the plants in all cases, and typically less than 40%. The nonextractable ^{14}C-radioactivity in 30 day rotational barley grain and straw samples harvested 125 days after treatment was characterized as biopolymers of glucose. Aqueous extracts of the rotational crop tissues contained less than 0.02 mg/kg glyphosate in all cases.

The results of this study demonstrate that only very low levels of glyphosate or glyphosate metabolites are present in the soil and plant tissues of rotational crops planted after treatment of a primary crop with glyphosate. The only metabolite of glyphosate found was AMPA. The majority of glyphosate derived radioactivity in the soil and plant tissues has been attributed to natural products derived by incorporation of one carbon compounds such as CO_2 into natural metabolic pools. The distribution of radioactivity in rotational crops was found to be similar to the distribution found in plants exposed to $^{14}CO_2$. The results of these studies show that glyphosate residues in emergency replant and rotational crops will be less than those found in the primary crop.

Conclusions from the 2001 EU evaluation: Glyphosate-Trimesium Monograph
A confined rotational crop study was included in the glyphosate-trimesium monograph ([redacted] 1993, RIP95-00018; [redacted] 1994, RIP95-00019). ^{14}C-Glyphosate-trimesium (labelled in the glyphosate portion) was applied either as a single or as sequential applications, at a total rate equivalent to 3.9 – 6.6 kg/ha of glyphosate acid. Soybeans were planted as the primary crop. Lettuce, wheat and radishes were planted as the rotational crops, at 35 days, 125 and 370 days after the initial application.
There was minimal uptake of residues in the samples. Glyphosate residue levels were <0.01 mg/kg in all samples, and the maximum AMPA residues were 0.03 mg/kg. All other extractable and unextractable radioactivity was associated with [^{14}C] incorporated or bound to natural products.

These data have been confirmed by two field studies which demonstrate that the residues in following crops are close to or below the limit of determination.

Studies added to the Submission
There is an additional rotational crop study not included in the glyphosate or glyphosate-trimesium monograph but submitted prior to ECCO review. The results are comparable to those included in the monographs. The summary is provided.

Annex point	Author(s)	Year	Study title
IIA 6.6.2/01	[redacted]	1998	LX1146-02 (Glyphosate technical) confined rotational crop study on lettuce, radish, and wheat in California [redacted] Study No.: 1651-91-146-01-09B-17 [redacted] Doc. No.: 459 GLY 20 April 1998 GLP: yes unpublished
Guideline:			EPA Guideline (165-1) Subdivision N of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)
Deviations:			None
Dates of experimental work:			July 1991 to April 1998
Study owner:			Cheminova

Executive Summary

Crop rotation experiments were performed with [^{14}C] glyphosate on lettuce, radish and wheat - crops considered to be representative of leafy, root, and cereal crops, respectively.
The active substance was applied to sandy loam soil at an application rate of 6.5 kg/ha, which is about 1.5X the maximum annual application rate of 4.32 kg/ha. After the application, soil was aged 30, 120, and 365 days prior to planting. Soil samples were taken after application and after harvest of the mature crops for each plant back interval.
Parent glyphosate residues above the LOQ were not found in any plant parts destined for human consumption. AMPA residues were found in the first and second planting of wheat. The residues in grains were 0.40 and 0.20 mg/kg, respectively. In the third planting no residues of AMPA were found in any wheat matrices.
No residues of parent glyphosate or AMPA were found in any of the mature radish and lettuce samples harvested from any of the planting intervals. This indicates that glyphosate and AMPA do not accumulate in rotational crops tested and that the majority of carbon which was initially part of the glyphosate molecules applied to the soil that is taken up by these plants becomes incorporated into plant components or is converted into compounds other than glyphosate and AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** [^{14}C] Glyphosate
   - Description: aqueous solution
   - Lot/Batch #: CFQ-6477
   - Purity: Radio purity 99%
   - CAS # :
   - Stability of test compound: Stocks of [^{14}C] glyphosate should be stored at -20 °C. The rate of decomposition under these conditions is not expected to be greater than 2% per annum

2. **Test Commodity:**

Crop:	Lettuce	Radish	Wheat
Type:	Leafy vegetable	Root and tuber vegetable	Cereals
Variety:	Waldmann's Green	Cherry Balle	Yecora Rojo
Botanical name:	*Lactuca sativa*	*Raphanus sativus*	*Triticum aestivum*

3. **Soil:** A sandy loam soil was used for all experiments. The soil physicochemical properties are described below in Table 6.6.2-1.

Table 6.6.2-1: Soil physicochemical properties

Soil characterisation results	Non-treated	Treated
Soil classification	sandy loam	sandy loam
pH	7.90	8.00
OM (%)	1.04	0.88
Sand (%)	64.20	62.20
Silt (%)	29.00	31.00
Clay (%)	6.80	6.80
Water holding capacity (%) at 1/3 Bar	9.35	10.17
Water holding capacity (%) at 15 Bar	3.10	3.30
CEC (meq/100 g)	5.07	5.62
Bulk density (g/cc)	1.55	1.51

B. STUDY DESIGN

The study was conducted during the period July 1991 to April 1998 by the [redacted]

1. Test procedure

Crop rotation experiments were performed with [^{14}C] glyphosate on lettuce, radish and wheat - crops considered to be representative of leafy, root, and cereal crops, respectively.

The rotational crops were grown in plastic pots with 30.5 cm diameter filled with sandy loam soil. Before sowing or planting, the active substance corresponding to an application rate of 6.5 kg/ha was applied. After the application, soil was aged 30, 120, and 365 days prior to planting.

2. Sampling

Soil samples were taken on the day of treatment, at the cultivation of the follow-up crop (30, 120 and 365 days after treatment (DAT) and at harvest time of the follow-up crop. Mature and immature lettuce, radish and wheat crop samples were obtained from sowing intervals of 30, 120, and 365 DAT.

3. Analysis

After homogenisation of soil and crop samples, duplicate subsamples were each oxidised for collecting $^{14}CO_2$ using a scintillation cocktail (Oxosol C^{14}). Assay of radioactivity was completed by liquid scintillation spectrometry.

All crop samples with significant levels of total radioactivity (> 0.01 ppm) were analysed for the residue contents of glyphosate and its metabolite AMPA.
For the determination of glyphosate and its metabolite AMPA the samples were extracted with chloroform and 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 Limit of Quantification (LOQ) for the method was 0.05 mg/kg for both analytes glyphosate and AMPA.

II. RESULTS AND DISCUSSION

Following the application of glyphosate to soil at an application rate of 6.5 kg a.s./ha, the total ^{14}C residue (TRR) was measured in soil. Measurements were carried out directly after application and after harvest of the individual mature crops for each plant back interval (see Table 6.6.2-2).

The residue level in the top layer of the soil had an average concentration of 4.5 mg/kg directly after application. After harvest of the mature crops, the soil residue levels decreased to 2.3 mg/kg (75/120 DAT), to 1.8 mg/kg (165/210 DAT) and to 0.6 mg/kg (410/455 DAT). Generally, residue levels in the soil decreased from sowing to harvest.

Furthermore, the total radioactive residues (TRR) were measured in wheat matrices, in lettuce and in radish leaf and root after each plant back interval. The TRR values are given in Table 6.6.2-3.
In the edible part of wheat, grains, residue levels were 2.0 mg/kg at a plant back interval of 30 DAT and decreased to 0.16 mg/kg at a plant back intervals of 365 DAT.

The TRR in mature lettuce (30 DAT) amounted to 0.34 mg/kg. After a plant back interval of 120 DAT, the TRR in lettuce amounted to 0.25 mg/kg and declined further to 0.02 mg/kg after a plant back interval 365 days. The total radioactive residues in radish roots were 0.24 mg/kg at a plant back interval of 30 days, decreasing to 0.15 mg/kg after 120 days and to 0.05 mg/kg after 365 days of soil aging.

Parent glyphosate and AMPA residues above the LOQ of 0.05 mg/kg were found only in wheat samples. Glyphosate was found in the mature samples of wheat forage (0.40 mg/kg) and wheat chaff (0.30 and 0.06 mg/kg). AMPA residues were found in the first (30 DAT) and second (120 DAT) planting of wheat. The residues ranged from 0.10 to 0.40 mg/kg. In the third planting no residues of AMPA were found in any wheat matrices.

Mature radish and lettuce samples harvested from any of the planting intervals did not contain any residues of parent glyphosate or AMPA. The details are given in Table 6.6.2-4.

Table 6.6.2-2: [^{14}C] glyphosate equivalents in wet and dry soil after plant back intervals of 30, 120 and 365 days

Crop		TRR (mg/kg)	Days after soil treatment	Soil concentration (mg/kg)			
				Wet soil		Dry soil	
				0-7.6 cm	7.6-15.2 cm	0-7.6 cm	7.6-15.2 cm
Soil			0	2.6[1]	ND[1]	3.2	ND
Soil			0	6.4[2]	0.1[2]	7.9	0.1
Radish	Immature	2.2	55	2.2	0.1	2.5	0.1
	Mature	4.8	75	2.1	0.04	2.3	0.04
	Immature	0.33	145	1.0	0.9	1.1	1.0
	Mature	0.17	165	2.0	0.09	2.2	0.1
	Immature	0.01	390	0.9	0.03	1.0	0.03
	Mature	0.02	410	0.7	0.08	0.8	0.09
Lettuce	Immature	0.46	55	2.5	0.04	3.0	0.05
	Mature	0.34	75	2.5	0.02	2.8	0.02
	Immature	0.68	145	3.2	0.05	3.6	0.06
	Mature	0.25	165	1.5	0.1	1.8	0.1
	Immature	0.02	390	1.1	0.04	1.2	0.04
	Mature	0.02	410	0.7	0.06	0.8	0.07
Wheat forage	Immature	0.46	60	1.6	0.03	1.8	0.03
	Mature	1.3	120	2.2	0.1	2.6	0.1
	Immature	0.45	150	1.8	0.2	2.4	0.3
	Mature	1.4	210	1.9	0.1	2.1	0.1
	Immature	0.01	395	0.7	0.04	0.8	0.04
	Mature	0.08	455	0.6	0.09	0.6	0.1

[1] Pot sample
[2] Core sample

Table 6.6.2-3: Total radioactive residues of [^{14}C] glyphosate equivalents in immature and mature crops after plant back intervals of 30, 120 and 365 days

Crop	Commodity	Immature crop		Mature Crop	
		TRR (mg/kg)	Days after seeding	TRR (mg/kg)	Days after seeding
Plant back interval: 30 DAT					
Radish	Leaf	2.2	25	4.8	45
	Root	0.38	25	0.24	45
Lettuce	Leaf	0.46	25	0.34	45
Wheat	Forage	0.46	30	1.3	90
	Chaff[1]	-	-	1.6	90
	Grain[1]	-	-	2.0	90
Plant back interval: 120 DAT					
Radish	Leaf	0.33	25	0.17	45
	Root	0.71	25	0.15	45
Lettuce	Leaf	0.68	25	0.25	45
Wheat	Forage	0.45	30	1.4	90
	Chaff[1]	-	-	1.0	90
	Grain[1]	-	-	0.7	90
Plant back interval: 365 DAT					
Radish	Leaf	0.01	25	0.02	45
	Root	0.06	25	0.05	45
Lettuce	Leaf	0.02	25	0.02	45
Wheat	Forage	0.01	30	0.08	90
	Chaff[1]	-	-	0.19	90
	Grain[1]	-	-	0.16	90

[1] Chaff and seed samples had not yet developed at the immature stage

Table 6.6.2-4: Determination of glyphosate and its metabolite AMPA in rotational crops harvested at maturity

Crop	[14C] glyphosate equivalents	Glyphosate	AMPA	Total
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Radish Leaf				
Plant back interval: 30 DAT	4.8	<0.05	<0.05	-
Plant back interval: 120 DAT	0.17	<0.05	<0.05	-
Plant back interval: 365 DAT	0.02	<0.05	<0.05	-
Radish Root				
Plant back interval: 30 DAT	0.24	<0.05	<0.05	-
Plant back interval: 120 DAT	0.15	<0.05	<0.05	-
Plant back interval: 365 DAT	0.05	<0.05	<0.05	-
Lettuce Leaf				
Plant back interval: 30 DAT	0.34	<0.05	<0.05	-
Plant back interval: 120 DAT	0.25	<0.05	<0.05	-
Plant back interval: 365 DAT	0.02	<0.05	<0.05	-
Wheat Forage				
Plant back interval: 30 DAT	1.3	<0.05	0.20	0.20
Plant back interval: 120 DAT	1.4	0.40	0.10	0.50
Plant back interval: 365 DAT	0.08	<0.05	<0.05	-
Wheat Chaff				
Plant back interval: 30 DAT	1.6	<0.05	0.40	0.40
Plant back interval: 120 DAT	1.0	0.30	0.20	0.50
Plant back interval: 365 DAT	0.19	0.06	<0.05	0.06
Wheat Grain				
Plant back interval: 30 DAT	2.0	<0.05	0.30	0.30
Plant back interval: 120 DAT	0.7	<0.05	0.20	0.20
Plant back interval: 365 DAT	0.16	<0.05	<0.05	-

III. CONCLUSIONS

The distribution of radioactive residues from soil into plant was investigated at three replant intervals in radish, lettuce and wheat. At the end of the trial TRRs (referring to glyphosate equivalents) of between 0.02 mg/kg (lettuce, 365 days) and 2.0 mg/kg (wheat grain, 30 days) were found in the plant parts destined for human consumption.

Parent glyphosate residues above the LOQ were not found in any plant parts destined for human consumption. AMPA residues were found in the first (30 DAT) and second (120 DAT) planting of wheat. The residues in grains were 0.30 and 0.20 mg/kg, respectively. In the third planting (365 DAT) no residues of AMPA were found in any wheat matrices.
Mature radish and lettuce samples harvested from any of the planting intervals did not contain any residues of parent glyphosate or AMPA.

This indicates that glyphosate and AMPA do not accumulate in rotational crops tested and that the majority of carbon which was initially part of the glyphosate molecules applied to the soil that is taken up by these plants becomes incorporated into plant components or is converted into compounds other than glyphosate and AMPA.

IIA 6.6.3 Field trials on representative crops

The conclusions from the 2001 EU evaluation as well as the supporting studies still apply.

Rotational crop studies were included in the initial glyphosate and glyphosate trimesium dossiers, or submitted prior to ECCO review ([redacted] 1998, this study is added to the submission).

Three rotational crop studies using ^{14}C-glyphosate have been conducted to determine the potential for glyphosate residues to be present in emergency replant and rotational crops.

The results of these studies demonstrate that only very low levels of glyphosate or its metabolites are present in the soil and plant tissues of rotational crops planted after treatment of a primary crop with glyphosate. The only metabolite of glyphosate found was aminomethylphosphonic acid (AMPA). The majority of glyphosate derived radioactivity in the soil and plant tissues has been attributed to natural products derived by incorporation of one carbon compounds such as CO_2 into natural metabolic pools. The distribution of radioactivity in the rotation crops was found to be similar to the distribution found in plants exposed to $^{14}CO_2$. The results of these studies show that glyphosate residues in emergency replant and rotational crops will be less than those found in the primary crop.

IIA 6.7 Proposed residue definition and maximum residue levels

IIA 6.7.1 Proposed residue definition

The current residue definition for enforcement for glyphosate was established in the 2001 EU evaluation. Plant metabolism studies demonstrated that glyphosate is the primary residue in crop commodities, AMPA is the major metabolite and in most cases the residues of AMPA are not significant. Radiolabelled studies in lactating goats and laying hens following oral administrations of glyphosate and AMPA showed that metabolites resulting from the degradation of these compounds in edible tissues, milk and eggs were either insignificant or entirely absent.

Glyphosate is the primary residue in plant and animal commodities and it was concluded that the residue definition for enforcement should be: **glyphosate**.

In 2009, under the framework of Article 10 of Regulation (EC) No 396/2005 the metabolism of glyphosate in genetically modified soya bean and maize containing the glyphosate-N-acetyl transferase (GAT) gene was assessed[1]. Submitted studies indicated that the metabolism of glyphosate in these transgenic crops proceeds in a different pathway, producing two additional metabolites, N-acetyl-glyphosate and N-acetyl-AMPA.

Several options for the definition of the residue for enforcement were proposed by EFSA, including maintaining the current definition. No change is currently proposed, so the definition of the residue for enforcement for both plant and animal products should be: **glyphosate**.

Taking into account the differences in metabolism in crops containing the GAT gene, the definition of the residue for risk assessment for plants and animal products was recently amended to be: **the sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA, calculated as glyphosate.**

[1] EFSA (European Food Safety Authority), 2009. Reasoned opinion on the modification of the residue definition of glyphosate in genetically modified maize grain and soybeans, and in products of animal origin. EFSA Journal 2009; 7(9):1310, 42 pp.

IIA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed, including details of statistical analyses used

Table 6.7.2-1 lists the MRLs as presented in the Commission Regulation 839/2008/EC. All MRLs for raw agricultural commodities were determined from the results of supervised field trials conducted in Europe, with the exception of soybeans and tea (for which import tolerances are recommended). For soybeans, data for MRL determinations were derived from supervised field trials conducted in the United States. For tea, data for MRL determinations were derived from supervised field trials conducted in Taiwan and Sri Lanka. In all cases, MRLs for raw agricultural commodities are based on currently approved, critical Good Agricultural Practices in the European Union.

For the estimation of the residues in animal products, the STMR of cereal grain and straw was used as proposed by the JMPR FAO panel, resulting in very low residue situation expected for all products of concern. Therefore, the MRLs for foodstuff of animal origin have been revised in Regulation 839/2008/EC.

No new MRLs are being proposed as part of this submission.

Table 6.7.2-1: Maximum Residue Limits (MRL) for glyphosate in the EU
(established under Commission Regulation 839/2008/EC)

Crop/Tissue	**MRL (mg/kg)**
1. FRUIT FRESH OR FROZEN; NUTS	
(i) Citrus fruit	
Grapefruit (Shaddocks, pomelos, sweeties, tangelo (except mineola), ugli and other hybrids)	0.1*
Oranges (Bergamot, bitter orange, chinotto and other hybrids)	0.5
Lemons (Citron, lemon)	0.1*
Limes	0.1*
Mandarins (Clementine, tangerine, mineola and other hybrids)	0.5
Others	0.1*
(ii) Tree nuts (shelled or unshelled)	0.1*
(iii) Pome fruit	0.1*
(iv) Stone fruit	0.1*
(v) Berries & small fruit	
(a) Table and wine grapes	0.5
(b) Strawberries	0.1*
(c) Cane fruit	0.1*
(d) Other small fruit & berries	0.1*
(vi) Miscellaneous fruit	
(a) Edible peel	
Dates	0.1*
Figs	0.1*
Table olives	1
Kumquats (Marumi kumquats, nagami kumquats, limequats (Citrus aurantifolia x Fortunella spp.))	0.1*
Carambola (Bilimbi)	0.1*
Persimmon	0.1*
Jambolan (java plum) (Java apple (water apple), pomerac, rose apple, Brazilean cherry Surinam cherry (grumichama Eugenia uniflora))	0.1*
Others	0.1*
(b) Inedible peel, small	0.1*
(c) Inedible peel, large	0.1*

Table 6.7.2-1: Maximum Residue Limits (MRL) for glyphosate in the EU
(established under Commission Regulation 839/2008/EC)

Crop/Tissue	MRL (mg/kg)
2. VEGETABLES FRESH OR FROZEN	
(i) Root and tuber vegetables	0.1*
(ii) Bulb vegetables	0.1*
(iii) Fruiting vegetables	0.1*
(iv) Brassica vegetables	0.1*
(v) Leaf vegetables & fresh herbs	0.1*
(vi) Legume vegetables (fresh)	0.1*
(vii) Stem vegetables (fresh)	0.1*
(viii) Fungi	
Cultivated (Common mushroom, Oyster mushroom, Shi-take)	0.1*
Wild (Chanterelle, Truffle, Morel, Cep)	50
Others	0.1*
(ix) Sea weeds	
3. PULSES, DRY	
Beans (Broad beans, navy beans, flageolets, jack beans, lima beans, field beans, cowpeas)	2
Lentils	0.1*
Peas (Chickpeas, field peas, chickling vetch)	10
Lupins	10
Others	0.1*
4. OILSEEDS AND OILFRUITS	
(i) Oilseeds	
Linseed	10
Peanuts	0.1*
Poppy seed	0.1*
Sesame seed	0.1*
Sunflower seed	20
Rape seed (Bird rapeseed, turnip rape)	10
Soya bean	20
Mustard seed	10
Cotton seed	10
Pumpkin seeds (Other seeds of cucurbitacea)	0.1*
Safflower	0.1*
Borage	0.1
Gold of pleasure	0.1
Hempseed	0.1*
Castor bean	0.1
Others	0.1*
(ii) Oilfruits	
Olives for oil production	1
Palm nuts (palmoil kernels)	0.1
Palmfruit	0.1
Kapok	0.1
Others	0.1*

Table 6.7.2-1: Maximum Residue Limits (MRL) for glyphosate in the EU
(established under Commission Regulation 839/2008/EC)

Crop/Tissue	MRL (mg/kg)
5. CEREALS	
Barley	20
Buckwheat (Amaranthus, quinoa)	0.1*
Maize	1
Millet (Foxtail millet, teff)	0.1*
Oats	20
Rice	0.1*
Rye	10
Sorghum	20
Wheat (Spelt, triticale)	10
Others	0.1*
6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	
(i) Tea (dried leaves and stalks, fermented or otherwise of Camellia sinensis)	2
(ii) Coffee beans	0.1
(iii) Herbal infusions (dried)	2
(iv) Cocoa (fermented beans)	0.1*
(v) Carob (st johns bread)	0.1*
7. HOPS (dried), including hop pellets and unconcentrated powder	0.1*
8. SPICES	0.1*
9. SUGAR PLANTS	
Sugar beet (root)	1*
Sugar cane	0.1*
Chicory roots	0.1*
Others	0.1*
10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
(i) Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals other processed products such as sausages and food preparations based on these	
(a) Swine	
Meat	0.05*
Fat free of lean meat	0.05*
Liver	0.05*
Kidney	0.5
Edible offal	0.05*
Others	0.05*
(b) Bovine	
Meat	0.05*
Fat	0.05*
Liver	0.2
Kidney	2
Edible offal	0.05*
Others	0.05*
(c) Sheep	0.05*
(d) Goat	0.05*
(e) Horses, asses, mules or hinnies	0.05*

Table 6.7.2-1: Maximum Residue Limits (MRL) for glyphosate in the EU
(established under Commission Regulation 839/2008/EC)

Crop/Tissue	MRL (mg/kg)
(f) Poultry -chicken, geese, duck, turkey and Guinea fowl-, ostrich, pigeon	
Meat	0.05*
Fat	0.05*
Liver	0.05*
Kidney	0.1*
Edible offal	0.05*
Others	0.05*
(g) Other farm animals (Rabbit, Kangaroo)	0.05*
Meat	0.05*
Fat	0.05*
Liver	0.05*
Kidney	0.05
Edible offal	0.05
Others	0.05
(ii) Milk and cream, not concentrated, nor containing added sugar or sweetening matter, butter and other fats derived from milk, cheese and curd	0.05*
(iii) Birds' eggs, fresh preserved or cooked Shelled eggs and egg yolks fresh, dried, cooked by steaming or boiling in water, moulded, frozen or otherwise preserved whether or not containing added sugar or sweetening matter	0.05*
(iv) Honey (Royal jelly, pollen)	
(v) Amphibians and reptiles (Frog legs, crocodiles)	
(vi) Snails	
(vii) Other terrestrial animal products	

* indicates lower limit of analytical determination

IIA 6.8 Proposed pre-harvest intervals, re-entry intervals or withholding periods to minimize residues in crops, plants, plant products, treated areas or spaces and a justification for each proposal

IIA 6.8.1 Pre-harvest interval (in days) for each relevant crop

Table 6.8.1-1: Proposed minimum pre-harvest intervals (PHI) for registered uses

Crop	Type of application	Minimum PHI (days)
Pre-plant of crop	Overall spray	N/A
Post planting/ pre emergence of crop	Overall spray	N/A
Cereals	Pre-harvest (in-crop)	7
Oilseeds	Pre-harvest (in-crop)	14
Orchard crops, vines, including citrus & tree nuts	Weed control in orchards	N/A

IIA 6.8.2 Re-entry period (in days) for livestock, to areas to be grazed

- 5 days

IIA 6.8.3 Re-entry period (in hours or days) for man to crops, buildings or spaces treated

The result of the risk assessment indicates that re-entry of treated field crops is possible after the spray solution has completely dried up. The assessment is detailed under M-III/7.5.1.

IIA 6.8.4 Withholding period (in days) for animal feeding stuffs

Feed items of the target crops are side products of food products. Feed items proposed for feeding-stuffs will therefore be harvested at or beyond the pre-harvest interval.

IIA 6.8.5 Waiting period (in days) between last application and sowing or planting the crop to be protected

- Pre-drilling of seed (for instance stubble treatments, post-cultivation treatments or pre-plant treatments):

  The limiting factor is the time taken for glyphosate to be absorbed by and translocated into the weeds. Glyphosate is adsorbed by the soil, therefore residues in succeeding crops are not a concern. Typical recommendations: 2-3 days before planting

- Pre-planting of transplanted crops (plugs or bare root):

  The limiting factor is to ensure that moist plugs or bare roots do not come into contact with the treated vegetation (weeds) or with glyphosate in solution. Experience has shown that a waiting period of 3 days is sufficient after spraying.

- Post-drilling pre-emergence:

  The limiting factor is to treat before crop emergence. Typically there is no restriction on application after drilling except to avoid crop emergence.

IIA 6.8.6 Waiting period (in days) between application and handling treated product

Not relevant, since a post-harvest treatment is not intended.

IIA 6.8.7 Waiting period (in days) between last application and sowing or planting succeeding crops

The results of the rotational crop studies show that glyphosate residues in emergency replant and rotational crops will be less than those found in the primary crop. Therefore, no limitation concerning the succeeding crops is necessary.

IIA 6.9 Estimation of the potential and actual exposure through diet and other means

IIA 6.9.1 TMDI calculations

Long-term consumer exposure to potential glyphosate residues is estimated according to the EFSA Primo model[2] for chronic risk assessment.

The most recent chronic risk assessment for glyphosate was published by EFSA in January 2012 in support of the application to set an import tolerance for glyphosate in lentils[3]. In that assessment, EFSA used the MRL values for most crops, and added the median residue value of 1.47 mg/kg for lentils, based on data in the import tolerance petition.

[2] Revision 2.0 of the EFSA model, downloaded Sep 2011. Reasoned Opinion on the Potential Chronic and Acute Risk to Consumers' Health Arising from Proposed Temporary EU MRLs According to Regulation (EC) No 396/2005 on Maximum Residue Levels of Pesticides in Food and Feed of Plant and Animal Origin, European Food Safety Authority, 15 March 2007

[3] European Food Safety Authority; Modification of the existing MRL for glyphosate in lentils. EFSA Journal 2012;10(1):2550. [25 pp.] doi:10.2903/j.efsa.2012.2550. Available online: www.efsa.europa.eu/efsajournal

Residue input values for several glyphosate-tolerant crops were conservatively calculated as the sum of the glyphosate MRL and a proposed AMPA MRL, expressed as glyphosate. These calculated residue input values were: rape seed (10.8 mg/kg), soybean (28.4 mg/kg) and maize (2.6 mg/kg). The AMPA MRLs were proposed in the 2000 Germany peer review[4] but were not included in the MRL legislation.

Using the above input values and the current established ADI of 0.3 mg/kg, the total calculated intake values accounted for up to 46.7% of the ADI (WHO Cluster B).

Based on toxicology data presented in this dossier, the proposed ADI for glyphosate has been increased to 3.0 mg/kg bw/day. A revised chronic risk assessment has been conducted using the proposed ADI. The residue level present in each commodity is set at the MRL (see Table 6.7.2-1). In addition, the proposed MRL of 10 mg/kg in lentils (see Document E-2) is also included in the assessment.

The TMDI calculation gives an unrealistic worst-case estimate of intake, because it assumes that 100% of crops with established and proposed uses will contain residues at the MRL. No account is taken of the potential reduction in residues during transport and storage or during commercial and domestic processing. In practice, the actual intake is likely to be much lower than the calculated values. Details of TMDI calculations for glyphosate are presented in Table 6.9.1-1.

For all population groups in all models the estimated TMDI is at or below 4.4% of the ADI. The results indicate that there is no unacceptable chronic risk to human health from the consumption of commodities treated with glyphosate according to the uses considered.

[4] Germany, 2000. Complete list of end points (available on CIRCA in "Archive individual substances/glyphosate")

Table 6.9.1-1: TMDI calculation of glyphosate (EFSA model rev. 2), based on EU MRLs

Glyphosate			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):		proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	3.0	ARfD (mg/kg bw):	n.n.
Source of ADI:	2 year rat study	Source of ARfD:	
Year of evaluation:	2012 submission	Year of evaluation:	

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the current established EU MRLs as of March 2012, plus a proposed import MRL for lentils (dry) at 10 mg/kg. A proposed ADI of 3.0 mg/kg bw/day was used.

Chronic risk assessment - refined calculations

	TMDI (range) in % of ADI	
	0	4
No of diets exceeding ADI:	---	0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
4.4	WHO Cluster diet B	2.8	Wheat	0.5	Sunflower seed	0.4	Soya bean	0.0
3.7	DK child	1.8	Wheat	1.5	Rye	0.3	Oats	0.0
3.3	WHO cluster diet D	2.2	Wheat	0.3	Sunflower seed	0.2	Soya bean	0.0
3.1	WHO cluster diet E	1.3	Wheat	0.5	Barley	0.4	Soya bean	0.0
2.7	WHO Cluster diet F	1.2	Wheat	0.4	Soya bean	0.4	Barley	0.0
2.3	IE adult	0.8	Barley	0.8	Wheat	0.1	Sunflower seed	0.0
2.3	UK Toddler	1.3	Wheat	0.8	Sugar beet (root)	0.1	Potatoes	0.0
2.3	IT kids/toddler	2.2	Wheat	0.0	Potatoes	0.0	Wild fungi	0.0
2.1	DE child	1.4	Wheat	0.3	Rye	0.1	Oats	0.0
2.1	NL child	1.6	Wheat	0.1	Potatoes	0.1	Oats	0.0
2.0	PT General population	1.3	Wheat	0.2	Soya bean	0.2	Sunflower seed	0.0
1.8	ES child	1.5	Wheat	0.1	Sunflower seed	0.1	Lentils	0.0
1.6	UK Infant	0.9	Wheat	0.3	Sugar beet (root)	0.2	Oats	0.0
1.6	WHO regional European diet	1.0	Wheat	0.2	Barley	0.1	Sunflower seed	0.0
1.5	IT adult	1.4	Wheat	0.0	Wild fungi	0.0	Potatoes	0.0
1.4	FR all population	1.1	Wheat	0.2	Sunflower seed	0.1	Wine grapes	0.0
1.3	ES adult	0.8	Wheat	0.3	Barley	0.1	Sunflower seed	0.0
1.3	SE general population 90th percentil	1.1	Wheat	0.1	Rye	0.1	Potatoes	0.0
1.2	FR toddler	0.9	Wheat	0.1	Sunflower seed	0.1	Potatoes	0.0
1.1	NL general	0.7	Wheat	0.2	Barley	0.0	Potatoes	0.0
1.1	DK adult	0.7	Wheat	0.2	Rye	0.1	Oats	0.0
1.0	UK vegetarian	0.7	Wheat	0.1	Sugar beet (root)	0.0	Oats	0.0
0.9	LT adult	0.4	Rye	0.4	Wheat	0.1	Oats	0.0
0.8	UK Adult	0.6	Wheat	0.1	Sugar beet (root)	0.0	Potatoes	0.0
0.7	FI adult	0.3	Wheat	0.2	Rye	0.1	Oats	0.0
0.5	FR infant	0.3	Wheat	0.1	Potatoes	0.0	Milk and cream,	0.0
0.1	PL general population	0.1	Potatoes	0.0	Peas	0.0	Apples	0.0

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Glyphosate is unlikely to present a public health concern.

IIA 6.9.2 NEDI calculations

Refined NEDI calculations are not necessary since the unrefined TMDI for glyphosate based on MRLs was below 100% of the ADI.

IIA 6.9.3 NESTI calculations

Since no acute reference dose has been set or proposed for glyphosate, acute risk assessments are not required.

IIA 6.10 Other/special studies

IIA 6.10.1 Literature Review

Literature Search Methodology

Monsanto Company has been conducting routine surveillance of technical literature for glyphosate-related publications in a structured fashion since early 1997. During the period from 1997 to the present time, the search process and the literature databases used have been modified as new resources and technology became readily available. The technical databases that are used for the search include: Web of Science^{SM}, BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches are done on glyphosate acid, glyphosate salts (including isopropyl amine, potassium, ammonium, and methylamine), and AMPA, and their related chemical names and CAS numbers. Searches based on these search terms will also identify publications that consider glyphosate and surfactants, (such as polyoxyethylenealkylamines, or POEA), in the context of glyphosate formulations.

Starting from the ongoing Monsanto literature database, all the peer-reviewed publications covering the time period from 2001 through 2010 that relate to the four key disciplines addressing exposure and hazard (toxicology, ecotoxicology, residues and environmental fate) were assessed within the appropriate discipline for inclusion in the literature review for the submission. Some publications address more than one discipline, and are included in each relevant discipline. More recent publications have continued to be reviewed up to shortly before submission, and selected publications have been included.

At the request of the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), additional publications cited in a recent document prepared by Earth Open Source[5] have also been included in the literature review. Many of the cited peer-reviewed publications were already included, but others were not within the scope of this literature review, primarily because the publication date was prior to 2001. The additional peer-reviewed publications have been included and are discussed within the appropriate discipline.

The peer-reviewed publications identified for inclusion during the literature search were reviewed within each discipline and classified into one of the categories listed below.

- **Category 0 publications**: These are publications in which glyphosate is only mentioned as an example substance or is discussed/studied in a context that is not relevant or related to any of the

[5] Earth Open Source report. 2011. Roundup and birth defects: Is the public being kept in the dark? Authored by Antoniou M, Habib MEEM, Howard CV, Jennings RC, Leifert C, Nodari RO, C Robinson, Fagan J. Available from: http://www.earthopensource.org/files/pdfs/Roundup-and-birth-defects/RoundupandBirthDefectsv5.pdf

regulatory sections or the exposure/hazard assessments within this submission; the publication is therefore outside of the scope of this submission.

- **Category 1 publications**: These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and the conclusions fall within the conclusions of the exposure/hazard assessment. The publication is submitted with minimal or no comment or discussion.
- **Category 2 publications**: These are publications which discuss glyphosate in a context relevant or related to the regulatory dossier sections and have conclusions that call into question the endpoints/conclusions in the exposure/hazard assessment. Additionally, Category 2 also includes publications with conclusions that support the risk/hazard assessment, and may be included in discussion of other relevant publications. For selected Category 2 publications, an OECD Tier-II type summary is provided in addition to a reliability assessment (Klimisch rating, see Klimisch et al. 1997); limited comments and critical remarks are provided, as appropriate.
- **Category 3 publications**: These are publications that discuss glyphosate in a context relevant or related to (1) non-regulatory endpoints that need to be addressed as per new Regulation (EC) 1107/2009; or (2) in a context relevant to sensitive allegations that have emerged or could emerge in the media; or (3) in a context relevant to the regulatory dossier sections and have conclusions that are in disagreement with endpoints/conclusions in the exposure/hazard assessment (although the experimental design seems relevant at first glance). An OECD Tier-II type summary is provided and a Klimisch rating assigned, and supplemented with critical review and discussion.
- **Category 'E' publications**: These are peer-reviewed publications that were cited in the Earth Open Source document. This category includes publications that were already captured by the literature search and are addressed within the appropriate discipline, as well as publications that were out of scope of the search (primarily as a result of being published prior to 2001). Publications already captured in the literature search were assigned a Category 1, 2 or 3 rating (as appropriate) in addition to a Category 'E' rating. An OECD Tier-II type summary has been prepared and a Klimisch rating assigned for each of the Category E publications. All Category 'E' publications are reviewed within the appropriate discipline, with most of the reviews provided within the toxicology dossier under Section IIA 5.10.

Approximately 2000 peer-reviewed publications from the Monsanto technical literature database were assessed, and of those about 1000 were assigned a Category 1, 2 or 3 and selected for inclusion in the submission.

A full description of the literature search methodology is provided in a separate document (Carr and Bleeke, 2012).

The publications selected for inclusion are listed in Document L for each respective section, under the Annex point for 'Other/Special Studies': Point IIA 5.10 (Toxicology), Point IIA 6.10 (Metabolism and Residue), Point IIA 7.13 (Environmental Fate), and Point IIA 8.16 (Ecotoxicology). Under each point, the list of Other/Special Studies is presented in three tables:

- Table 1 lists other relevant studies conducted by the Glyphosate Task Force or member companies in support of the submission, that do not fit within any other dossier points .
- Table 2 lists all the relevant peer-reviewed publications from the literature that were selected for inclusion in the submission. For each publication it is noted whether or not a Klimisch rating is included in the review.

- Table 3 lists the publications and other documents that are cited within the discussion of the literature. These include documents such as government or company reports; publications that are included in the literature review under another section of the dossier; and publications that are outside the scope of the literature review.

Overview of Residue Literature

There have been a number of articles on glyphosate that have been published since the last submission that are related to residues. None of these contain information that are counter to the conclusions drawn in the dossier. A brief over the relevant literature is given, followed by a more detailed summary and Klimisch rating for one publication. A list of references is provided at the end.

Several recent publications addressed various aspects of glyphosate metabolism in plants. Glyphosate tolerant (GT) soybeans was shown to metabolize glyphosate to AMPA (Duke, 2011). Glutathione transferase activity in maize increased following application of glyphosate, suggesting (but not confirming) it may be involved in the degradation of glyphosate in maize (Cataneo et al., 2003). Recent work on velvet bean, which has a high innate tolerance to glyphosate, investigated the uptake, translocation and metabolism of glyphosate in the plant, and concluded that the tolerance may due to a combination of limited uptake, impaired translocation and enhanced degradation (Rojano-Delgado et al., 2012). A metabolic scheme involved degradation of glyphosate to sarcosine and glycine, which has been observed in microbial degradation but not in plants, was proposed, based on chromatographic retention times of the products.

The uptake of glyphosate via the roots was explored. Corn seedlings grown in hydroponic solution took up glyphosate through the roots, with the apex as the principal sink following translocation (Wagner et al., 2003). In another publication, rape and barley seeds planted into soil 6.5 months after application of glyphosate to the soil took up very low levels (0.002-0.005%) of the applied glyphosate (Simonsen et al., 2008).

Most of the work involving field trials and analysis of residues was done with GT soybeans (Arregui et al., 2004; Bohm et al., 2008; Duke et al., 2003; Reddy et al., 2008). Additional studies examined residues in immature GT and non-GT soybeans (Lorenzatti et al., 2004), peas, barley and flax following a preharvest application (Cessna et al., 2002), and plant materials gathered from the forest following forestry applications of glyphosate (Ando et al., 2003).

Cereal samples collected in Denmark and analyzed for glyphosate and two plant growth regulators showed the presence of glyphosate in over half the cereal samples, averaging 0.08-0.11 mg over the two years they were analyzed (1998-1999) (Granby and Vahl, 2001). All residues were below the MRL.

One study looked at the effect of breadmaking on residues of glyphosate in wheat (Low et al., 2005), and showed a partial degradation of glyphosate during the fermentation cycle. Use of glyphosate in preharvest wheat can lead to higher residues of shikimic acid in grain and flour when applied at the soft-dough stage, 21 days prior to harvest. Other studies looked at the effects of glyphosate residues on the malting of barley (Caierao and Acosta, 2007) and rumen fermentation in sheep (Huther et al., 2005), and found no effect of glyphosate on the processes.

Finally, several publications determined the dietary exposure to glyphosate residues in Cameroon (Gimou et al., 2008), the EU (Harris and Gaston, 2004), and France (Nougadère et al., 2011), with exposure well with the ADI in all cases.

Detailed Summary and Klimisch Rating

Annex point	Author(s)	Year	Study title
AII 6.10.1	Rojano-Delgado, A.M., Cruz-Hipolito, H., De Prado, R., Luque de Castro, M.D., Rodriguez Franco, A.	2012	Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in *Mucuna pruriens* var. *utilis* plants. Phytochemistry Volume: 73 Pages: 34-41 DOI: 10.1016/j.phytochem.2011.09.007 ISSN: 0031-9422

Abstract[6]

Velvet bean (*Mucuna pruriens*, Fabaceae) plants exhibits an innate, very high resistance (i.e., tolerance) to glyphosate similar to that of plants which have acquired resistance to this herbicide as a trait. We analyzed the uptake of [^{14}C]-glyphosate by leaves and its translocation to meristematic tissues, and used scanning electron micrographs to further analyze the cuticle and 3D capillary electrophoresis to investigate a putative metabolism capable of degrading the herbicide. Velvet bean exhibited limited uptake of glyphosate and impaired translocation of the compound to meristematic tissues. Also, for the first time in a higher plant, two concurrent pathways capable of degrading glyphosate to AMPA, Pi, glyoxylate, sarcosine and formaldehyde as end products were identified. Based on the results, the innate tolerance of velvet bean to glyphosate is possibly a result of the combined action of the previous three traits, namely: limited uptake, impaired translocation and enhanced degradation.

MATERIALS AND METHODS

1. Test material:

Test item: Roundup ®
Active substance(s): Glyphosate
Adjuvant: Not stated
Description: none
Source of test substance: Monsanto
Lot/Batch #: Not stated
Purity: Not stated

2. Vehicle and/or positive control: Glyphosate[glycine-2-^{14}C] (specific activity 273.8 MBq/mmol) as marker for glyphosate uptake and translocation assays

3. Test organism:

Species: *Mucuna pruriens* (glyphosate tolerant plant)
Amaranthus retroflexus (glyphosate susceptible)
Source: Seeds were collected in 2009 in Martinez de la Torre, Veracruz, Mexico.
Holding conditions prior to exposure: Seeds were germinated in pots containing peat and sandy loam (1:2 v/v) in a growth chamber at 28/18 °C with a 16 h photoperiod under 850 µmol/m²·s and 80% relative humidity.

[6] Quoted from article

Crop growth stage at treatment:	Third pair of true leaves present

4. Test system:

Study type:	Four different experimental setups
Guideline:	None.
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Dose response assay: 21 days Whole plant shikimic acid, [^{14}C] glyphosate and translocation assay: 96 h Metabolism: 672 h
Treatments:	Dose response, shikimic acid assay and glyphosate metabolism: Treatments were applied to the third pair of true leaves with laboratory track sprayer with TeeJet 80.02.E.VS flat fan nozzle delivering 200 L/ha at 200 kPa. [^{14}C]-glyphosate uptake and translocation assays: [^{14}C]-glyphosate was mixed with formulated glyphosate to prepare emulsions with a specific activity of 1.85 kBq/µL. The test item was applied to the axial surface of second leaf of each plant in four 0.5 µL droplets using a PB 600 TA micro applicator.
Replicates per concentration/harvest time:	Dose response assay: 4 per treatment and each test was conducted three times Shikimic acid assay: 10 per harvest time and each test was conducted three times [^{14}C]-glyphosate uptake and translocation assays: 3 Glyphosate metabolism: 5 per harvest time
Plants per replicate:	Dose response assay: 3 plants/pot Shikimic acid assay: 'several tissue samples' Glyphosate metabolism: 4

Parameters measured:	Dose response assay: Shoot fresh weight after 21 days Shikimic acid assay: Shikimic acid accumulation was determined spectrophotometrically 6, 12, 24, 48, 72 and 96 h after treatment. [^{14}C]-glyphosate uptake and translocation assays: Radioactivity was quantified by LSS in dried samples collected 12, 24, 48 and 96 h after application. Translocation was determined after samples were pressed for 6 h on phosphor storage film and scanned for radiolabel dispersion. Data were compared via ANOVA followed by Tukey's HSD test as a post-hoc test. Glyphosate metabolism: Glyphosate and its metabolites (AMPA, glyoxylate, sarcosine and formaldehyde) were determined by electrophoresis from samples collected 0, 72, 96, 168, 336, 504 and 672 h after treatment.
Test concentrations:	Dose response assay: *A. retroflexus*: 0, 12, 25, 50, 100 and 200 g a.e./ha *M. pruriens*: 0, 350, 400, 450, 500, 550, 600 g a.e./ha Shikimic acid assay: 500 g a.e./ha [^{14}C]-glyphosate uptake and translocation assays: 3.6 g a.i./L, corresponding to 720 g a.i./ha at 200 L/ha. Glyphosate metabolism: 500 g a.e./ha
Analytical determination of test concentrations:	Not measured
5. Environmental conditions:	Not specified

KLIMISCH EVALUTION

1. Reliability of study: **Reliable with restrictions**.

Comment:

- Unclear, whether same formulation was used for all four experiments
- Wavelengths for spectrophotometrical measurements not stated.
- Unclear, on which plants in terms of exposure SEM data were collected.
- No analytical verification of test substance
- Characterization of new pathway in plants based only on identification of metabolites by retention time in single method.
- Metabolites formed in new proposed pathway (sarcosine, formaldehyde and glycine) are natural products. Analysis does not distinguish between glyphosate-derived and plant-derived metabolites.
- Formulation of unknown origin/content of adjuvants or surfactants.

2. Relevance of study: **Not relevant**

Comment: Unclear which formulation was tested, test concentrations are not reproducible, and glyphosate metabolic pathway not verified.

Hence, study is not considered to be relevant.

3. Klimisch code: **Klimisch rating of 3.**

References

Ando, C., R. Segawa, C. Gana, L. Li, J. Walters, R. Sava, T. Barry, K.S. Goh, P. Lee, D. Tran, J. White, and J. Hsu. 2003. Dissipation and offsite movement of forestry herbicides in plants of importance to native Americans in California National Forests. Bulletin of Environmental Contamination and Toxicology 71:354-361.

Arregui, M.C., A. Lenardon, D. Sanchez, M.I. Maitre, R. Scotta, and S. Enrique. 2004. Monitoring glyphosate residues in transgenic glyphosate-resistant soybean. Pest Management Science 60:163-166.

Bohm, G.M.B., M.I. Genovese, G. Pigosso, D. Trichez, and C.V. Rombaldi. 2008. Residues of glyphosate and aminomethylphosphonic acid and levels of isoflavones in BRS 244 RR and BRS 154 soybean. Ciência e Tecnologia de Alimentos 28:192-197.

Caierao, E., and A.D.S. Acosta. 2007. Industrial suitability for malting of grains from desiccated pre-harvest barley. Pesquisa Agropecuaria Brasileira 42:1277-1282.

Cataneo, A.C., G.F.G. Déstro, L.C. Ferreira, K.L. Chamma, and D.C.F. Sousa. 2003. Glutathione S-transferase activity on the degradation of the herbicide glyphosate in maize (Zea mays) plants. Planta Daninha 21:307-312.

Cessna, A.J., A.L. Darwent, L. Townley-Smith, K.N. Harker, and K.J. Kirkland. 2002. Residues of glyphosate and its metabolite AMPA in field pea, barley and flax seed following preharvest applications. Canadian Journal of Plant Science 82:485-489.

Duke, S.O. 2011. Glyphosate Degradation in Glyphosate-Resistant and -Susceptible Crops and Weeds. J Agric Food Chem 59:5835-5841.

Duke, S.O., A.M. Rimando, P.F. Pace, K.N. Reddy, and R.J. Smeda. 2003. Isoflavone, glyphosate, and aminomethylphosphonic acid levels in seeds of glyphosate-treated, glyphosate-resistant soybean. Journal of Agricultural and Food Chemistry 51:340-344.

Gimou, M.M., U.R. Charrondiere, J.C. Leblanc, and R. Pouillot. 2008. Dietary exposure to pesticide residues in Yaounde: the Cameroonian total diet study. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25:458-471.

Granby, K., and M. Vahl. 2001. Investigation of the herbicide glyphosate and the plant growth regulators chlormequat and mepiquat in cereals produced in Denmark. Food Additives and Contaminants 18:898-905.

Harris, C.A., and C.P. Gaston. 2004. Effects of refining predicted chronic dietary intakes of pesticide residues: a case study using glyphosate. Food Additives and Contaminants 21:857-864.

Huther, L., S. Drebes, and P. Lebzien. 2005. Effect of glyphosate contaminated feed on rumen fermentation parameters and in sacco degradation of grass hay and corn grain. Archives of Animal Nutrition 59:73-79.

Lorenzatti, E., M.I. Maitre, L. Argelia, R. Lajmanovich, P. Peltzer, and M. Anglada. 2004. Pesticide residues in immature soybeans of Argentina croplands. Fresenius Environmental Bulletin 13:675-678.

Low, F.L., I.C. Shaw, and J.A. Gerrard. 2005. The effect of Saccharomyces cerevisiae on the stability of the herbicide glyphosate during bread leavening. Letters in Applied Microbiology 40:133-137.

Nougadère, A., J.-C. Reninger, J.-L. Volatier, and J.-C. Leblanc. 2011. Chronic dietary risk characterization for pesticide residues: A ranking and scoring method integrating agricultural uses and food contamination data. Food and Chemical Toxicology 49:1484-1510.

Reddy, K.N., A.M. Rimando, S.O. Duke, and V.K. Nandula. 2008. Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. J Agric Food Chem 56:2125-2130.

Rojano-Delgado, A.M., H. Cruz-Hipolito, R. De Prado, M.D. Luque de Castro, and A.R. Franco. 2012. Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in Mucuna pruriens var. utilis plants. Phytochemistry 73:34-41.

Simonsen, L., I.S. Fomsgaard, B. Svensmark, and N.H. Spliid. 2008. Fate and availability of glyphosate and AMPA in agricultural soil. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes 43:365 - 375.

Wagner, R., M. Kogan, and A.M. Parada. 2003. Phytotoxic activity of root absorbed glyphosate in corn seedlings (Zea mays L.). Weed Biology and Management 3:228-232.

IIA 6.11 Summary and evaluation of residue behaviour, reasonable grounds in support of the petition

IIA 6.11.1 Summary and evaluation of residue behaviour

The results of numerous plant uptake and metabolism studies demonstrate that glyphosate is slowly metabolised in plants to AMPA. With only a few exceptions (some soybean commodities and hydroponically-grown maize forage where AMPA levels were comparable to or greater than glyphosate levels), glyphosate is the major compound present in plant tissues. In all cases, AMPA accounts for less than 27% of the radioactive residues, and typically is less than 10%. With the exception of AMPA, no other metabolites of glyphosate are detected that account for greater than 5% of the total radioactive residues.

Numerous supervised residue trials have been conducted to establish MRLs for glyphosate. In cases where residues resulting from different glyphosate formulations have been compared in side-by-side field trials, no differences were been found. Thus, it is possible to extrapolate from data obtained on the active substance in accordance with the requirements of Annex II 6.3.

Good agricultural practices for the application of glyphosate can be grouped into six categories based on the types of applications:

a. Pre-harvest broadcast applications yielding detectable glyphosate residues that require establishment of MRLs.
b. Applications prior to crop emergence that result in undetectable glyphosate residues.
c. Grassland applications.
d. Directed spray applications underneath the foliage of existing crops (post-directed applications).
e. Selective equipment applications (e.g. recirculating sprayer and wiper applicator applications).
f. Forestry applications.

In-crop, pre-harvest applications are currently approved in various European Union Member States for cereals (wheat, barley, oats, and rye), pulses (beans and peas), oil seed crops and forage grasses. Maximum glyphosate residues in grain and seed resulting from pre-harvest applications according to approved uses reached 20 mg/kg.

A major method of glyphosate application is a pre-plant or pre-emergence treatment that does not result in significant residues.

Upon review of the database supporting the current uses, it was determined that while there were numerous residue studies of pre-plant and pre-emergence applications in a variety of crops, many were older, non-GLP studies and did not always represent the current GAP. In order to provide an up-to-date set of studies, a representative set of trials was recently conducted. The glyphosate and AMPA residues for all trials of all crops were below the LOQ (<0.05 mg/kg), and therefore support the existing MRLs of 0.1 mg/kg for pre-plant/pre-emergence uses.

EU MRLs were adopted and included in Annex II of Regulation (EC) No 396/2005, which adequately support claimed uses (**COMMISSION REGULATION (EC) No 839/2008 of 31 July 2008 and COMMISSION REGULATION (EC) No 149/2008 of 29 January 2008**).

The ADI for glyphosate has been proposed at 3.0 mg/kg bw/day. Since no acute reference dose has been set or proposed for glyphosate, acute risk assessments are not required.

Theoretical Maximum Daily Intakes (TMDI) calculations using the EFSA model rev. 2 were conducted to assess the chronic dietary exposure.

TMDI calculation resulted in an ADI utilisation of 4.4% (EFSA model) indicating that there is no chronic risk for any population group. Since the calculations are based on 100% market share for glyphosate in all target crops, the assessments represent an unrealistic worst case. The actual consumer risk is considerably lower.

IIA 6.11.2 Reasonable grounds in support of the petition

No EC data requirement.