GLYPHOSATE (158)

EXPLANATION

Glyphosate was first evaluated by the JMPR in 1986, and reviewed in 1987 and 1988.

The 1987 JMPR examined data on glyphosate residues in soya beans following in-crop directed applications to weeds. MRLs for soya bean (dry), soya bean fodder and soya bean forage (green) of 5, 20 and 5 mg/kg respectively were recommended. The CXLs for soya bean and related items do not accommodate the current use of glyphosate as a desiccant close to harvest.

This new use, coupled with existing approved in-crop applications with selective equipment and by spot treatment, results in higher residue levels of glyphosate in soya beans treated according to label directions and good agricultural practices. Information for the evaluation of glyphosate residues in soya bean commodities as a result of these new uses was submitted to the Meeting.

The 1988 JMPR re-examined data on residues in wheat resulting from the pre-harvest use of glyphosate, and recommended MRLs for wheat and wheat bran of 5 and 40 mg/kg respectively.

At the 26th (1994) Session of the CCPR it was suggested by several countries that the proposed MRL for unprocessed wheat bran should be reconsidered because it was too high. The Committee lowered the draft MRL to 20 mg/kg but referred the matter to the JMPR for further consideration. Milling data on wheat were made available to the meeting.

METHODS OF RESIDUE ANALYSIS

Analytical methods

Glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA) can be determined by HPLC.

A residue method based on HPLC with post-column reaction detection was validated in a previous inter-laboratory study (Cowell *et al*.,1986). The procedure can determine trace quantities of glyphosate and AMPA in various substrates.

The sample is extracted with chloroform/hydrochloric acid (0.1N). The diluted aqueous extract is cleaned up by elution through Chelex 100 resin in the Fe (III) form. The glyphosate and AMPA are eluted from the resin with hydrochloric acid and the iron is removed with an anion-exchange resin (AG 1X8). After concentration to dryness to remove the hydrochloric acid, samples are analyzed by two-column switched HPLC with a post-column reactor.

Determination involves post-column hypochlorite oxidation, and reaction of the amine

product with *o*-phthalaldehyde and mercaptoethanol to produce a fluorescent derivative which is measured by a fluorescent detector at 340 nm excitation and 455nm emission wavelengths.

It was found that the use of a buffer solution at pH 2.0 gave better resolution of glyphosate and AMPA with the columns and system used.

The LOD of the method is 0.05 mg/kg for glyphosate and AMPA in wheat and soya bean commodities. It has been validated from 0.05 to 250 mg/kg for both glyphosate and AMPA in various crop samples, and was evaluated by the 1987 JMPR.

Stability of pesticide residues in stored analytical samples

Samples collected in the field were frozen in dry ice as soon as possible, and once at the laboratory stored in a freezer at <-18ºC. Residues of glyphosate and AMPA were stable in plant tissues held in frozen storage up to seven months.

USE PATTERN

Glyphosate is formulated as a soluble concentrate of the isopropylamine salt of *N*-(phosphonomethyl)glycine (glyphosate) containing 356 g/l of glyphosate free acid.

Glyphosate is a broad-spectrum herbicide which has a high activity when applied to foliage. It is strongly bound to soil particles and is not taken up by the roots of the plants. It is used post-emergence but not as a pre-emergence or soil-applied herbicide.

Glyphosate is readily translocated from treated foliage to other parts of the plant, which makes it very effective against some perennial weeds. Residues from treated weeds passing into the soil are not taken up by other plants.

Information on major international use patterns was made available to the 1986 JMPR.

Glyphosate has been approved in the United States and Canada for application as an in-crop spot treatment. Application in growing soya bean crops must be made before initial pod set.

More recently, pre-harvest applications of glyphosate to soya beans have been approved for use in both Canada and the USA. See Table 1.

Glyphosate may be applied using selective application equipment: recirculating sprayers, shielded applicators and wiper applicators. All these methods of application minimize contact of the herbicide solution with desirable vegetation. Nonetheless, some does contact the crop owing to splattering, applicator drip, spray drift, or physical contact of the treated weeds with the crop.

When sequential selective applications are combined with a pre-harvest application, residue levels of glyphosate in soya beans increase.

Table 1. Summary of GAP for glyphosate in soya beans. (See endnotes for explanation of abbreviations).

Country	Uses	Application		Rate		PHI, days	Remarks
		Type	No.	kg ai/ha	l/ha		
Canada	Pre-plant	BS GA	NR 1	0.27-4.3	50-300		
	In-crop ST	GA BS	NR 1	0.27-4.3	1-2%		Application can be made up to initial pod set
	In-crop WA	GA WA	NR 1-3		5-33%		Application can be made up to initial pod set
	In-crop PH	GA BS	NR 1	0.89	50-100	7-14	Apply when crop has <30% grain moisture
USA	Prior crop emergency	GA	NR 1	0.2-4.2	29-374		
	In-crop ST	GA BS	NR 1	0.2-4.2	29-374	7	≤10% of the total field area to be harvested
	RC	GA	NR 1-3	0.2-4.2	29-374	7	
	In-crop WA	GA	NR 1-3		33 to 100% soln	7	
	In-crop PH	GA BS	NR 1	≤5.0	29-374	7	Apply after pods have set and lost all green colour

Pre-plan Pre-planting
BS Boom sprayer application
GA Ground application
RC Recirculating sprayer
ST Spot treatment
WA Wiper application
PH Pre harvest application
NR No restriction

RESIDUES RESULTING FROM SUPERVISED TRIALS

Soya beans. Data on the residues of glyphosate in soya bean grain and fodder resulting from selective equipment, spot treatment and pre-harvest applications were available to the Meeting for re-evaluation. The residues arising from treatments according to GAP covered a wide range depending on the type (selective equipment, spot treatment or pre-harvest) and total number of applications, rates and timings.

Previous metabolism studies in various crops have demonstrated that the principal metabolite of glyphosate in plants is aminomethylphosphonic acid (AMPA). Consequently, the studies submitted to the Meeting focused on the HPLC determination of glyphosate and AMPA residues in soya beans, grain and hay following in-crop applications of glyphosate according to label directions and good agricultural practices.

Supervised trials were conducted in Canada (London and Caledonia) to investigate the residue levels of glyphosate and AMPA in soya beans following the pre-harvest application of glyphosate, after 90% leaf drop. In these studies the analytical recoveries of glyphosate and AMPA from control samples fortified over the range 0.05 to 1.0 mg/kg and analyzed concomitantly with the field-treated samples averaged 85.6% (67.9-98.9%) and 82.3% (68.0-91.2%) respectively. The results are shown in Table 2.

Table 2. Residues of glyphosate in soya beans (grain) from pre-harvest applications of 0.36 kg ai/l. Supervised trials in Canada (Mueth, 1988). Duplicate plots sampled in each trial.

Location, year	kg ai/ha	Glyphosate, mg/kg[1], range	AMPA, mg/kg[1]	PHI (days)
Caledonia, ON 1985-1986 (2 trials)	0.45	<0.05	<0.05	7-8
	0.90	<0.05	<0.05	7-8
	1.8	<0.05-0.08	<0.05	7-8
London, ON 1986 (2 trials)	0.45	<0.05	<0.05	12
	0.90	<0.05	<0.05	12
	1.8	<0.05-0.08	<0.05	12

[1] Values are the average of duplicate analyses and are not corrected for recovery

Supervised trials were conducted in the USA to determine glyphosate residues in soya bean seeds following pre-harvest applications. The herbicide was ground-applied at 5.0 kg ai/ha according to label directions as a single treatment seven to nine days before harvest in ten soya bean plots. Two test plots were established at each site: one treated and one untreated control.

Grain samples were collected at normal harvest from each plot and maintained in frozen storage until analysis. All samples were analyzed by HPLC with some modifications that improved the resolution of glyphosate and AMPA with the columns and systems used. Analytical recoveries of glyphosate and AMPA from control samples fortified over the range 0.05 to 40.0 mg/kg and analyzed concomitantly with field-treated samples averaged 93.3% (73.5-122.6%) and 85.3% (70.0-103.8%) respectively. The trial was in compliance with Good Laboratory Practice Standards. Results are given in Table 3.

The maximum glyphosate and AMPA residues were 2.97 and 0.39 mg/kg, not corrected for recovery.

Table 3**.** Residues in soya beans in supervised trials in the USA (1993) from the pre-harvest use of glyphosate (Oppenhuizen, 1994).

Location	kg ai/ha	Glyphosate, mg/kg[1]	AMPA, mg/kg[1]	PHI (days)
Arkansas	5.0	0.56	0.39	7
Georgia	5.1	0.24	0.07	7
Illinois	5.1	0.22	<0.05	7

Location	kg ai/ha	Glyphosate, mg/kg[1]	AMPA, mg/kg[1]	PHI (days)
Iowa	5.0	1.60	<0.05	7
Louisiana	5.0	0.93	0.06	9
Maryland	5.0	0.14	<0.05	7
Minnesota	5.1	0.12	<0.05	8
Missouri	5.0	0.16	<0.05	7
Ohio	5.0	0.69	0.07	8
Tennessee	5.0	2.97	0.11	7

[1] Values are the average of duplicate analyses and are not corrected for recovery

Supervised field trials were conducted at four locations in the USA to investigate the residues of glyphosate and AMPA in soya beans and soya bean hay after sequential treatment of the crop with selective and pre-harvest applications of glyphosate according to label directions and good agricultural practices (Tables 4 and 5).

The samples were analyzed by HPLC using a ninhydrin-based post-column reactor.

The average analytical recoveries of glyphosate and AMPA in the beans over the range 0.05 to 20 mg/kg were 97.3% (117.2-70.8%) and 94.2% (105.0-75.6%) respectively.

Analytical recoveries of glyphosate and AMPA from control hay samples, fortified over the range 0.05 to 20 mg/kg and analyzed concomitantly with field treated samples, averaged 99.1% (90.8-105.6%) and 93.9% (86.6-99.1%) respectively.

Table 4. Glyphosate and AMPA residues in soya beans from supervised trials in the USA. Recirculating sprayer applications as in-crop spot treatment combined with pre-harvest applications (Kunstman, 1983).

Location (No. of replicates)	kg ai/ha	Glyphosate, mg/kg[1], range	AMPA, mg/kg[1], range	PHI (days)
Banks, MS Grain moisture 19.6% at pre-harvest application	2.1 (RC)			95
	2.1 (RC)			37
	0.8 (PH)	9.2	1.5	15
	1.7 (PH)	11.9	1.3	15
	3.4 (PH)	11.4	1.8	15
	5.0 (PH)	16.9	1.8	15
Bruins, AR (3)	2.5 (RC)			80-89

Location (No. of replicates)	kg ai/ha	Glyphosate, mg/kg[1], range	AMPA, mg/kg[1], range	PHI (days)
Grain moisture 10-22% at pre-harvest application				
	2.5 (RC)			64-73
	0.8 (PH)	0.4-0.7	0.3-0.5	7-16
	1.7 (PH)	0.4-1.1	0.2-0.5	7-16
	3.4 (PH)	0.8-1.2	0.4-0.5	7-16
	5.0 (PH)	0.4-0.5	0.2-0.3	7-16
Princeton, KY (3) Grain moisture 10-20% at pre-harvest application	5.0 (RC)			112-126
	5.0 (RC)			81-95
	5.0 (RC)			48-62
	0.8 (PH)	1.3-2.8	0.5-7.6	11-25
	1.7 (PH)	1.7-3.3	0.6-0.9	11-25
	3.4 (PH)	0.4-8.8	0.2-1.2	11-25
	5.0 (PH)	3.1-6.2	0.7-1.3	11-25
Adel, IA (2) Grain moisture 14-20% at pre-harvest application	5.0 (RC)			122
	5.0 (RC)			102
	5.0 (RC)			75
	0.8 (PH)	<0.05-0.06	<0.05	8-11
	1.7 (PH)	0.09-0.1	<0.05	8-11
	3.4 (PH)	0.1-0.2	<0.05	8-11
	5.0 (PH)	0.4-0.7	<0.05	8-11

[1] Values are the average of duplicate analyses and are not corrected for recovery
(RC) Recirculating shielded sprayer application
(PH) Pre-harvest application (as desiccant)

Table 5. Glyphosate and AMPA residues in soya bean hay from the same supervised trials as Table 4.

Location (No. of replicates)	kg ai/ha	Glyphosate, mg/kg[1], range	AMPA, mg/kg[1], range	PHI (days)
Banks, MS (2) Grain moisture 11.6-19.6% at pre-harvest application	2.1 (RC)			95
	2.1 (RC)			37
	0.8 (PH)	3.4-7.1	0.2-0.3	9-15
	1.7(PH)	7.9-14.8	0.4-0.5	9-15
	3.4 (PH)	10.1-21.3	0.3-0.4	9-15
	5.0 (PH)	9.9-28.6	0.4-0.5	9-15
Bruins, AR (3) Grain moisture 10-22% at pre-harvest application	2.5 (RC)			80-89
	2.5 (RC)			64-73
	0.8 (PH)	1.8-7.2	0.1-0.2	7-16
	1.7 (PH)	5.0-14.2	0.2-0.3	7-16
	3.4 (PH)	11.9-34.0	0.3-0.5	7-16
	5.0 (PH)	20.4-31.4	0.3-0.5	7-16
Princeton, KY (3) Grain moisture 10-20% at pre-harvest application	5.0 (RC)			112-126
	5.0 (RC)			81-95
	5.0 (RC)			48-62
	0.8 (PH)	2.5-3.1	0.1-0.2	11-25
	1.7 (PH)	3.6-6.5	0.2-0.3	11-25
	3.4 (PH)	6.98-17.4	0.3-0.7	11-25
	5.0 (PH)	13.9-29.8	0.3-0.8	11-25
Adel, IA (2) Grain moisture 14-20% at pre-harvest application	5.0 (RC)			122

Location (No. of replicates)	kg ai/ha	Glyphosate, mg/kg[1], range	AMPA, mg/kg[1], range	PHI (days)
	5.0 (RC)			102
	5.0 (RC)			75
	0.8 (PH)	9.9-12.5	0.1-0.1	8-11
	1.7 (PH)	41.8-48.8	0.3-0.4	8-11
	3.4 (PH)	87.2-88.7	0.7-0.8	8-11
	5.0 (PH)	194.7-202	1.5-1.7	8-11

[1] Values are the average of duplicate analyses and are not corrected for recovery
(RC) Recirculating shielded sprayer application
(PH) Pre-harvest application (desiccant)

The 1986 JMPR evaluated studies in which pigs, chickens and cattle were fed with diets containing 100 ppm of glyphosate and AMPA. No residues of glyphosate or AMPA were detectable in muscle, fat, milk or eggs.

Wheat and wheat bran. In residues trials at 15 sites in production areas across the United States wheat plots were treated 3 or 7 days before harvest with glyphosate herbicide at rates of 0.4 or 0.8 kg ai/ha of active ingredient. Applications were made aerially at eleven sites and by ground boom at four sites. Glyphosate formulated with surfactant was also applied at 0.8 kg ai/ha at seven locations.

Wheat grain and straw from the regular harvest of these treated plots were analyzed for residues of glyphosate and AMPA. Residues in the grain are shown in Table 6 and in the straw in Table 7.

Glyphosate residues in wheat grain from treatments at 0.8 kg ai/ha ranged from <0.05 to 2.9 mg/kg. Residues after 3 days were generally slightly higher than after 7 days.

Glyphosate residues in wheat straw from 0.4 kg ai/ha ranged from 0.5 to 61 mg/kg, and from 0.8 kg ai/ha from 0.68 to 81 mg/kg. Glyphosate residues in wheat grain and straw from treatments with and without surfactant were comparable at the same rate and pre-harvest interval. AMPA residues were usually between 1 and 10% of the glyphosate residues.

Table 6. Glyphosate and AMPA residues in wheat grain in the USA from supervised trials with pre-harvest applications (Allin, 1989).

No of trials	Rate, kg ai/ha	Days after treatment	Residues, mg/kg	
			Glyphosate, range	AMPA, range
15	0.4	3	<0.05-0.44	<0.05-0.07
15	0.4	7	<0.05-2.5	<0.05-0.09
15	0.8	3	0.18-2.9	<0.05-0.17
15	0.8	7	<0.05-2.5	<0.05-0.06

Table 7. Glyphosate and AMPA residues in wheat straw from the same trials as Table 6.

No of trials	Rate, kg ai/ha	Days after treatment	Residues, mg/kg	
			Glyphosate, range	AMPA, range
15	0.4	3	0.74-61	<0.05-0.7
15	0.4	7	0.51-29	<0.05-1.2
15	0.8	3	4.5-81	0.1-1.3
15	0.8	7	0.67-69	<0.05-2.6

These residues in wheat grain and straw are within the current CXLs for wheat grain and cereal straws.

Trials were carried out in the USA to determine the fate of residues of glyphosate and AMPA during the milling and fractionation of topically treated wheat grain.

Wheat grain samples taken 7 days after treatment were milled. They contained 0.67 mg/kg glyphosate and <0.05 mg/kg AMPA from treatment at 0.4 kg ai/ha, and 67 mg/kg glyphosate and 1.8 mg/kg AMPA from treatment at 3.4 kg ai/ha. A Brabender Quadrumat Senior Mill, an automatic pilot mill to simulate the commercial preparation of flour, was used to prepare the grain fractions. The samples were dried to the usual moisture content of 14-15%. The samples were analyzed by HPLC. Residues of glyphosate and AMPA in the grain and bran are shown in Table 8, and those of glyphosate in the milling fractions in Table 9.

The 1987 JMPR evaluated studies to determine residues of glyphosate and AMPA in milling fractions of wheat grain. Residues levels in bran were 2-4 times as high as in the original grain.

Table 8. Residues of glyphosate and AMPA in treated wheat grain and bran. PHI 7 days. USA.

Location	Rate, kg ai/ha	Glyphosate, mg/kg[1]		AMPA, mg/kg		Factor B/A
		Grain[2] (A)	Bran (B)	Grain[2]	Bran	
Saltillo	0.4	0.67	1.7	<0.05	0.07	2.5
Mayview	3.4	67	121	1.8	2.6	1.8

[1] Corrected for mean recovery of 80.2%
[2] Before drying

Table 9. Residues of glyphosate in milling fractions of the wheat of Table 8.

Fraction	Fraction weight (g)	Fraction wt. as % of total	Glyphosate in fraction		
			mg/kg	mg	% of total glyphosatee
Wheat treated at 0.4 kg ai/ha					
Bran	639	33.3	1.7	1.09	84.5
Break flour	1029	53.6	0.14	0.14	10.8
Reduction flour	198	10.3	0.12	0.024	1.8

Fraction	Fraction weight (g)	Fraction wt. as % of total	Glyphosate in fraction		
			mg/kg	mg	% of total glyphosatee
Shorts	54	2.8	1.2	0.065	5.0
TOTAL	1,920	100.0		1.32	102
Wheat treated at 3.4 kg ai/ha					
Bran	334	39.3	121	40.4	76.7
Break flour	414	48.8	22.8	9.4	17.8
Reduction flour	93	10.9	22.8	2.1	4.0
Shorts	8.4	1.0	94.7	0.8	1.5
TOTAL	849	100.0		52.7	100.0

NATIONAL MAXIMUM RESIDUE LIMITS

The following national MRLs were reported. They refer to the combined residues of glyphosate and its metabolite aminomethylphosphonic acid (AMPA).

Country	Commodity	MRL, mg/kg
USA	Soya beans	20
	Soya bean forage	15
	Soya bean hay (fodder)	200
	Wheat milling fractions, excluding flour	20
Canada	Soya beans	6

APPRAISAL

Glyphosate was first evaluated by the 1986 JMPR and subsequently in 1987 and 1988.

The Meeting received a proposal to revise the MRLs for soya bean and soya bean fodder on the grounds that the present Codex MRL for soya beans (5 mg/kg) is inadequate to cover the residues that result from the use of glyphosate as a desiccant, which involves application close to harvest.

At the 26th (1994) Session of the CCPR it was suggested (ALINORM 95/24, para 261) that the proposed MRL in unprocessed wheat bran (40 mg/kg) was too high and should be reconsidered by the JMPR. The 26th Session amended the draft MRL to 20 mg/kg pending the JMPR review.

Several residue studies with in-crop applications of glyphosate to soya beans were submitted to the Meeting for review of the MRLs for soya beans and soya bean fodder according to the new use pattern as a pre-harvest desiccant.

The use of glyphosate for in-crop weed control in soya beans varies greatly depending

on the nature of the weeds (annual or perennial), extent of infestation, stage of crop growth and application equipment used.

More recently the pre-harvest application of glyphosate as a desiccant has been approved both in Canada and the USA. Two separate studies have investigated the residue levels of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in soya bean grain.

The first study was conducted at four supervised trial sites in Canada. For all treatment rates (0.45, 0.9 and 1.8 kg ai/ha) the combined residue levels of glyphosate and AMPA were less than 0.1 mg/kg.

In the second study where ten test sites were selected in the USA, glyphosate and AMPA residues ranged from 0.12 to 3.0 and <0.05 to 0.39 mg/kg respectively.

Data from supervised trials on soya bean crops in the USA were submitted to the Meeting. They demonstrated that when a sequential application of glyphosate in the crop is combined with a pre-harvest application, the glyphosate and AMPA residues in the beans vary considerably, depending on the application rate and the timing of the application.

In these studies the maximum residue levels of glyphosate and AMPA in soya beans following the application of glyphosate at the maximum label use rate as a desiccant according to good agricultural practices were 16.9 and 1.8 mg/kg respectively. In soya bean hay, the maximum residues of glyphosate and AMPA were 202 and 1.7 mg/kg.

The Meeting estimated maximum residue levels of 20 mg/kg for soya bean (dry) and 200 mg/kg for soya bean fodder to replace the previous recommendations (5 and 20 mg/kg respectively).

Glyphosate residues in pigs, poultry and cattle were evaluated by the 1986 JMPR. When administered to animals glyphosate is rapidly excreted without degradation.

Residues in cattle, pig and poultry meat, eggs and milk were negligible after the animals were fed with a diet containing 100 ppm glyphosate and AMPA. The highest residues were found in pig liver and kidney (up to 0.16 and 0.91 mg/kg respectively) and cattle kidney (up to 1.4 mg/kg).

Residue levels of glyphosate in wheat treated according to the proposed label directions for the pre-harvest use of glyphosate were within the Codex MRL (5 mg/kg).

The Meeting received details of a processing study on wheat to determine the fate of glyphosate and AMPA residues during milling. In two trials with incurred residues of glyphosate and AMPA the concentration factor for residues in the production of unprocessed wheat bran from grain ranged from 1.8 to 2.5 and averaged about 2.0.

On the basis of studies evaluated by the JMPR in 1987 and the new study described above, the Meeting confirmed that a concentration factor of 4.0 was adequate and therefore estimated a maximum residue level of 20 mg/kg for unprocessed wheat bran to replace the recommendation of the 1988 Meeting (40 mg/kg).

Residues were determined by HPLC with fluorescence detection after post-column derivatization with *o*-phthalaldehyde. The method has been validated from 0.05 to 250 mg/kg for

both glyphosate and AMPA in various crop samples.

RECOMMENDATIONS

The Meeting estimated the maximum residue levels shown below, which are recommended for use as MRLs.

Definition of the residue: glyphosate

Commodity		Recommended MRL (mg/kg)		PHI, days
CCN	Name	New	Previous	
VD 0541	Soya bean (dry)	20	5	7-14
Al 0541	Soya bean fodder	200	20	7-14
CM 0654	Wheat bran, unprocessed	20	40[1]	

[1]40 mg/kg was recommended by the 1988 JMPR, but the draft MRL at this level was lowered to 20 mg/kg by the 1994 CCPR.

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