GLYPHOSATE (158)

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EXPLANATION

Glyphosate was listed in the Periodic Re-evaluation Programme of the Thirty-fourth Session of the CCPR for residue review by 2005 JMPR. It has been reviewed by the JMPR in 1986 (TR), 1987 (R), 1988 (R), 1994 (R), 1997 (TR), 2004 (T), 2005 (R) and 2011 (R).

For the current evaluation the Meeting received critical data required for the estimation of MRLs for glyphosate tolerant rape (with the *gat* trait).

The glyphosate tolerant crops with the *gat* trait have been inserted with a glyphosate N-acetyltransferase gene which inactivates glyphosate by converting it to N-acetylglyphosate, making it the main metabolite in plant commodities. The Meeting received data on glyphosate metabolism in glyphosate tolerant rape crops, methods of residue analysis, storage stability, use patterns (USA and Canada), data from supervised residue trials and processing studies.

Trivial and systematic chemical names of all glyphosate related compounds referenced in the study reports submitted for the evaluation of the use of glyphosate on maize and soya bean with the *gat* trait are shown below in Table 1. These genetically modified crops lead to a different metabolic pathway of glyphosate in plants than in conventional crops or crops genetically modified to contain the CP4-EPSPS gene making them tolerant for glyphosate.

Table 1 List of reference compounds used in various study reports, including reports evaluated in previous JMPR reports

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports	Found as or in
Gly	metabolite 1/parent glyphosate N-(phosphonomethyl)glycine C3H8NO5P DPX-B2856	Rat, goat, hen
AMPA	metabolite 2 AMPA (aminomethyl)phosphonic acid H2NCH2PO3H IN-YB726	Rat, goat, hen
NA-AMPA	metabolite 6 N-acetyl AMPA [(acetylamonio)methyl]phosphonic acid CH3C(O)NHCH2PO3H2 IN-EY252	Rat, goat, hen
NA-Gly	metabolite 9 N-acetylglyphosate N-acetyl-N-(phosphonomethyl)glycine IN-MCX20	Rat, goat, hen

METABOLISM AND ENVIRONMENTAL FATE

Plant metabolism

The Meeting received information on the fate of glyphosate after single pre-emergence application followed by three foliar applications on rape crops containing the *gat* gene. Studies were conducted with [phosphonomethyl-^{14}C] glyphosate under glasshouse conditions at a total rate of approximately 7.5 kg ai/ha.

The metabolism of [^{14}C] glyphosate was investigated in *0827* rape plants containing the *gat* gene [Chapleo & McLachlan, 2010, DuPont-26109]. A single pre-emergence soil application (4.5 kg ai/ha) followed by three foliar applications (each 1.0 kg ai/ha at the 2 and 5 leaf stage and 1 week before maturity) were made of an aqueous solution containing [^{14}C] glyphosate (SL formulation and 2% ammonium sulphate salt). The intervals between the four applications were 48, 9 and 83 days, respectively. Rape plants were harvested as immature foliage (midway between the 2nd and 3rd foliar applications), immediately prior to the last application (pods with seeds, foliage) and finally at maturity whereupon plants were separated into seeds and foliage fractions. After homogenization tissues were extracted with 0.1% formic acid (aqueous):methanol (96:4, v/v) followed by enzyme (α-amylase then amyloglucosidase and cellulase), alkaline (NaOH, 0.1 N, 60 °C, 6 hours) then acid (HCl, 1.0 N, 60 °C, 6 hours) digestion. Extracts containing ≥ 0.01 mg/kg were analysed by HPLC and the identification of residues accomplished with reference to authenticated reference standards.

Table 2 Identification of radioactivity in plants and seed

	Harvest 1		Harvest 2				Harvest 3	
	Foliage		Immature pods (with seeds)		Foliage		Mature seeds	
TRR (mg equiv/kg)	5.98		1.27		1.55		2.15	
				%TRR				
Extracted	97		80		96		96	
glyphosate		3.0		ND		ND		21
N-acetylglyphosate		90		80		93		51
AMPA		1.4		ND		ND		1.9
N-acetyl AMPA		3.4		ND		ND		15
Unidentified		ND		ND		ND		7.1 [a]
Unextracted	2.7		20		3.6		3.5	

ND = not detected

[a] Multiple components, no single component > 1% TRR or 0.022 mg equiv/kg.

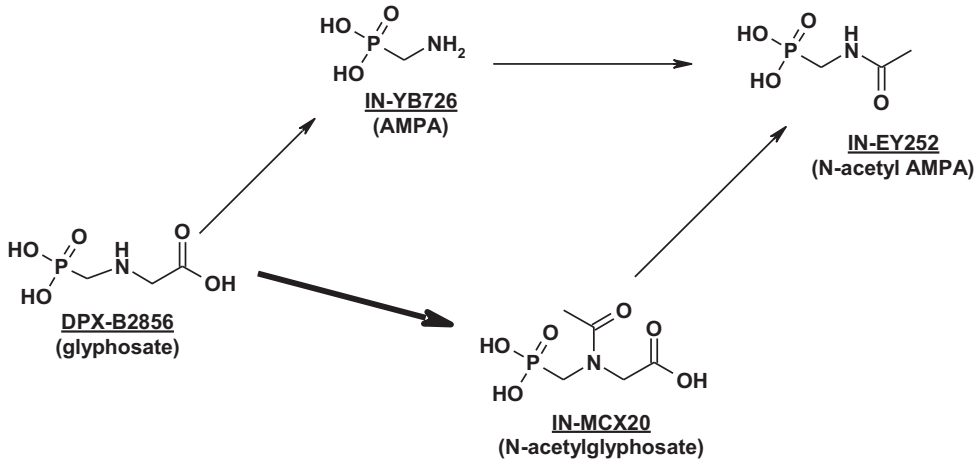

Figure 1 Proposed metabolic pathway for [^{14}C] glyphosate in 0827 rape seed (canola) plants containing the *gat* gene

RESIDUE ANALYSIS

Analytical methods

The Meeting received information on methods for the determination of glyphosate and some of its metabolites in rape commodities (DuPont 27816 Rev 1). The methods were similar to those reviewed by the 2011 JMPR and are suitable for measuring residues in rape commodities.

Residues of glyphosate, N-acetylglyphosate, N-acetyl AMPA, and AMPA were extracted from forage, seed, and meal in 96% aqueous 0.1% formic acid/4% methanol. Clean-up included partitioning with methylene chloride and passage through a C_{18} SPE cartridge. A portion of the C_{18} purified sample was further cleaned-up on an anion exchange cartridge before analysis for glyphosate, N-acetylglyphosate and N-acetyl AMPA with glyphosate internal standard. Another aliquot of the C_{18} purified extract was further cleaned-up on a cation exchange cartridge before analysis for AMPA with AMPA internal standard.

Residues of glyphosate, N-acetylglyphosate, N-acetyl AMPA, and AMPA were extracted from oil via direct partition into 0.02 M phosphoric acid, with methylene chloride present to retain the oily components. An aliquot of the aqueous sample was diluted prior to analysis for glyphosate, N-acetylglyphosate, N-acetyl AMPA, and AMPA after addition of both glyphosate and AMPA internal standards to each sample. The sample was injected twice, once for analysis of glyphosate, N-acetylglyphosate and N-acetyl AMPA and a second time for AMPA. The LOQ was 0.05 mg/kg for each analyte (glyphosate and metabolites). Mean recoveries for forage, seed, refined oil and meal samples fortified at levels that ranged from 0.05 to 30 mg/kg were acceptable ranging from 78 ± 9.7% to 97 ± 14% for the various analytes.

Stability of pesticide residues in stored analytical samples

The 2005 and 2011 JMPR meetings evaluated data on the storage stability of glyphosate residues (and metabolites) in plant commodities that included commodities with high oil content (corn and soya bean), and in animal commodities. The studies concluded residues in high oil commodities are stable for more than 12 months freezer storage. The longest storage interval in the current trials was 302 days.

USE PATTERNS

Glyphosate is registered as an herbicide on a wide variety of crops. Information on registered uses was made available to the Meeting and those uses of relevance to this evaluation, which are supported by supervised residue trials and based on label information provided by the manufacturers, are summarized in Table 3.

Table 3 Table of use patterns

Crop	Country	Method	Growth stage	Rate kg ai/ha	No.	L/ha	PHI (days)	Comment
Rape seed tolerant varieties, *gat* gene	Canada	Broadcast spray (ground or aerial)	Pre-emergence	0.68	–		na	
		Broadcast spray (ground or aerial)	0–6 leaf stage	0.3–0.68	–	50–100	7	
		Broadcast spray (ground or aerial)	Pre-harvest	0.9	–	50–100	7 [a]	
Rape seed tolerant varieties, *gat* gene	USA (pending)	Broadcast spray (ground or aerial)	pre-emergence (A)	≤ 1.7 (seasonal max 1.7 kg/ha pre-emergence)	≥ 1			The combined total per year for pre- and post-emergent (A+B) = (1.7 + 0.84) =
		Broadcast	from crop	≤ 0.84	–single or		60	

Crop	Country	Method	Growth stage	Rate kg ai/ha	No.	L/ha	PHI (days)	Comment
		spray, in crop (over-the-top), ground or aerial	emergence to 6 leaf stage (B)	If two applications 0.42 at the 1–3 leaf stage + 0.42 at < 6 leaf stage but > 10 days apart.	split			2.5 kg ai/ha. The combined total max of post emergence applications = 0.84 kg ai/ha/year

[a] Pods are green to yellow; most seeds are yellow to brown. Apply when crop has < 30% grain moisture, typically 7–14 days before harvest

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on supervised residue trials of foliar treatments of glyphosate for the following crops:

Application rates were reported as glyphosate acid equivalents (parent). Unquantifiable residues are shown as below the reported LOQ (e.g. < 0.05 mg/kg). Residues, application rates and spray concentrations have been rounded to two figures. Residue data are recorded unadjusted for percentage recoveries or for residue values in control samples. Where multiple analyses were conducted on a single sample, the average value is reported. Where results from separate plots with distinguishing characteristics such as different formulations, varieties or treatment schedules were reported, results are listed for each plot.

Residues from the trials conducted according to critical GAP have been used for the estimation of maximum residue levels, STMR and HR values. Those results are underlined.

The Meeting received supervised residue trials on glyphosate tolerant rape containing the *gat* trait. Supervised residue trials on glyphosate tolerant rape (containing the *gat* trait) were conducted in the USA and Canada in 2009 (DuPont 27816 Rev 1). Residues in seeds are shown in Table 5. The maximum frozen storage interval was 302 days. Oil and meal processing samples were stored for 34 and 46 days, respectively.

Table 4 Summary of sprayers, plot sizes and field sample sizes in the supervised trials

Crop	Location	Year	Sprayer	Plot size	Sample size
Rape	Canada (Portage la Praine)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.5–0.6 kg seed 1.3–1.4 kg forage
Rape	Canada (Dundurn)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.5–0.55 kg seed 1.2–4.5 kg forage
Rape	Canada (Gibbons)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.5–0.9 kg seed 1.0–1.8 kg forage
Rape	Canada (Fort Sasks)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.7–1.0 kg seed 1.4–1.8 kg forage
Rape	Canada (Waldheim)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.7–1.3 kg seed 1.0–1.4 kg forage
Rape	Canada (Wakaw)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.15–0.65 kg seed 1.0–1.4 kg forage
Rape	Canada (Rosthern)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.55–0.7 kg seed 1.2–1.9 kg forage
Rape	Canada (Wellwood)	2009	ATV mounted CO_2 boom 6 nozzles	3.6 × 20 m^2	0.5–0.8 kg seed 1.0–1.3 kg forage
Rape	Canada (Wellwood)	2009	Tractor mounted/ATV mounted CO_2 boom 6 nozzles	3.6 × 20 m^2 3 × 20 m^2	0.5–1.4 kg seed 1.0–1.7 kg forage
Rape	Canada (Franklin)	2009	Tractor mounted/ATV mounted CO_2 boom 6 nozzles	3 × 20 m^2	0.6–1.0 kg seed 1.0–1.9 kg forage
Rape	USA (Frenchtown)	2009	Backpack CO_2 boom 4 nozzles	7.3 × 22 m^2	0.9–1.6 kg seed 2.0–2.2 kg forage
Rape	USA (Erie)	2009	Backpack CO_2 boom 4 nozzles	6 × 15 m^2 12 × 24 m^2	0.5 or 28 for oil pro kg seed 1.1 kg forage

Crop	Location	Year	Sprayer	Plot size	Sample size
Rape	USA (Perley)	2009	Backpack CO_2 boom 4 nozzles	12 × 18 m^2	0.5–1.0 kg seed 1.0–1.5 kg forage
Rape	USA (Jamestown)	2009	Backpack CO_2 boom 4 nozzles	6 × 15 m^2 12 × 24 m^2	1.0 or 28 for oil pro kg seed 1.1–1.2 kg forage
Rape	USA (Montpellier)	2009	Backpack CO_2 boom 4 nozzles	6 × 15 m^2	1.0–1.1 kg seed 1.2 kg forage
Rape	USA (Jerome)	2009	Tractor/ATV mounted boom	7.3 × 15 m^2 7.3 × 38 m^2	1.0 or 27 for oil pro kg seed 1.3–1.4 kg forage
Rape	USA (Ephrata)	2009	ATV mounted CO_2 boom 6 nozzles	3 × 61 m^2	1.3–2.8 kg seed 1.0–2.1 kg forage

Table 5 Residues of glyphosate and its metabolites in seeds of glyphosate tolerant rape (containing the *gat* trait). All residue concentrations are expressed in glyphosate equivalents (DuPont 27816 Rev 1)

Trial, state, country, year (variety)	N	kg ai/ha	L/ha	last treatment, timing	DALA	matrix	Gly	NA-gly	AMPA	NA-AMPA	Tot MRL	Tot diet
Portage la Praine, MB Canada 2009 DP- Ø73496-4	3 (30 75)	0.685 0.68 0.885	100 100 90	PRE BBCH 14 BBCH 87	8	seed	1.3	0.46	< 0.05	0.05	1.8	1.8
Dundurn, SK Canada 2009 DP-Ø73496-4	3 (42 89)	0.69 0.67 0.90	100 100 100	PRE BBCH 14–16 BBCH 89	7	seed	9.0	0.22	0.05	< 0.05	9.2	9.2
Gibbons, AB Canada 2009 DP-Ø73496-4	3 (44 78)	0.66 0.66 0.91	100 100 100	PRE BBCH 14–16 BBCH 83–84	7	seed	2.1	0.30	< 0.05	< 0.05	2.4	2.4
	3 (44 78)	0.67 0.67 0.93	100 100 100	PRE BBCH 14–16 BBCH 83–84	7 11 12 18 21 28	seed	2.3 2.2 2.3 3.5 c0.06 3.2 4.5	0.48 0.37 0.38 0.39 0.26 0.41	< 0.05 < 0.05 ND < 0.05 ND < 0.05	< 0.05 ND < 0.05 < 0.05 ND < 0.05	2.8 2.6 2.7 3.9 3.5 4.9	2.8 2.6 2.7 3.9 3.5 4.9
Fort Saskatchewan, AB Canada 2009 DP-Ø73496-4	3 (48 72)	0.67 0.68 0.88	100 100 100	PRE BBCH 14–16 BBCH 84	7	seed	0.82	0.61	ND	< 0.05	1.4	1.5
	3 (48 72)	0.67 0.67 1.2	100 100 100	PRE BBCH 14–16 BBCH 81	7	seed	1.4	0.96	ND	< 0.05	2.4	2.4
										Mean	1.9	1.95
Waldheim, SK Canada 2009 DP-Ø73496-4	3 (41 90)	0.70 0.69 0.92	100 100 100	PRE BBCH 16 BBCH 89	7	Seed	2.2	0.46	ND	< 0.05	2.7	2.7
	3 (41 90)	0.70 0.68 0.92	100 100 100	PRE BBCH 16 BBCH 88–89	7	Seed	2.7 c0.05	0.34	ND	< 0.05	3.0	3.1
										Mean	2.85	2.9
Wakaw, SK Canada 2009 DP-Ø73496-4	3 (50 93)	0.69 0.67 0.91	100 100 50	PRE BBCH 16 BBCH 80	6	Seed	4.9 c0.27	0.69	0.06	ND	5.6	5.6
	3 (48 87)	0.70 0.70 0.90	100 100 100	PRE BBCH 14–51	6	Seed	7.2 c0.13	3.1	0.08	< 0.05	10	10

Trial, state, country, year (variety)	N	kg ai/ha	L/ha	last treatment, timing	DALA	matrix	Gly	NA-gly	AMPA	NA-AMPA	Tot MRL	Tot diet
				BBCH 80								
										Mean	7.8	7.8
Rosthern, SK Canada 2009 DP-Ø73496-4	3 (42 93)	0.69 0.68 0.92	100 100 100	PRE BBCH 15–16 BBCH 85	6	Seed	3.1	0.29	< 0.05	< 0.05	3.4	3.5
	3 (42 93)	0.69 0.68 0.91	100 100 100	PRE BBCH 15–16 BBCH 85	6	Seed	2.4	0.28	< 0.05	< 0.05	2.7	2.7
										Mean	3.05	3.1
Wellwood, MB Canada 2009 DP-Ø73496-4	3 (34 87)	0.68 0.68 0.89	100 100 90	PRE BBCH 15 BBCH 89	6	Seed	0.91	2.8	< 0.05	< 0.05	3.7	3.8
	3 (34 87)	0.68 0.67 0.90	100 100 90	PRE BBCH 15 BBCH 89	6	Seed	0.82	1.6	ND	< 0.05	2.4	2.4
										Mean	3.05	3.1
Franklin, MB Canada 2009 DP-Ø73496-4	3 (34 87)	0.70 0.67 0.88	100 100 91	PRE BBCH 15 BBCH 89	6	Seed	0.41	0.34	ND	ND	0.75	0.77
	3 (34 87)	0.68 0.69 0.91	100 100 91	PRE BBCH 15 BBCH 89	6	Seed	0.46	0.34	ND	ND	0.80	0.82
										Mean	0.775	0.795
Frenchtown, NJ USA 2009 DP-Ø73496-4	2 (34)	1.8 0.63	281 280	PRE 6 leaf	55	Seed	ND	0.30	ND	0.05	0.31	0.38
Erie, ND USA 2009 DP-Ø73496-4	2 (28)	1.75 0.63	281 280	PRE BBCH 15	62	Seed	ND	0.52 c0.05	ND	0.10	0.53	0.66
	3 (28 55)	0.70 0.71 4.5	94 94 94	PRE BBCH 15 BBCH 85	7	Seed	0.61	56	< 0.05	1.1	57	57
Perley, MN USA 2009 DP-Ø73496-4	2 (28)	1.79 0.62	94 94	PRE BBCH 15	61 64 66 71 76 82	Seed	0.36 c1.5 0.37 0.05 ND 0.05 ND	1.4 c2.2 1.9 1.2 0.96 c1.7 1.3 1.1	ND c0.08 ND ND ND ND ND	0.08 0.08 0.08 0.08 0.08 0.05	1.8 2.3 1.3 1.0 1.3 1.1	1.9 2.3 1.4 1.1 1.4 1.1
Jamestown, ND USA 2009 DP-Ø73496-4	2 (33)	1.75 0.64	94 94	PRE BBCH 16	60	Seed	< 0.05	1.9	ND	0.55	1.9	2.5
	3 (33 54)	0.72 0.72 4.6	187 94 187	PRE BBCH 16 BBCH 85	6	Seed	4.8	12	< 0.05	0.27	17	17
Montpelier, ND USA 2009 DP-Ø73496-4	2 (33)	1.73 0.64	94 94	PRE BBCH 15	60	seed	< 0.05 c0.08	0.31	ND	0.06	0.35	0.42
	3 (33 54)	0.72 0.72 0.93	47 47 187	PRE BBCH 15 BBCH 85	6	Seed	0.71	14	ND	0.34	15	15
Jerome, ID USA 2009 DP-Ø73496-4	2 (32)	1.8 0.64	178 186	PRE BBCH14–16	69	Seed	ND	0.32	ND	< 0.05	0.33	0.38
	3 (32, 62)	0.67 0.68 4.5	176 186 196	PRE BBCH14–16 BBCH 89	7	Seed	14	0.47	0.13	ND	14.5	15

Trial, state, country, year (variety)	N	kg ai/ha	L/ha	last treatment, timing	DALA	matrix	Gly	NA-gly	AMPA	NA-AMPA	Tot MRL	Tot diet
Ephrata, WA USA 2009 DP-Ø73496-4	2 (20)	1.8 0.62	140 140	PRE 6 leaf	77 80 83 87 91 98	Seed	ND ND ND ND ND ND	0.26 0.23 0.23 0.24 0.20 0.19	ND ND ND ND ND ND	< 0.05 < 0.05 < 0.05 < 0.05 < 0.05 < 0.05	0.27 0.24 0.24 0.25 0.21 0.20	0.32 0.29 0.29 0.30 0.26 0.26
	2 (20)	1.7 0.62	47 47	PRE 6 leaf	66	Seed	ND	0.08	ND	ND	0.09	0.11

For additional containment, tents were used to cover the field plots at the Canadian locations. The tents were a white mesh material that reflected some light, resulting in taller, thinner plants than typical. The tents also created a warmer, more humid environment which served to prolong flowering and to delay maturity beyond what was normal for un-tented canola in the area. Consequently, the trials ran late into the season, it was harder to determine staging and timing, and in some provinces the crops were affected by frost before sampling occurred, despite efforts to protect the plants from frost damage by re-covering the plots with the tents following the third application. The use of mesh tents is unlikely to significantly affect residues in seed or forage.

Samples of forage were collected at times after planting typical for use of the crop as forage.

Table 6 Residues of glyphosate and its metabolites in forage of glyphosate tolerant rape (containing the *gat* trait). All residue concentrations are expressed in glyphosate equivalents (DuPont 27816 Rev 1).

Trial, state, country, year (variety)	N	kg ai/ha	L/ha	last treatment, timing	DALA	matrix	Gly	NA-gly	AMPA	NA-AMPA	Tot MRL	%moisture (corrected)
Portage la Praine, MB Canada 2009 DP- Ø73496-4	2 (30)	0.685 0.68	100 100 90	PRE BBCH 14	35	forage	ND	0.32	ND	ND	0.32	85 (2.1DM)
Dundurn, SK Canada 2009 DP-Ø73496-4	2 (42)	0.69 0.67	100 100 100	PRE BBCH 14–16	70	Forage	ND	0.19	< 0.05	ND	0.24	78 (1.1DM)
Gibbons, AB Canada 2009 DP-Ø73496-4	2 (44)	0.66 0.66	100 100	PRE BBCH 14–16	62	Forage	ND	0.42	ND	ND	0.42	79
	2 (44)	0.67 0.67	100 100	PRE BBCH 14–16	35 38 41 48 55 62	Forage	ND ND ND ND ND ND	0.94 0.38 0.73 0.45 0.39 0.50	ND ND ND ND ND ND	< 0.05 ND ND ND ND ND	0.99 0.38 0.73 0.45 0.39 0.50	79 (4.7DM)
Fort Saskatchewan, AB Canada 2009 DP-Ø73496-4	2 (48)	0.67 0.68	100 100	PRE BBCH 14–16	64	Forage	ND	0.43	ND	ND	0.43	79
	2 (48)	0.67 0.67	100 100	PRE BBCH 14–16	64	Forage	ND	0.25	ND	ND	0.25	76
										Mean	0.34	(1.5DM)
Waldheim, SK Canada 2009 DP-Ø73496-4	2 (41)	0.70 0.69	100 100	PRE BBCH 16	76	Forage	ND	0.74	ND	ND	0.74	66
	2 (41)	0.70 0.68	100 100	PRE BBCH 16	76	Forage	ND	1.3	ND	< 0.05	1.4	68
										Mean	1.1	(3.3DM)
Wakaw, SK Canada 2009 DP-Ø73496-4	2 (50)	0.69 0.67	100 100	PRE BBCH 16	82	Forage	ND	0.14	ND	ND	0.14	71 (0.48DM)
	2 (48)	0.70 0.70	100 100	PRE BBCH 14–51	76	Forage	ND	ND	ND	ND	< 0.05	71

Trial, state, country, year (variety)	N	kg ai/ha	L/ha	last treatment, timing	DALA	matrix	Gly	NA-gly	AMPA	NA-AMPA	Tot MRL	%moisture (corrected)
Rosthern, SK Canada 2009 DP-Ø73496-4	2 (42)	0.69 0.68	100 100	PRE BBCH 15–16	82	Forage	0.17	0.19	ND	ND	0.36	74
	2 (42)	0.69 0.68	100 100	PRE BBCH 15–16	82	Forage	< 0.05	0.10	ND	ND	0.15	75
										Mean	0.26	(1.0DM)
Wellwood, MB Canada 2009 DP-Ø73496-4	2 (34)	0.68 0.68	100 100	PRE BBCH 15	35	Forage	ND	0.79	ND	ND	0.79	79
	2 (34)	0.68 0.67	100 100	PRE BBCH 15	35	Forage	ND	0.60	ND	ND	0.60	82
										Mean	0.70	(3.6DM)
Franklin, MB Canada 2009 DP-Ø73496-4	2 (34)	0.70 0.67	100 100	PRE BBCH 15	35	Forage	ND	0.48	ND	ND	0.48	82
	2 (34)	0.68 0.69	100 100	PRE BBCH 15	35	Forage	ND	0.40	ND	ND	0.40	81
										Mean	0.44	(2.4DM)
Frenchtown, NJ USA 2009 DP-Ø73496-4	2 (34)	1.8 0.63	281 280	PRE 6 leaf	40	Forage	ND	0.54	ND	ND	0.54	78 (2.5DM)
Erie, ND USA 2009 DP-Ø73496-4	2 (28)	1.75 0.63	281 280	PRE BBCH 15	30	Forage	ND	0.91	ND	ND	0.91	82 (5.1DM)
Perley, MN USA 2009 DP-Ø73496-4	2 (28)	1.79 0.62	94 94	PRE BBCH 15	27 30 33 41 48 55	Forage	ND ND ND ND ND 0.19	1.2 0.98 0.66 0.90 0.58 0.81	ND ND ND ND ND ND	< 0.05 ND < 0.05 ND ND ND	1.2 0.98 0.71 0.90 0.58 1.0	85 (8.0DM)
Jamestown, ND USA 2009 DP-Ø73496-4	2 (33)	1.75 0.64	94 94	PRE BBCH 16	32	Forage	< 0.05	3.4	ND	0.13	3.6	78 (16.4DM)
Montpelier, ND USA 2009 DP-Ø73496-4	2 (33)	1.73 0.64	94 94	PRE BBCH 15	32	Forage	ND	0.08	ND	ND	0.08	80 (0.4DM)
Jerome, ID USA 2009 DP-Ø73496-4	2 (32)	1.8 0.64	178 186	PRE BBCH14–16	45	Forage	ND	0.41	ND	ND	0.41	75 (1.6DM)
Ephrata, WA USA 2009 DP-Ø73496-4	2 (20)	1.8 0.62	140 140	PRE 6 leaf	55 58 62 69 76 83	Forage	ND ND ND ND ND ND	0.18 0.10 0.09 0.14 0.38 0.56	ND ND ND ND ND ND	ND ND ND ND ND < 0.05	0.18 0.10 0.09 0.14 0.38 0.61	76 (2.5DM)
	2 (20)	1.7 0.62	47 47	PRE 6 leaf	55	Forage	ND	0.08	ND	ND	0.08	77

For additional containment, tents were used to cover the field plots at the Canadian locations. The tents were a white mesh material that reflected some light, resulting in taller, thinner plants than typical. The tents also created a warmer, more humid environment which served to prolong flowering and to delay maturity beyond what was normal for un-tented canola in the area. Consequently, the trials ran late into the season, it was harder to determine staging and timing, and in some provinces the crops were affected by frost before sampling occurred, despite efforts to protect the plants from frost damage by re-covering the plots with the tents following the third application. The use of mesh tents is unlikely to significantly affect residues in seed or forage.

Processing studies on grain

A processing study was undertaken in rape seed from rape plants containing event DP-Ø73496-4 modified with the *gat* gene (Shepard, 2013; DuPont-27816 Rev 1). Rape plots were treated with

approximately 5.85 kg ai/ha, as applications of 0.68, 0.68 and 4.5 kg ai/ha, with application timings pre-emergence, 6 leaf stage (BBCH 16) and 7 days per-harvest. Two different processing procedures (cold-pressing and solvent extraction) were trialled and samples of refined oil and meal collected for analysis.

Cold pressing

Rape seed samples were pressed in an expeller to mechanically remove a portion of the crude oil. Resulting products were meal (cold press) and expelled crude oil. Cold press meal was collected and placed in frozen storage. Crude oil was filtered and processed in the same manner as the combined crude oil from the solvent extraction procedure described below. Cold press refined oil was collected separately and placed into frozen storage.

Solvent Extraction Procedure

Rape seed/kernel was flaked in a flaking roll with a gap setting of 0.28 to 0.38 mm. Flakes were heated to 82–99 C and held for 10 to 15 minutes in the temperature range. Flakes were then pressed (expelled) in an expeller to mechanically remove a portion of the crude oil. Resulting products were presscake and expelled crude oil. Residual crude oil remaining in the solid material (presscake) exiting the expeller was extracted with hexane.

Presscake was placed in stainless steel batch extractors and submerged in 49–60 °C solvent (hexane). After 30 minutes, the miscella (hexane and crude oil) was drained and fresh hexane added to repeat the cycle two more times. The final two washes were for 15–30 minutes each. After the final draining, the solvent was removed from extracted presscake (meal) using a stream of warm air. Meal was collected and placed into frozen storage.

Miscella was passed through a laboratory vacuum evaporator to separate the crude oil and hexane. Crude oil was heated to 91–96 °C for hexane removal.

Crude oil samples recovered from the expeller and solvent extraction were filtered and combined into a container. Percent free fatty acid (FFA) of the crude oil was determined. Oil was placed in a water bath after pre-treatment with 85% phosphoric acid. Oil was mixed for 29–31 minutes at 40–44 °C. After the pre-treatment, an amount of 12 N baumé sodium hydroxide was added to the oil. Samples were mixed for 19 to 21 minutes at 40–44 °C and then for 9–11 minutes at 65–70 °C. Neutralized oil was then centrifuged to separate the refined oil and soapstock. Refined oil was decanted and filtered. Refined oil was collected and placed in frozen storage.

There was marked reduction in canola oil to non-detectable levels from the residues on the raw agricultural commodity. There was concentration in meal up to 1.8× for glyphosate and N-acetylglyphosate residues. Concentration and reduction of residues was the similar for both cold press and solvent extraction processes. Average meal processing factors were 0.787 and 0.563 for cold press and solvent extraction processes for glyphosate residues and 1.45 and 1.10 for N-acetylglyphosate residues.

Table 7 Results of processing rape seed into oil

Location	kg ai/ha	DALA		Gly	NA-gly	AMPA	NA-AMPA	Total	PF_{total}
Erie, ND USA 2009 DP-Ø73496-4	0.70 0.71 4.5	7	RAC	0.61 c0.05	56 c0.09	0.023 c0.05	1.1 c0.05	57.73	–
			Refined oil—cold press	ND	ND	ND	ND	< 0.05	< 0.0009
			Refined oil—solvent ext	ND	ND	ND	ND	< 0.05	< 0.0009
			Meal—cold press	0.93	88	< 0.05	1.9	90.86	1.57
			Meal—solvent ext	0.75	80	ND	1.6	82.35	1.43

Location	kg ai/ha	DALA		Gly	NA-gly	AMPA	NA-AMPA	Total	PF_{total}
Jamestown, ND USA 2009 DP-Ø73496-4	0.72 0.72 4.6	6	RAC	4.8	12	< 0.05	0.27	17.11	
			Refined oil—cold press	ND	ND c0.05	ND	ND	< 0.05	< 0.003
			Refined oil—solvent ext	ND	ND	ND	ND	< 0.05	< 0.003
			Meal—cold press	2.5	22	< 0.05	0.66	25.19	1.47
			Meal—solvent ext	1.7	17	< 0.05	0.62	19.34	1.13
Jerome, ID USA 2009 DP-Ø73496-4	0.67 0.68 4.5	7	RAC	14	0.47	0.13	ND	14.6	
			Refined oil—cold press	ND	ND	ND	ND	< 0.05	< 0.003
			Refined oil—solvent ext	ND	ND	ND	ND	< 0.05	< 0.003
			Meal—cold press	4.0	0.45	0.05	< 0.05	4.539	0.311
			Meal—solvent ext	1.3	0.24	ND	< 0.05	1.563	0.107

Calculated processing factors for processed rape seed are summarized in Table 8. Processing factors are based on total glyphosate free acid equivalents

Table 8 Summary of calculated processing factors

Commodity	PF_{total}	Best estimate PF_{total}
Refined oil—cold press	< 0.0009 < 0.003 < 0.003	< 0.003
Refined oil—solvent extracted	< 0.0009 < 0.003 < 0.003	< 0.003
Meal—cold pressed	0.311 1.47 1.57	1.47
Meal—solvent extracted	0.107 1.13 1.43	1.13

Residues in the edible portion of food commodities

No data submitted.

National residue definition

Not applicable.

APPRAISAL

Glyphosate is an herbicide with uses on many crops, conventional and glyphosate tolerant. Glyphosate has been evaluated several times with the initial evaluation in 1986 and the latest in 2011 where the use of glyphosate on glyphosate tolerant crops was reviewed.

The 2011 JMPR established a residue definition for compliance with MRLs for plant commodities as the sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate in the case of soya bean and maize and glyphosate for other crops. The definition of the residue for compliance with MRL for animal commodities is the sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate. For estimation of dietary intake it is the sum of glyphosate, AMPA, N-acetyl-glyphosate

and N-acetyl AMPA, expressed as glyphosate. The toxicology of glyphosate was re-evaluated by the 2011 JMPR which estimated group ADI of 0–1 mg/kg bw for the sum of glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl-AMPA. The same Meeting confirmed that an ARfD was unnecessary.

For the current evaluation data have been submitted covering the use on genetically modified rape crops containing the *gat* trait (glyphosate-N-acetyl transferase or GAT gene). These crops inactivate glyphosate by converting it to *N*-acetyl-glyphosate. The Meeting received information on glyphosate metabolism in genetically modified rape containing the *gat* trait, methods of residue analysis, GAP information, supervised residue trials on *gat* rape crops and the fate of residue during storage and processing.

To assist uniform interpretation of GAP application rates have been expressed in terms of glyphosate acid equivalents (ae), unless indicated otherwise.

Metabolites referred to in the appraisal were addressed by their common names:

N-acetyl-glyphosate	*N*-acetyl-*N*-(phosphonomethyl)glycine
AMPA	aminomethyl phosphonic acid
N-acetyl-AMPA	[(acetylamino)methyl]phosphonic acid.

Plant metabolism

The metabolic fate of [^{14}C] glyphosate in *gat* rape plants was examined following a single pre-emergence soil application of 4.5 kg ae/ha, followed by three foliar applications at 1.0 kg ae/ha at three different growth stages (2 and 5 leaf stage and 1 week before harvest). Rape plants were harvested as immature foliage, immediately prior to the final application and at maturity (PHI 7 days).

N-acetyl-glyphosate was the major metabolite in immature foliage (90% TRR; 5.4 mg/kg glyphosate equivalents). Glyphosate, AMPA and *N*-acetyl-AMPA were also detected accounting for 3.0, 1.4 and 3.4% of TRR respectively. At the intermediate harvest prior to the final application the only glyphosate related compound detected in foliage and immature pods (with seeds) was N-acetyl-glyphosate representing 93% TRR in foliage and 80% TRR in immature pods (with seeds). The residue in seeds at harvest, seven days after the final application, comprised glyphosate (21% TRR; 0.45 mg/kg), N-acetyl-glyphosate (51% TRR; 1.1 mg/kg), AMPA (1.9% TRR; 0.04 mg/kg) and N-acetyl-AMPA (15% TRR; 0.32 mg/kg).

The proposed pathway of glyphosate in rape plants with the *gat* trait is deactivation to *N*-acetyl-glyphosate which can be further metabolized to *N*-acetyl-AMPA and AMPA. The metabolism is similar to that observed for maize and soya bean crops with the *gat* trait previously reviewed by the 2011 JMPR.

Methods of Analysis

The Meeting received description and validation data for analytical methods for residue analysis of glyphosate and its metabolites in various plant commodities using LC-MS/MS. The LOQs are 0.05 mg/kg.

Stability of pesticide residues in stored analytical samples

No new information was received on the stability of glyphosate and its residues in samples stored frozen. The periods of demonstrated stability reported by the 2011 JMPR for other high oil and high protein crops cover the frozen storage intervals used in the residue studies.

Definition of the residue

The 2011 JMPR reviewed glyphosate metabolism studies in tolerant maize and soya bean containing the *gat* trait. Glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA were the major components of the residue in both maize and soya bean. In seeds from *gat* rape, *N*-acetyl-glyphosate

was the major component of the residue (51% TRR) followed by glyphosate (21% TRR) and N-acetyl AMPA (15% TRR). In *gat* rape forage, N-acetyl glyphosate was the major metabolite (90–93% TRR).

To accommodate the use of glyphosate on rape plants containing the *gat* trait the Meeting concluded that the previously established residue definition for enforcement in plants of "glyphosate" should be replaced by "*the sum of glyphosate and N-acetyl-glyphosate expressed as glyphosate* for soya bean, maize and rape crops and remain "*glyphosate*" for all other crops.

Based on the above the Meeting agreed to amend the previous definition for glyphosate for compliance with MRL for plant commodities as follows:

Definition of the residue for compliance with MRL (for plant commodities): for soya bean, maize and rape - *sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate*, and for other crops - *glyphosate*.

The Meeting confirmed the residue definition for estimation of dietary intake as (for plant and animal commodities: *glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl AMPA, expressed as glyphosate*.

Results of supervised residue trials on crops

The Meeting received supervised residue trial data for glyphosate on glyphosate-tolerant rape (*gat* trait).

For estimation of maximum residue levels for rape crops glyphosate and *N*-acetyl glyphosate levels are summed and expressed as glyphosate equivalents.

For estimation of the residue levels for dietary risk assessment of glyphosate in *gat* crops, in general all four analytes may be present in significant amounts. In the *gat* modified rape, *N*-acetyl glyphosate is the major residue found in rape seed, followed by glyphosate and *N*-acetyl AMPA. AMPA is a minor component of the residue and is included in the sum of residues when AMPA is reported as < LOQ.

The current Meeting received field trials performed in the USA and Canada involving glyphosate tolerant rape containing the *gat* trait. GAP for Canada is for application pre-emergence at 0.68 kg ae/ha, post-emergence at the 0–6 leaf stage at 0.3–0.68 kg ae/ha followed by a pre-harvest application at 0.9 kg ae/ha (PHI 7 days).

Residues of glyphosate and N-acetyl-glyphosate in rape seed trials matching Canada GAP were: 0.775, 1.8, 1.9, 2.4, 2.85, 3.05, 3.05, 7.8, 9.2 and 15 mg/kg (n=10). The Meeting estimated a maximum residue level of 30 mg/kg for glyphosate in rape seed to replace its previous recommendation of 20 mg/kg.

Corresponding total residues, for dietary intake estimation, were: 0.795, 1.8, 1.95, 2.4, 2.9, 3.1, 3.1, 7.8, 9.2 and 15 mg/kg. The Meeting estimated an STMR for glyphosate in rape seed of 3.0 mg/kg.

It was assumed that rape forage is plant material available from 25 days after planting. Residues in rape forage matching Canada GAP were: 0.4, 0.48, 1.0, 1.1, 1.5, 1.6, 2.1, 2.4, 2.5, 2.5, 3.3, 3.6, 4.7, 5.1, 8.0 and 16 mg/kg (dry matter basis). The Meeting estimated median and highest residues for glyphosate in rape forage of 2.25 and 16 mg/kg respectively, both on a dry-matter basis.

Fate of residues during processing

The Meeting received information on the nature of residues under simulated processing condition on the fate of incurred residues of glyphosate during the processing of rape seeds. Calculated processing factors for total glyphosate acid equivalents (combined results of the four metabolites) are summarized below.

Summary of calculated processing factors and estimated STMR-P values

Commodity	PF_{total}	Best estimate PF_{total}	$STMR_{RAC}$ (mg/kg)	$STMR_{RAC}$×PF

				(mg/kg)
Refined oil-cold press	< 0.0009 < 0.003 < 0.003	< 0.003	3.0	< 0.009
Refined oil-solvent extracted	< 0.0009 < 0.003 < 0.003	< 0.003		< 0.009
Meal-cold pressed	0.311 1.47 1.57	1.47		4.41
Meal-solvent extracted	0.107 1.13 1.43	1.13		3.39

The estimated STMR-P values in oil are lower than the previous values reported by the 2011 JMPR (previous 0.093 mg/kg) while the STMR-P estimated for rape seed meal is higher (previous 2.3 mg/kg).

Residues in animal commodities

Animal commodity maximum residue levels

The current evaluation has not led to recommendations that would alter the dietary burdens calculated using the livestock intake figures employed by the 2011 JMPR. The glyphosate dietary burdens for cattle (dairy and beef) were based on grass, cotton seed and barley grain while those for poultry were based on barley, soya bean grain and soya bean hulls and as such do not require a re-evaluation of animal commodity maximum residues levels.

RECOMMENDATIONS

On the basis of the data obtained from supervised residue trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IEDI assessment.

Definition of the residue for compliance with MRL (for plant commodities) for soya bean, maize and rape: *sum of glyphosate and N-acetyl-glyphosate, expressed as glyphosate*, and for other crops - *glyphosate*.

The Meeting confirmed the residue definition for estimation of dietary intake as (for plant and animal commodities: *glyphosate, N-acetyl-glyphosate, AMPA and N-acetyl AMPA, expressed as glyphosate*.

Definition of the residue for compliance with MRL (for animal commodities): *sum of glyphosate and N-acetylglyphosate, expressed as glyphosate*.

Definition of the residue for estimation of dietary intake (for plant and animal commodities): *glyphosate, N-acetylglyphosate, AMPA and N-acetyl AMPA, expressed as glyphosate*.

The residue is not fat soluble.

Table of recommendations

Commodity		Recommended MRL (mg/kg)		STMR or STMR-P (mg/kg)
CCN	Name	New	Previous	
SO 0495	Rape seed	30	20	3.0
	Rape forage			2.25 (highest residue 16)
OR 0495	Rape seed oil, edible			0.009
	Rape seed meal			4.41

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDI) of glyphosate for the 13 GEMS/Food regional diets, based on estimated STMRs were in the range 0–1% of the maximum ADI of 1 mg/kg bw for the sum of glyphosate, *N*-acetyl glyphosate, AMPA and *N*-acetyl AMPA, expressed as glyphosate. The Meeting concluded that the long-term intake of residues of glyphosate, *N*-acetyl glyphosate, AMPA and *N*-acetyl AMPA from uses that have been considered by the JMPR is unlikely to present a public health concern. The results are shown in Annex 4 of the JMPR 2013 Report.

Short-term intake

The International Estimated Short Term Intake (IESTI) of glyphosate was not calculated. The 2004 and 2005 JMPR concluded that it was unnecessary to establish an ARfD for glyphosate. The Meeting therefore concluded that short-term dietary of glyphosate residues is unlikely to present a risk to consumers.

REFERENCES

Code	Author	Year	Title, Institute & Report reference
DuPont 26109	Chapleo, S and McLachlan, T	2010	The metabolism of [^{14}C] glyphosate in 0827 canola. E. I. du Pont de Nemours and Company. DuPont Report No. DuPont 26109. Unpublished.
DuPont 27816 Rev 1	Shepard, E	2013	Magnitude and decline of glyphosate related residues in forage and seed of genetically modified canola event DP-Ø73496-4 and magnitude of glyphosate related residues in canola event DP-Ø73496-4 seed process fractions following applications of Touchdown Total® Herbicide—Locations in the United States and Canada, season 2009. E. I. du Pont de Nemours and Company. DuPont Report No. DuPont 27816, Revision No. 1. Unpublished.