

## GLYPHOSATE (158)

### EXPLANATION

Glyphosate was first evaluated by the 1986 JMPR; an ADI of 0.3 mg/kg bw was allocated and maximum residue levels were estimated for some cereal grains and straw, vegetables, oilseeds and animal products.

In 1987 a revised analytical method for glyphosate residues and residue data on milled products and processed cereal commodities were evaluated. MRLs were recommended for kiwifruit, soya beans, soya bean fodder and forage, wheat and wheat bran.

The 1988 JMPR re-evaluated data on wheat, barely and oats resulting from pre-harvest applications of glyphosate and recommended new or revised MRLs for wheat and its milling fractions.

The 1994 JMPR evaluated data on residues resulting from new pre-harvest uses of glyphosate and recommended revised MRLs for soya bean, soya bean fodder, and unprocessed wheat bran.

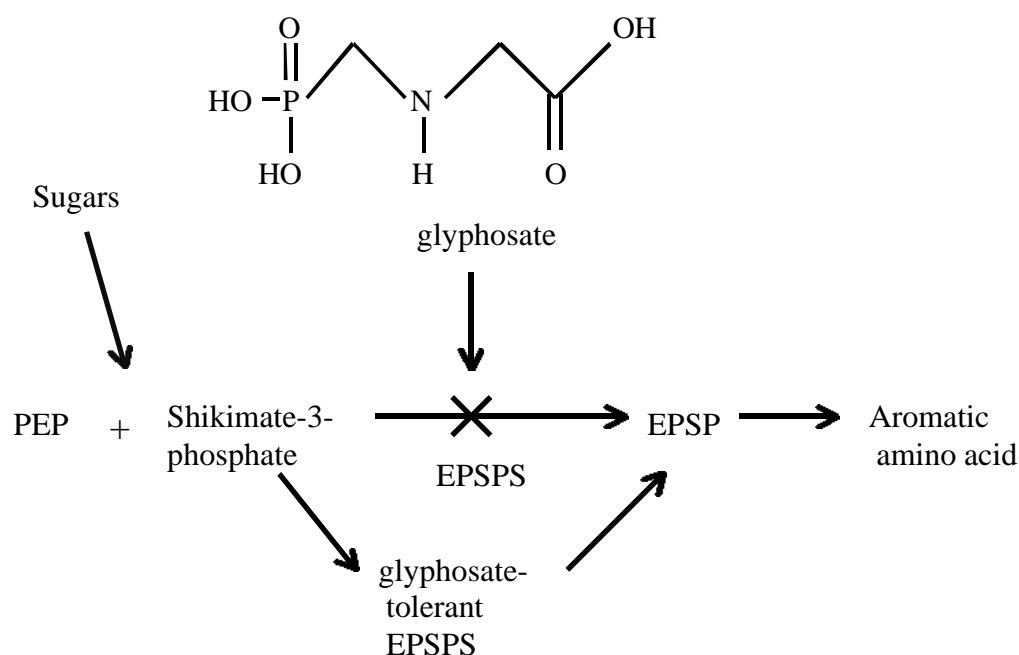
The 1997 JMPR was requested to evaluate the new uses of glyphosate on cotton, maize and sorghum according to GAP. These new uses are (1) pre-harvest topical applications and (2) in-crop applications to cotton and maize crops which have been genetically modified to be resistant to glyphosate. Relevant data on metabolism and residue trials were submitted to the Meeting.

### Genetic modification of crops

Glyphosate binds to and blocks the activity of 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), an enzyme of the aromatic amino acid biosynthetic pathway. The inhibition of EPSPS by glyphosate prevents the plant from producing the aromatic amino acids essential for protein synthesis. EPSPS is present in all plants, bacteria, and fungi but not in animals, which do not synthesize aromatic amino acids, but receive them from food.

The development of glyphosate-resistant (glyphosate-tolerant) crops has been in progress since the early 1980s, using a “target-site modification” approach in which a glyphosate-resistant EPSPS was identified and its expression induced in plants by genetic manipulation. Glyphosate treatment leaves the plant unaffected because the continued action of the glyphosate-resistant EPSPS enzyme supplies the plant’s need for aromatic amino acids (Figure 1).

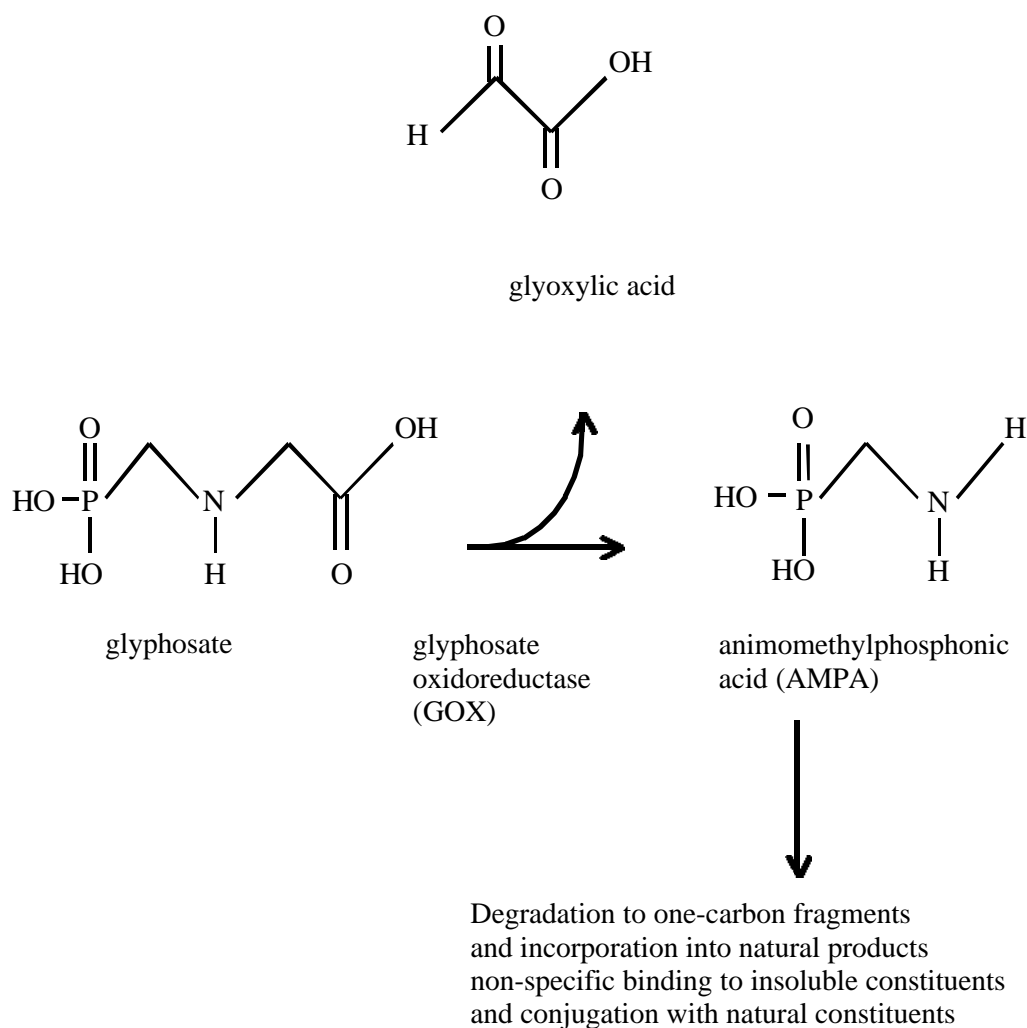
Figure 1. Action point of glyphosate and mechanism of glyphosate resistance.



A search was instituted to identify naturally-occurring EPSPS with not only a high degree of glyphosate resistance, but also tight binding of the substrate phosphoenolpyruvate (PEP). The EPSPS enzyme identified is derived from *Agrobacterium* sp. Strain (CP4 EPSPS), and has been used to develop glyphosate-resistant crops.

While the CP4 EPSPS enzyme has been successful in providing glyphosate resistance in cotton, the activity of CP4 EPSPS alone has been insufficient to ensure adequate resistance in other crops. In maize, a second mechanism of resistance has been developed to allow applications of glyphosate at rates of use necessary for effective weed control. The second mechanism is glyphosate inactivation, which reduces cellular levels of glyphosate by converting it to aminomethylphosphonic acid (AMPA). The inactivating enzyme is glyphosate oxidoreductase (*gox*). The gene encoding *gox* was isolated from a naturally-occurring bacterium, *Achromobacter* sp., and has been modified to optimize its expression in plants. The inactivation process is shown in Figure 2.

Figure 2. Mechanism of glyphosate inactivation in plants.



## PLANT METABOLISM

A number of metabolism studies with vegetables, orchard tree, nut and pasture crops were reported to the 1986 JMPR. The 1986 Meeting concluded that glyphosate applied to the soil was absorbed to a very small extent or not at all by the crops and conversion of glyphosate to aminomethylphosphonic acid (AMPA), which is the primary metabolite, was not observed.

Hydroponic administration allows sufficient uptake of glyphosate to elucidate the metabolic transformation in plants. Metabolic studies with the hydroponic administration of glyphosate to maize, wheat, cotton and soya beans showed the conversion of glyphosate to AMPA and further degradation in plant tissues.

Metabolic studies with plants that had been genetically modified to be resistant to glyphosate showed that the metabolism was the same as in susceptible plants: glyphosate is metabolized to AMPA, which is either non-selectively bound to natural plant constituents, further degraded to one-carbon fragments that are incorporated into natural products, or conjugated with naturally-occurring organic acids to give trace-level metabolites. The metabolites are the same in resistant and non-resistant crops; only the relative distribution varies, depending on the speed and extent to which glyphosate is converted to AMPA.

### Metabolism in susceptible plants

Maize, cotton, wheat and soya beans were treated with [ $^{14}\text{C}$ ]glyphosate, labelled in the phosphonomethyl group, added to the hydroponic solution (Rueppel, 1973). In order to define the metabolism more completely, glyphosate labelled at the carboxyl- and  $\alpha$ -carbon atoms has also been hydroponically administered to soya beans.

In the hydroponic uptake experiments, which lasted for 28 days for all crops except wheat (10 days), the composition of the  $^{14}\text{C}$ -labelled residue in both the aqueous plant extracts and the nutrient media has been identified as a function of time on the basis of TLC and/or column chromatography with LSC.

The forage from all four crops could be efficiently extracted with water; 70-90% of the radioactivity was extractable, so the isolated compounds probably include all the major metabolites. The main  $^{14}\text{C}$ -labelled compound was glyphosate in all the forage except maize.

The major metabolite (4-28%) of [ $^{14}\text{C}$ ]glyphosate was chromatographically identified as *N*-methylaminomethylphosphonic acid, but its presence was considered to be an artifact on the basis of uptake studies using highly purified [*phosphonomethyl*- $^{14}\text{C}$ ]glyphosate.

The conclusions were as follows.

1. Significant degradation of glyphosate occurs in plants; AMPA also appears to be degraded. The high extractability indicates that conjugation of glyphosate and AMPA with natural plant components represents a minor pathway at most.
2. The major metabolic pathway of degradation of glyphosate probably involves the formation of AMPA and glyoxylate by enzymatic cleavage of the C-N bond. There is significant incorporation of glyoxylate, aminomethylphosphonate fragments, and/or  $\text{CO}_2$  into natural products.

The residues found in maize, wheat, cotton and soya bean forage are shown in Table 1.

Table 1. Metabolites found in maize, wheat, cotton and soya bean forage after hydroponic application of [ $^{14}\text{C}$ ]glyphosate.

Crop	% of the TRR					
	Glyphosate <sup>1</sup>	AMPA <sup>1</sup>	N-MAPA <sup>1,2</sup>	Natural products	Unidentified <sup>2</sup>	Unextractable
Maize	21.1	27.9	NA	4.0	20.0	26.6
Wheat	55.3	4.2	NA	1.0	8.0	31.5
Cotton	61.5	6.8	2.0	8.8	10.9	10.0
Soya bean	69.2	9.0	1.1	9.0	2.3	9.5

<sup>1</sup>*N*-methylaminomethylphosphonic acid

<sup>2</sup>Defined as extractable  $^{14}\text{C}$  lost during the analytical procedure

### Metabolism in resistant cotton

Cotton that has been genetically modified to be resistant to glyphosate contains the CP4 EPSPS gene. In a study of the metabolism of glyphosate in resistant cotton plants two application of [ $^{14}\text{C}$ ]glyphosate were made to test plots at the 3-4 leaf stage (42 days after planting) and at the 5-6 leaf stage (51 days after planting). The target rates were 0.93 kg/ha for the first application and 1.26 kg/ha for the second application (Bleeke, 1997). The timing and application rates were according to expected GAP for over-the-top treatments. In order to distinguish between radioactive residues resulting from the metabolism of [ $^{14}\text{C}$ ]glyphosate within the plant and those resulting from the uptake of  $^{14}\text{CO}_2$  formed by microbial

degradation of [ $^{14}\text{C}$ ]glyphosate in the soil, the plants were divided into two treatment groups. In one group the soil was covered during application to minimize contact of the test substance with the soil, but was left uncovered in the other.

Crop samples were collected at the forage stage (78 days after planting, or 27 days after the second application) and at maturity (209 days after planting, or 158 days after the second application). At the final harvest the plants were separated into seed, lint, and stalk fractions. The forage samples were extracted with water and the seed samples first with hexane to remove oil, then with 50% acetonitrile in water. The total radioactivity in the forage and seed and its distribution after extraction are shown in Table 2.

Table 2. Distribution of radioactivity in extracts of forage and seed of glyphosate-resistant cotton after treatment with [ $^{14}\text{C}$ ]glyphosate.

Sample	PHI, days	TRR, mg/kg, as glyphosate	[ $^{14}\text{C}$ ], mg/kg (% of TRR)			Total Recovery, %
			Hexane fraction	Aqueous fraction	Unextracted	
TP forage	27	30.4	NA	30.0 (98.5)	0.447 (1.5)	100.0
TU forage	27	15.2	NA	14.7 (96.9)	0.708 (4.7)	101.6
CP forage	27	0.008	NA	NA	NA	NA
CU forage	27	0.039	NA	NA	NA	NA
TP seed	158	0.107	0.012 (11.3)	0.034 (31.9)	0.058 (54.1)	97.3
TU seed	158	0.181	0.027 (14.7)	0.034 (18.6)	0.136 (75.4)	108.7
CP seed	158	0.018	NA	NA	NA	NA
CU seed	158	0.070	0.016 (22.9)	0.006 (8.8)	0.053 (76.1)	107.8

TP: treated protected TU: treated unprotected

CP: control protected CU: control unprotected

NA: not analysed

The total radioactive residues in the forage were 15-30 mg/kg (glyphosate equivalents), but in the seed they were <0.2 mg/kg. There were also relatively high levels of radioactivity in the final harvest control samples (treated with unlabelled glyphosate), particularly in those from the unprotected plots. The level of  $^{14}\text{C}$  in the control samples indicated that the uptake of soil-generated  $^{14}\text{CO}_2$  and its incorporation into the plant made a significant contribution to the total radioactive residues at harvest.

In the plants grown on protected soil the total radioactive residues in the forage were about 30 mg/kg and in the seed about 0.1 mg/kg. In the forage >95% of the TRR was extractable and in the seed hexane and water extracted about 11% and 32% respectively; the unextractable residues accounted for 54%. The extracts were analysed by HPLC with the results shown in Table 3.

Table 3. Compounds identified and characterized in the forage and seed of glyphosate-resistant cotton after foliar application of [ $^{14}\text{C}$ ]glyphosate.

Sample	Compound/or type	TP forage		TP seed	
		% of TRR	mg/kg <sup>1</sup>	% of TRR	mg/kg <sup>1</sup>
Hexane extract	Saponifiable fatty acids	NA	NA	10.4	0.011
	Total	NA	NA	11.3	0.012
	Glyphosate	95.7	29.1	23.7	0.025
	AMPA	0.66	0.201	1.38	0.001
Aqueous extract	Conjugates	0.29	0.087	NA	NA
	Natural products	0.40	0.123	6.93	0.007
	Total	98.5	30.0	31.9	0.034
Seed after hexane and aqueous extraction	Base-extractable	NA	NA	10.7	0.011
	Unextracted	NA	NA	43.4	0.047
	Total	NA	NA	54.1	0.058

<sup>1</sup>As glyphosate

The primary route of metabolism in glyphosate-resistant cotton plants is the gradual conversion of glyphosate to aminomethylphosphonic acid (AMPA), as in susceptible crops. In the forage, glyphosate accounted for almost all the residue with small amounts of AMPA, and in the seed glyphosate constituted more than 64% of the water-extractable residues, or 12-25% of the total radioactive residues. The radioactivity found in the oil and bound in the seed was characterized as being associated with natural products.

#### Metabolism in resistant maize

Maize that has been genetically modified to be resistant to glyphosate contains both the CP4 EPSPS and *gox* genes. To study its metabolism of glyphosate two post-emergence applications of 0.9 and 0.84 kg glyphosate/ha were made at 43 and 73 days after planting (George, 1995). The timing and application rates are expected to become GAP for over-the-top treatments. Again some plots were protected to prevent uptake by the plants of  $^{14}\text{Co}_2$  formed by microbial degradation of [ $^{14}\text{C}$ ]glyphosate in the soil.

Crop samples were collected according to normal agricultural practices. Forage and silage samples were collected 3 and 49-53 days after the second application respectively, and fodder and grain samples 83 days after the second application at normal harvest. Ground forage, silage and fodder samples were extracted with water. Ground grain samples were extracted first with hexane to remove oil, then with water. The total radioactivity found in the forage, silage, fodder and grain and its distribution between extracted and unextracted fractions are shown in Table 4. No significant differences were found between the uncovered and covered soil groups, indicating that the radioactivity in the plants was mainly derived from the applied [ $^{14}\text{C}$ ]glyphosate.

Table 4. Distribution of radioactivity in forage, silage, fodder and grain of glyphosate-resistant maize after treatment with [ $^{14}\text{C}$ ]glyphosate.

Sample	PHI, days	TRR, mg/kg	mg/kg, % of TRR		Total recovery, %
			Extracted	Unextracted	
TP forage	3	13.3	12.8 (96.2)	0.38 (2.9)	99.1
TP silage	49-53	9.11	8.52 (93.5)	0.40 (4.4)	97.9
TP fodder	83	14.9	14.2 (95.2)	0.68 (4.5)	99.7
TP grain	83	0.685	0.54 (79.2)	0.14 (20.9)	100.1
TU forage	3	10.8	10.0 (93.0)	0.31 (2.8)	95.8

Sample	PHI, days	TRR, mg/kg	mg/kg, % of TRR		Total recovery, %
			Extracted	Unextracted	
TU silage	49-53	9.59	8.3 (86.8)	0.43 (4.5)	91.3
TU fodder	83	19.1	18.0 (94.4)	1.0 (5.4)	99.8
TU grain	83	1.04	0.84 (81.1)	0.24 (23.2)	104.3

TP: treated protected  
CP: control protected  
NA: not analysed

TU: treated unprotected  
CU: control unprotected

The total radioactive residues in the forage, silage and fodder of the two groups were 9.11 to 19.1 mg/kg glyphosate equivalents, and in the grain much lower at about 0.7 mg/kg. About 90% of the TRR could be extracted from all samples except grain from which hexane and water extracted about 1% and 80% respectively, with 20-23% remaining in the extracted grain. The aqueous extracts were analysed by HPLC with the results shown in Table 5.

Table 5. Components of the residue found in aqueous extracts of glyphosate-resistant maize forage, fodder and grain after foliar application of [ $^{14}\text{C}$ ]glyphosate.

Metabolite or characterization	mg/kg, (% of $^{14}\text{C}$ in extract)			
	Forage	Silage	Fodder	Grain
Glyphosate	7.77 (71.9)	6.43 (67.1)	14.27 (74.8)	0.03 (2.6)
Glyphosate conjugates	0.04 (0.4)	0.04 (0.4)	0.36 (1.9)	NA
AMPA	1.72 (15.9)	1.26 (13.1)	2.13 (11.2)	0.63 (60.3)
<i>N</i> -glyceryl-AMPA	0.06 (0.5)	0.14 (1.5)	0.31 (1.6)	0.07 (6.9)
Saponifiable fatty acids	NA	NA	NA	0.01 (1.0)
Starch	NA	NA	NA	0.22 (20.9)
Natural products	0.24 (2.2)	0.34 (3.5)	0.65 (3.4)	0.04 (3.6)
Total	9.8 (90.9)	8.2 (85.6)	17.7 (92.9)	1.0 (95.3)

The metabolism of glyphosate in resistant maize, as in cotton, follows the same pathway as in susceptible crops. The results of the study show that glyphosate is gradually metabolized to AMPA and low levels of AMPA conjugates. In grain, AMPA is the major component of the residue, with only trace levels of glyphosate remaining. Grain also contained significant levels of bound radioactivity (about 19% of the TRR). Because glyphosate is rapidly degraded to AMPA owing to the presence of the *gox* protein, further reactions of AMPA become important. These result in higher levels of AMPA conjugates, bound AMPA residues, and natural products derived from the degradation of AMPA to one-carbon fragments.

## METHODS OF RESIDUE ANALYSIS

Glyphosate and its major metabolite, AMPA, can be determined by GLC or HPLC after derivatization.

Samples from supervised trials on susceptible cotton were analysed by the GLC method employing anion and cation exchange resin and carbon clean-up, followed by trifluoroacetylation and methylation, which was evaluated by the 1986 JMPR.

The limit of determination was 0.05 mg/kg in cotton seed and hay and the recoveries at 0.05-0.4 mg/kg of glyphosate and AMPA respectively were 66.3-89.4% and 66.0-84.9% in the hay and 56.7-74.8% and 63.4-93.2% in the seed.

Samples from supervised trials on resistant cotton, resistant and susceptible maize, and susceptible sorghum were analysed by two-column switched HPLC with a post-column reactor. The method was evaluated by the 1994 JMPR.

The limit of determination was 0.05 mg/kg in all commodities and the mean recoveries were as shown in Table 6.

Table 6. Fortification levels and mean recoveries of glyphosate and AMPA in several commodities.

	Cotton <sup>1</sup>		Maize <sup>2</sup>		Maize <sup>1</sup>			Sorghum <sup>2</sup>		
	Seed	Gin	Grain	Fodder	Grain	Fodder	Forage	Grain	Fodder	Hay
Spike, mg/kg	0.06-50	0.03-100	0.05-2.0	0.05-200	0.05-25	0.05-50	0.05-30	0.05-3.0	0.05-20.0	0.05-10.0
Glyphosate mean recovery, %	86.33	84.59	77.36	82.30	86.28	88.38	83.43	87.24	83.70	83.60
AMPA Mean recovery, %	87.31	82.59	82.63	84.19	89.88	88.02	82.21	90.25	78.13	79.39

<sup>1</sup>Resistant

<sup>2</sup>Susceptible

## USE PATTERN

Glyphosate is a leaf-absorbed, non-selective systemic herbicide which is used for the control of unwanted vegetation. Major international use patterns include pre-planting application to most crops, directed spray in tree and vine crops, silvicultural site preparation and conifer release, fallow and reduced tillage systems, general land management in non-crop situations, and pre-harvest application to cereals and oilseeds. The rate of application depends on the type and size of the weeds and the degree of control required.

Table 7 shows the use patterns which have recently been approved in the USA for applications to cotton, maize and sorghum.

Table 7. Registered uses of glyphosate on cotton (susceptible and resistant), maize (susceptible and resistant) and sorghum (susceptible) in the USA.

Crop	Application			PHI, days	Remarks
	Type	No. <sup>1</sup>	Rate, kg/ha <sup>2</sup>		
Cotton	Pre-plant, pre-emergence at-planting		0.32-4.2		Applications must be made before emergence.
	Hooded sprayer selective equipment		0.32-4.2	7	
	Spot treatment		0.32-4.2	7	Apply before boll opening.
	Pre-harvest	1	0.32-4.2	7	Apply after sufficient bolls have developed to produce the desired yield of cotton.
Cotton (glyphosate resistant)	Pre-plant, pre-emergence at-planting		0.32-4.2		
	In-crop	2	0.84	7	Apply from ground cracking to pinhead square stage. Applications must be at least 10 days apart.
	Post-directed hooded sprayer	2	0.84	7	Applications must be at least 10 days apart.
	Pre-harvest	1	1.7	7	Applications must be at least 10 days apart.



Crop	Application			PHI, days	Remarks
	Type	No. <sup>1</sup>	Rate, kg/ha <sup>2</sup>		
Maize	Pre-plant, pre-emergence at-planting		0.32-4.2		Applications must be made before emergence.
	Spot treatment		0.32-4.2	7	Apply before silking of maize.
	Pre-harvest (ground)	1	2.5	7	Apply at 35% grain moisture or less.
	Pre-harvest (aerial)	1	0.84	7	Ensure that maximum kernel fill is complete and the maize is physiologically mature.
	Post-harvest		0.32-4.2		
Maize (glyphosate resistant)	Pre-plant, pre-emergence		0.32-4.2		
	In-crop	2	0.84	7 50	Apply from emergence to 12-leaf stage or 30 inches plant height. Applications must be at least 14 days apart. PHI of 50 days is for forage.
	Pre-harvest	1	0.84	7 50	PHI of 50 days is for forage.
	Post-harvest		0.32-4.2		
Sorghum	Pre-plant, pre-emergence at-planting		0.32-4.2		Applications must be made before emergence.
	Spot treatment, wiper		0.32-4.2	7 40	PHI of 40 days is for wiper application.
	Pre-harvest	1	1.7	7	Apply at 30% grain moisture or less.
	Post-harvest		0.32-4.2		

<sup>1</sup>The combined total of all treatments must not exceed 6.7 kg/ha per year if number of applications is not indicated

<sup>2</sup>Expressed as glyphosate acid (*N*-(phosphonomethyl)glycine)

## RESIDUES RESULTING FROM SUPERVISED TRIALS

The results of supervised trials on susceptible cotton, maize and sorghum are shown in Tables 8-15.

Cotton. Twelve supervised trials on susceptible cotton were reported to the Meeting. The results are shown in Table 8. The underlined residues are from treatments according to GAP and double-underlined residues have been used for the estimation of STMRs. The application patterns in the trials were as follows.

Application (glyphosate acid)	Pre-emergence, kg/ha	Post-emergence, kg/ha	Pre-harvest, kg/ha	Total application, kg/ha
Type 1	6.7	--	3.4	10
Type 2	6.7	2 x 5.0	3.4	20
Type 3	6.7	3 x 5.0	3.4	25
Type 4	2 x 6.7	8.9 (from 3 applicns.)	3.4	26

Table 8. Residues of glyphosate and AMPA in susceptible cotton seed and hay from supervised trials in the USA in 1974 and 1975. SL 41% formulation (Baszis, 1980).

Location	Application <sup>1</sup>			PHI, days	Residues, mg/kg					
	No. (treatment)	kg ai/ha <sup>2</sup>	kg ai/ha <sup>3</sup>		Glyphosate		AMPA		Total <sup>4</sup>	
					Seed	Hay	Seed	Hay	Seed	Hay
Malone, Florida	2 (1)	10	1.2	9	<u>0.54</u>	<u>15</u>	<u>&lt;0.05</u>	<u>0.11</u>	<u>0.62</u>	<u>15</u>
Ropesville, Texas	2 (1)	10	1.6	13	3.6	29	0.06	0.17	3.7	29
Sasser, Georgia	4 (2)	20	1.5	9	<u>2.7</u>	<u>7.4</u>	<u>0.08</u>	<u>0.08</u>	<u>2.8</u>	<u>7.5</u>
Mount Pleasant Mississippi	5 (3)	25	0.52	13	2.9	18	0.07	0.13	3.0	18
St. Joseph Louisiana	6 (type 4)	26	1.8	9	<u>5.9</u>	<u>33</u>	<u>0.07</u>	<u>0.24</u>	<u>6.0</u>	<u>33</u>
Five Points California	2 (1)	10	1.1	13	1.9	17	0.05	0.18	2.0	17
Kerman	1	3.7	1.5	3	1.5	98	0.05	0.46	1.6	99
California				10	0.49	84	0.06	0.45	0.58	85
Weldon	1	3.7	2.8	3	2.0	120	<0.05	0.83	2.1	120
North Carolina				10	0.92	11	0.20	0.09	1.2	11
Cheneyville	1	3.7	1.5	7	<u>2.3</u>	<u>24</u>	<u>&lt;0.05</u>	<u>0.20</u>	<u>2.4</u>	<u>24</u>
Louisiana				14	0.15	11	<0.05	0.10	0.23	11
Sledge	1	3.7	1.8	5	<u>0.63</u>	<u>20</u>	<u>&lt;0.05</u>	<u>0.18</u>	<u>0.71</u>	<u>20</u>
Mississippi				10	0.27	6.3	<0.05	0.09	<u>0.35</u>	6.4
Ropesville	1	3.7	1.5	3	0.47	60	<0.05	0.21	0.55	60
Texas				10	0.26	19	<0.05	0.20	0.34	19
Dawson	1	3.7	1.2	3	4.1	27	<0.05	0.44	4.2	28
Georgia				8	<u>2.9</u>	<u>3.8</u>	<u>0.07</u>	<u>0.21</u>	<u>3.0</u>	<u>4.1</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine<sup>2</sup>Total of all applications<sup>3</sup>Concentration of last application<sup>4</sup>Glyphosate + AMPA expressed as glyphosate

Forty eight supervised trials on susceptible cotton were reported to the Meeting. The results are shown in Tables 9 and 10. The application patterns were as shown below.

Application (glyphosate acid)	Pre-emergence, kg/ha 3-4 leaf	Post- emergence, kg/ha 5-6 leaf	Post- emergence, kg/ha 7-8 leaf	Post- emergence, kg/ha	Post- directed, kg/ha	Pre- harvest, kg/ha	Total Application, kg/ha
Type 1	3.36	1.26			1.26	1.68	7.56
Type 2	3.36	0.84	1.26		1.26	1.68	8.4
Type 3	3.36	0.84		1.26	1.68	1.68	8.82

Table 9. Residues of glyphosate and AMPA in seed and gin from susceptible cotton (genotype 1445) from supervised trials in the USA in 1994 (Oppenhuizen, 1995a). SL 41% formulation.

Location	Application <sup>1</sup>			PHI, days	Residues, mg/kg					
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hl <sup>3</sup>		Glyphosate		AMPA		Total <sup>4</sup>	
					Seed	Gin	Seed	Gin	Seed	Gin
Alabama	4 (1)	7.6	0.90	7	<u>0.46</u>	<u>9.7</u>	<u>&lt;0.05</u>	<u>0.09</u>	<u>0.54</u>	<u>9.8</u>
	5 (2)	8.5	0.90	7	<u>0.47</u>	<u>8.3</u>	<u>&lt;0.05</u>	<u>0.08</u>	<u>0.55</u>	8.4
	5 (3)	8.9	0.90	7	<u>0.44</u>	<u>16</u>	<u>&lt;0.05</u>	<u>0.18</u>	<u>0.52</u>	<u>16</u>
Arkansas	4 (1)	7.5	1.4	8	<u>0.41</u>	<u>4.3</u>	<u>0.21</u>	<u>0.05</u>	<u>0.73</u>	<u>4.4</u>
	5 (2)	8.1	1.4	8	<u>0.33</u>	<u>7.2</u>	<u>0.06</u>	<u>0.07</u>	<u>0.42</u>	<u>7.3</u>
	5 (3)	8.5	1.4	8	<u>0.43</u>	<u>8.6</u>	<u>&lt;0.05</u>	<u>0.08</u>	<u>0.51</u>	<u>8.7</u>
Arizona	4 (1)	7.1	1.0	7	<u>1.3</u>	<u>41</u>	<u>0.06</u>	<u>0.28</u>	<u>1.4</u>	<u>41</u>
	5 (2)	7.9	1.0	7	<u>0.43</u>	<u>23</u>	<u>&lt;0.05</u>	<u>0.20</u>	<u>0.51</u>	<u>23</u>
	5 (3)	8.3	1.0	7	<u>1.0</u>	<u>32</u>	<u>&lt;0.05</u>	<u>0.19</u>	<u>1.1</u>	<u>32</u>
California	4 (1)	7.7	1.3	6	<u>2.8</u>	<u>36</u>	<u>0.08</u>	<u>0.39</u>	<u>2.9</u>	<u>37</u>
	5 (2)	8.5	1.3	6	<u>2.0</u>	<u>26</u>	<u>0.07</u>	<u>0.25</u>	<u>2.1</u>	<u>26</u>
	5 (3)	9.0	1.3	6	<u>1.4</u>	<u>20</u>	<u>0.08</u>	<u>0.18</u>	<u>1.5</u>	<u>20</u>
Louisiana	4 (1)	7.6	1.8	17	0.43	1.2	<0.05	<0.05	0.51	1.3
	5 (2)	8.4	1.8	17	0.50	0.79	0.05	<0.05	0.58	0.87
	5 (3)	8.8	1.8	17	0.40	0.86	<0.05	<0.05	0.48	0.94
Mississippi	4 (1)	7.6	1.2	6	<u>3.6</u>	<u>30</u>	<u>0.07</u>	<u>0.26</u>	<u>3.7</u>	<u>30</u>
(Bolivar)	5 (2)	8.4	1.2	6	<u>3.4</u>	<u>31</u>	<u>0.05</u>	<u>0.23</u>	<u>3.5</u>	<u>31</u>
	5 (3)	8.9	1.2	6	<u>0.69</u>	<u>27</u>	<u>&lt;0.05</u>	<u>0.13</u>	<u>0.77</u>	<u>27</u>
Mississippi	4 (1)	7.7	1.2	9	<u>0.22</u>	<u>5.8</u>	<u>&lt;0.05</u>	<u>0.05</u>	<u>0.30</u>	<u>5.9</u>
(Washington)	5 (2)	8.5	1.2	9	<u>0.41</u>	<u>3.7</u>	<u>&lt;0.05</u>	<u>0.05</u>	<u>0.49</u>	<u>3.8</u>
	5 (3)	8.9	1.2	9	<u>0.13</u>	<u>4.0</u>	<u>&lt;0.05</u>	<u>0.05</u>	<u>0.21</u>	<u>4.1</u>
Tennessee	4 (1)	7.7	1.4	8	<u>3.6</u>	<u>34</u>	<u>0.10</u>	<u>0.45</u>	<u>3.8</u>	<u>35</u>
	5 (2)	8.4	1.4	8	<u>5.0</u>	<u>26</u>	<u>0.13</u>	<u>0.26</u>	<u>5.2</u>	<u>26</u>
	5 (3)	8.8	1.4	8	<u>2.5</u>	<u>29</u>	<u>&lt;0.05</u>	<u>0.27</u>	<u>2.6</u>	<u>29</u>
Texas	4 (1)	7.6	1.5	6	<u>2.0</u>	<u>33</u>	<u>&lt;0.05</u>	<u>0.30</u>	<u>2.1</u>	<u>33</u>
(Uvalde)	5 (2)	8.4	1.5	6	<u>2.2</u>	<u>84</u>	<u>&lt;0.05</u>	<u>0.84</u>	<u>2.3</u>	<u>85</u>
	5 (3)	8.9	1.5	6	<u>2.4</u>	<u>32</u>	<u>&lt;0.05</u>	<u>0.33</u>	<u>2.5</u>	<u>33</u>
Texas	4 (1)	7.9	0.92	8	<u>2.5</u>	<u>18</u>	<u>&lt;0.05</u>	<u>0.21</u>	<u>2.6</u>	<u>18</u>
(Willacy)	5 (2)	8.5	0.92	8	<u>2.7</u>	<u>21</u>	<u>&lt;0.05</u>	<u>0.31</u>	<u>2.8</u>	<u>22</u>
	5 (3)	9.0	0.92	8	<u>3.1</u>	<u>19</u>	<u>&lt;0.05</u>	<u>0.25</u>	<u>3.2</u>	<u>19</u>
Texas	4 (1)	7.5	1.2	7	<u>1.7</u>	<u>29</u>	<u>&lt;0.05</u>	<u>0.12</u>	<u>1.8</u>	<u>29</u>
(Hockley)	5 (2)	8.3	1.2	7	<u>2.8</u>	<u>42</u>	<u>&lt;0.05</u>	<u>0.25</u>	<u>2.9</u>	<u>42</u>
	5 (3)	8.7	1.2	7	<u>4.6</u>	<u>31</u>	<u>&lt;0.05</u>	<u>0.15</u>	<u>4.7</u>	<u>31</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine<sup>2</sup>Total of all applications<sup>3</sup>Concentration of last application<sup>4</sup>Glyphosate + AMPA expressed as glyphosate

Table 10. Residues of glyphosate and AMPA in seed and gin from resistant cotton (genotype 1698) from supervised trials in the USA in 1994 (Oppenhuizen, 1995a). SL 41% formulation.

Location	Application <sup>1</sup>			PHI, days	Residues (mg/kg)					
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hl <sup>3</sup>		Glyphosate		AMPA		Total	
					Seed	Gin	Seed	Gin	Seed	Gin
Arkansas	4 (1)	7.5	1.4	8	<u>0.67</u>	<u>4.0</u>	<u>&lt;0.05</u>	<u>0.08</u>	<u>0.75</u>	<u>4.1</u>
	5 (2)	8.1	1.4	8	<u>0.58</u>	<u>7.8</u>	<u>&lt;0.05</u>	<u>0.09</u>	<u>0.66</u>	<u>7.9</u>

Location	Application <sup>1</sup>			PHI, days	Residues (mg/kg)					
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hi <sup>3</sup>		Glyphosate		AMPA		Total	
					Seed	Gin	Seed	Gin	Seed	Gin
	5 (3)	8.5	1.4	8	<u>0.69</u>	<u>6.2</u>	<u>&lt;0.05</u>	<u>0.10</u>	<u>0.77</u>	<u>6.4</u>
Louisiana	4 (1)	7.6	1.8	17	0.44	2.2	<0.05	<0.05	0.52	2.3
	5 (2)	8.4	1.8	17	0.31	2.0	<0.05	<0.05	0.39	2.1
	5 (3)	8.7	1.8	17	0.30	0.99	<0.05	<0.05	0.38	1.1
Mississippi	4 (1)	7.7	1.2	9	<u>1.2</u>	<u>5.0</u>	<u>&lt;0.05</u>	<u>0.05</u>	<u>1.3</u>	<u>5.1</u>
(Washington)	5 (2)	8.5	1.2	9	<u>0.98</u>	<u>4.3</u>	<u>&lt;0.05</u>	<u>&lt;0.05</u>	<u>1.1</u>	<u>4.4</u>
	5 (3)	8.9	1.2	9	<u>0.60</u>	<u>5.1</u>	<u>&lt;0.05</u>	<u>0.05</u>	<u>0.68</u>	<u>5.2</u>
Texas	4 (1)	7.6	1.5	6	<u>2.5</u>	<u>26</u>	<u>&lt;0.05</u>	<u>0.31</u>	<u>2.6</u>	<u>26</u>
(Uvalde)	5 (2)	8.5	1.5	6	<u>2.1</u>	<u>65</u>	<u>&lt;0.05</u>	<u>0.54</u>	<u>2.2</u>	<u>66</u>
	5 (3)	8.9	1.5	6	<u>1.8</u>	<u>74</u>	<u>0.05</u>	<u>0.67</u>	<u>1.9</u>	<u>75</u>
Texas	4 (1)	7.6	0.92	8	<u>4.0</u>	<u>15</u>	<u>0.14</u>	<u>0.18</u>	<u>4.2</u>	<u>15</u>
(Willacy)	5 (2)	8.5	0.92	8	<u>4.2</u>	<u>16</u>	<u>0.14</u>	<u>0.15</u>	<u>4.4</u>	<u>16</u>
	5 (3)	9.0	0.92	8	<u>4.0</u>	<u>18</u>	<u>0.13</u>	<u>0.16</u>	<u>4.2</u>	<u>18</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine

<sup>2</sup>Total of all applications

<sup>3</sup>Concentration of last application

<sup>4</sup>Glyphosate + AMPA expressed as glyphosate

Twelve supervised trials on susceptible maize were carried out in the USA in 1993, with the results shown in Table 11

Table 11. Residues of glyphosate and AMPA in susceptible maize grain and fodder from supervised trials in the USA in 1993 (Oppenhuizen, 1995b). SL 41% formulation.

Location	Application			PHI, days	Residues, mg/kg					
	No.	kg ai/ha	kg ai/hl		Glyphosate		AMPA		Total	
					Grain	Fodder	Grain	Fodder	Grain	Fodder
Illinois	1	2.5	1.8	6	<u>0.07</u>	<u>23</u>	<u>0.08</u>	<u>0.66</u>	<u>0.19</u>	<u>24</u>
Indiana	1	2.5	2.1	7	<u>&lt;0.05</u>	<u>8.5</u>	<u>&lt;0.05</u>	<u>0.26</u>	<u>&lt;0.13</u>	<u>8.9</u>
Iowa	1	2.5	1.3	7	<u>&lt;0.05</u>	<u>28</u>	<u>0.13</u>	<u>0.41</u>	<u>0.25</u>	<u>29</u>
Kentucky	1	2.5	1.9	7	<u>0.05</u>	<u>43</u>	<u>0.06</u>	<u>0.52</u>	<u>0.14</u>	<u>44</u>
Michigan	1	2.6	1.7	6	<u>&lt;0.05</u>	<u>3.7</u>	<u>&lt;0.05</u>	<u>0.09</u>	<u>&lt;0.13</u>	<u>3.8</u>
Minnesota	1	2.5	1.3	6	<u>0.19</u>	<u>82</u>	<u>&lt;0.05</u>	<u>0.46</u>	<u>0.27</u>	<u>83</u>
Missouri	1	2.5	1.6	6	<u>0.05</u>	<u>8.8</u>	<u>&lt;0.05</u>	<u>0.13</u>	<u>0.13</u>	<u>9.0</u>
Nebraska	1	2.5	2.7	6	<u>&lt;0.05</u>	<u>92</u>	<u>&lt;0.05</u>	<u>0.81</u>	<u>&lt;0.13</u>	<u>93</u>
Ohio	1	2.5	1.6	6	<u>0.54</u>	<u>11</u>	<u>&lt;0.05</u>	<u>0.14</u>	<u>0.62</u>	<u>11</u>
South Dakota	1	2.5	2.5	7	<u>&lt;0.05</u>	<u>55</u>	<u>&lt;0.05</u>	<u>0.33</u>	<u>&lt;0.13</u>	<u>56</u>
Texas	1	2.5	1.4	6	<u>&lt;0.05</u>	<u>54</u>	<u>0.12</u>	<u>0.61</u>	<u>0.23</u>	<u>55</u>
Wisconsin	1	2.5	1.4	7	<u>&lt;0.05</u>	<u>18</u>	<u>&lt;0.05</u>	<u>0.12</u>	<u>&lt;0.13</u>	<u>18</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine

<sup>2</sup>Total of all applications

Sixty six supervised trials on resistant maize in the USA in 1994 were reported to the Meeting. The results are shown in Tables 12 and 13. The application patterns in the trials were as follows.

Treatment type	Pre-emergence, kg/ha	Early post-emergence, kg/ha	Late post-emergence, kg/ha	Pre-harvest, kg/ha	Total application, kg/ha
Type 1	6.38	0.84			7.2
Type 2	6.38	0.84	0.84		8.1
Type 3	6.38	0.84	0.84	0.84	8.9

Table 12. Residues of glyphosate and AMPA in resistant maize grain and fodder from supervised trials in the USA in 1994 (Oppenhuizen, 1995d). SL 41% formulation.

Location	Application <sup>1</sup>			PHI, days	Residues, mg/kg					
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hi <sup>3</sup>		glyphosate		AMPA		Total <sup>4</sup>	
					Grain	Fodder	Grain	Fodder	Grain	Fodder
Colorado	2 (1)	7.2	0.50	124	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
	3 (2)	8.1	0.53	99	<0.05	0.16	<0.05	0.11	<0.13	0.33
	4 (3)	8.9	0.50	6	<u>0.34</u>	<u>8.2</u>	<u>&lt;0.05</u>	<u>0.17</u>	<u>0.42</u>	<u>8.5</u>
Iowa	2 (1)	7.3	0.45	132	<0.05	<0.05	<0.05	0.05	<0.13	0.13
(Butler)	3 (2)	8.1	0.45	117	<0.05	0.09	0.08	0.06	0.17	0.18
	4 (3)	9.0	0.44	7	<u>&lt;0.05</u>	<u>20</u>	<u>0.11</u>	<u>0.35</u>	<u>0.22</u>	<u>21</u>
Iowa	2 (1)	7.2	0.45	143	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Hamilton)	3 (2)	8.1	0.45	130	<0.05	0.14	0.11	0.11	0.22	0.31
	4 (3)	8.9	0.47	7	<u>&lt;0.05</u>	<u>8.0</u>	<u>&lt;0.05</u>	<u>0.17</u>	<u>&lt;0.13</u>	<u>8.3</u>
Iowa	2 (1)	7.2	0.45	132	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Des Moines)	3 (2)	8.1	0.45	99	<0.05	0.12	0.13	0.29	0.25	0.56
	4 (3)	8.9	0.45	7	<u>&lt;0.05</u>	<u>12</u>	<u>0.21</u>	<u>0.50</u>	<u>0.37</u>	<u>13</u>
Illinois	2 (1)	7.2	0.45	124	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Henry)	3 (2)	8.1	0.45	104	<0.05	<0.05	0.13	<0.05	0.25	<0.13
	4 (3)	8.9	0.60	7	<u>&lt;0.05</u>	<u>7.8</u>	<u>0.12</u>	<u>0.12</u>	<u>0.23</u>	<u>8.0</u>
Illinois	2 (1)	7.2	0.62	134	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Clinton)	3 (2)	8.1	0.75	104	<0.05	0.38	0.45	0.34	0.73	0.90
	4 (3)	8.9	0.59	6	<u>&lt;0.05</u>	<u>3.0</u>	<u>0.30</u>	<u>0.41</u>	<u>0.51</u>	<u>3.6</u>
Illinois	2 (1)	7.2	0.51	153	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Warren)	3 (2)	8.1	0.75	121	<0.05	0.10	0.41	0.27	0.67	0.51
	4 (3)	8.9	0.83	7	<u>&lt;0.05</u>	<u>12</u>	<u>0.41</u>	<u>0.47</u>	<u>0.67</u>	<u>13</u>
Indiana	2 (1)	7.2	0.58	111	<0.05	<0.05	0.14	<0.05	0.26	<0.13
	3 (2)	8.1	0.86	97	<0.05	0.41	0.88	0.19	1.4	0.70
	4 (3)	8.9	0.60	6	<u>&lt;0.05</u>	<u>7.1</u>	<u>0.64</u>	<u>1.3</u>	<u>1.0</u>	<u>9.1</u>
Kansas	2 (1)	7.2	0.48	119	<0.05	<0.05	0.33	<0.05	0.55	<0.13
	3 (2)	8.1	0.46	91	<0.05	0.13	0.48	0.13	0.78	0.33
	4 (3)	8.9	0.45	8	<u>&lt;0.05</u>	<u>2.8</u>	<u>0.48</u>	<u>0.21</u>	<u>0.78</u>	<u>3.1</u>
Kentucky	2 (1)	7.2	0.52	109	<0.05	<0.05	0.20	0.13	0.35	0.25
	3 (2)	8.1	0.51	79	<0.05	1.3	1.3	2.74	2.0	5.4
	4 (3)	8.9	0.53	6	<u>0.06</u>	<u>41</u>	<u>1.4</u>	<u>4.7</u>	<u>2.2</u>	<u>48</u>
Michigan	2 (1)	7.2	0.53	135	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13

Location	Application <sup>1</sup>			PHI, days	Residues, mg/kg					
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hi <sup>3</sup>		glyphosate		AMPA		Total <sup>4</sup>	
					Grain	Fodder	Grain	Fodder	Grain	Fodder
	3 (2)	8.1	0.52	114	<0.05	0.48	0.47	0.13	0.76	0.68
	4 (3)	8.9	0.52	6	<u>&lt;0.05</u>	<u>12</u>	<u>0.36</u>	<u>0.42</u>	<u>0.60</u>	<u>13</u>
Minnesota	2 (1)	7.2	0.45	143	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Polk)	3 (2)	8.1	0.45	112	<0.05	0.30	0.28	0.31	0.48	0.77
	4 (3)	8.9	0.45	7	<u>&lt;0.05</u>	<u>2.0</u>	<u>0.30</u>	<u>0.27</u>	<u>0.51</u>	<u>2.4</u>
Minnesota	2 (1)	7.2	0.52	142	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Rock)	3 (2)	8.1	0.55	118	<0.05	0.14	0.13	0.11	0.25	0.31
	4 (3)	8.9	0.57	6	<u>0.06</u>	<u>34</u>	<u>0.21</u>	<u>0.28</u>	<u>0.38</u>	<u>34</u>
Missouri	2 (1)	7.2	0.65	110	<0.05	<0.05	0.12	<0.05	0.23	<0.13
	3 (2)	8.1	0.50	90	<0.05	0.54	1.33	0.69	2.1	1.6
	4 (3)	8.9	0.47	6	<u>&lt;0.05</u>	<u>14</u>	<u>1.00</u>	<u>0.48</u>	<u>1.6</u>	<u>15</u>
North Carolina	2 (1)	7.2	0.45	95	<0.05	<0.05	<0.05	0.30	<0.13	0.51
	3 (2)	8.1	0.45	76	<0.05	0.06	<0.05	0.39	<0.13	0.65
	4 (3)	8.9	0.45	7	<u>&lt;0.05</u>	<u>6.0</u>	<u>&lt;0.05</u>	<u>0.91</u>	<u>&lt;0.13</u>	<u>7.4</u>
Nebraska	2 (1)	7.2	0.45	128	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(York)	3 (2)	8.1	0.45	96	<0.05	0.05	0.24	0.08	0.41	0.17
	4 (3)	8.9	0.45	7	<u>&lt;0.05</u>	<u>1.8</u>	<u>0.32</u>	<u>0.13</u>	<u>0.54</u>	<u>2.0</u>
Nebraska	2 (1)	7.2	0.45	129	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
(Polk)	3 (2)	8.1	0.45	96	<0.05	0.16	0.20	0.15	0.35	0.39
	4 (3)	8.9	0.45	8	<u>&lt;0.05</u>	<u>1.9</u>	<u>0.24</u>	<u>0.21</u>	<u>0.41</u>	<u>2.2</u>
Ohio	2 (1)	7.2	0.48	145	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
	3 (2)	8.1	0.50	107	<0.05	0.10	0.35	0.31	0.58	0.57
	4 (3)	8.9	0.49	6	<u>&lt;0.05</u>	<u>8.4</u>	<u>0.22</u>	<u>0.44</u>	<u>0.38</u>	<u>9.1</u>
Pennsylvania	2 (1)	7.2	0.45	114	<0.05	<0.05	<0.05	0.05	<0.13	0.13
	3 (2)	8.1	0.42	99	<0.05	0.38	0.28	0.43	0.48	1.0
	4 (3)	8.9	0.55	6	<u>&lt;0.05</u>	<u>6.2</u>	<u>0.31</u>	<u>0.62</u>	<u>0.52</u>	<u>7.1</u>
South Dakota	2 (1)	7.2	0.51	142	0.08	<0.05	<0.05	<0.05	0.16	<0.13
	3 (2)	8.1	0.59	112	<0.05	18	0.33	0.40	0.55	19
	4 (3)	8.9	0.58	6	<u>&lt;0.05</u>	<u>20</u>	<u>0.32</u>	<u>0.45</u>	<u>0.54</u>	<u>21</u>
Texas	2 (1)	7.2	0.59	118	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
	3 (2)	8.2	0.61	83	<0.05	0.14	0.16	<0.05	0.29	0.22
	4 (3)	9.1	0.60	7	<u>&lt;0.05</u>	<u>18</u>	<u>0.11</u>	<u>&lt;0.05</u>	<u>0.22</u>	<u>18</u>
Wisconsin	2 (1)	7.2	0.56	118	<0.05	<0.05	<0.05	<0.05	<0.13	<0.13
	3 (2)	8.1	0.56	103	<0.05	0.18	0.10	0.37	0.20	0.74
	4 (3)	8.9	0.46	6	<u>&lt;0.05</u>	<u>6.7</u>	<u>0.14</u>	<u>0.20</u>	<u>0.26</u>	<u>7.0</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine<sup>2</sup>Total of all applications<sup>3</sup>Concentration of last application<sup>4</sup>Glyphosate + AMPA expressed as glyphosate

Table 13. Residues of glyphosate and AMPA in resistant maize forage from supervised trials in the USA in 1994 (Oppenhuizen, 1995d). SL 41% formulation.

Location	Application			PHI, days	Residues, mg/kg		
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hl <sup>3</sup>		glyphosate	AMPA	Total <sup>4</sup>
Colorado	2 (1)	7.2	0.50	80	<0.05	<0.05	<0.13
	3 (2)	8.1	0.53	55	<u>0.11, 0.05</u>	<u>0.16, 0.12</u>	<u>0.35, 0.23</u>
Iowa	2 (1)	7.3	0.45	77	<0.05	0.07	0.16
(Butler)	3 (2)	8.1	0.45	62	<u>&lt;0.05, 0.05</u>	<u>0.14, 0.12</u>	<u>0.26, 0.23</u>
Iowa	2 (1)	7.2	0.45	83	<0.05	<0.05	<0.13
(Hamilton)	3 (2)	8.1	0.45	70	0.08, 0.08	0.21, 0.22	0.40, 0.41
Iowa	2 (1)	7.2	0.45	96	<0.05	<0.05	<0.13
(Des Moines)	3 (2)	8.1	0.45	63	<u>0.11, 0.16</u>	<u>0.46, 0.32</u>	<u>0.81, 0.65</u>
Illinois	2 (1)	7.2	0.45	84	<0.05	<0.05	<0.13
(Henry)	3 (2)	8.1	0.45	64	<u>&lt;0.05, &lt;0.05</u>	<u>0.12, 0.12</u>	<u>0.23, 0.23</u>
Illinois	2 (1)	7.2	0.62	84	<0.05	<0.05	<0.13
(Clinton)	3 (2)	8.1	0.75	54	<u>0.27, 0.19</u>	<u>0.43, 0.45</u>	<u>0.92, 0.87</u>
Illinois	2 (1)	7.2	0.51	97	<0.05	<0.05	<0.13
(Warren)	3 (2)	8.1	0.75	65	<u>0.31, 0.08</u>	<u>0.36, 0.37</u>	<u>0.86, 0.64</u>
Indiana	2 (1)	7.2	0.58	75	<0.05	0.12	0.23
	3 (2)	8.1	0.86	61	<u>0.19, 0.52</u>	<u>0.83, 0.93</u>	<u>1.5, 1.9</u>
Kansas	2 (1)	7.2	0.48	76	<0.05	<0.05	<0.13
	3 (2)	8.1	0.46	48	<u>0.21, 0.21</u>	<u>0.52, 0.44</u>	<u>1.0, 0.88</u>
Kentucky	2 (1)	7.2	0.52	55	<0.05	0.19	0.34
	3 (2)	8.1	0.51	25	0.59, 0.73	2.3, 2.5	4.1, 4.5
Michigan	2 (1)	7.2	0.53	93	<0.05	<0.05	<0.13
	3 (2)	8.1	0.52	72	0.24, 0.23	0.35, 0.56	0.77, 1.1
Minnesota	2 (1)	7.2	0.45	98	<0.05	0.05	0.13
(Polk)	3 (2)	8.1	0.45	67	0.12, 0.14	0.37, 0.44	0.68, 0.81
Minnesota	2 (1)	7.2	0.52	93	<0.05	<0.05	<0.13
(Rock)	3 (2)	8.1	0.55	69	0.10, 0.10	0.20, 0.18	0.40, 0.37
Missouri	2 (1)	7.2	0.65	69	<0.05	0.09	0.19
	3 (2)	8.1	0.50	49	<u>0.10, 0.11</u>	<u>0.90, 1.1</u>	<u>1.5, 1.8</u>
North Carolina	2 (1)	7.2	0.45	67	<0.05	0.11	0.22
	3 (2)	8.1	0.45	48	<u>0.05, 0.05</u>	<u>0.33, 0.32</u>	<u>0.55, 0.54</u>
Nebraska	2 (1)	7.2	0.45	93	<0.05	<0.05	<0.13
(York)	3 (2)	8.1	0.45	61	<u>0.12, 0.13</u>	<u>0.32, 0.26</u>	<u>0.61, 0.53</u>
Nebraska	2 (1)	7.2	0.45	96	<0.05	<0.05	<0.13
(Polk)	3 (2)	8.1	0.45	63	<u>0.08, 0.06</u>	<u>0.37, 0.24</u>	<u>0.64, 0.42</u>
Ohio	2 (1)	7.2	0.48	88	<0.05	<0.05	<0.13
	3 (2)	8.1	0.50	50	<u>0.13, 0.24</u>	<u>0.41, 0.55</u>	<u>0.75, 1.1</u>
Pennsylvania	2 (1)	7.2	0.45	86	<0.05	0.07	0.16
	3 (2)	8.1	0.42	71	0.18, 0.18	0.70, 0.57	1.2, 1.1
South Dakota	2 (1)	7.2	0.51	93	<0.05	0.05	0.13

Location	Application			PHI, days	Residues, mg/kg		
	No. (type)	kg ai/ha <sup>2</sup>	kg ai/hl <sup>3</sup>		glyphosate	AMPA	Total <sup>4</sup>
	3 (2)	8.1	0.59	63	<u>0.20, 0.18</u>	<u>0.47, 0.54</u>	<u>0.91, 1.0</u>
Texas	2 (1)	7.2	0.59	84	<0.05	<0.05	<0.13
	3 (2)	8.2	0.61	49	<u>0.09, 0.05</u>	<u>0.06, 0.05</u>	<u>0.18, 0.13</u>
Wisconsin	2 (1)	7.2	0.56	86	0.10	0.11	0.27
	3 (2)	8.1	0.56	71	0.18, 0.14	0.41, 0.26	0.80, 0.54

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine

<sup>2</sup>Total of all applications

<sup>3</sup>Concentration of last application

<sup>4</sup>Glyphosate + AMPA expressed as glyphosate

Eight supervised trials on susceptible sorghum in the USA were reported in 1994. The results are shown in Tables 14 and 15.

Table 14. Residues of glyphosate and AMPA in susceptible sorghum grain and fodder from supervised trials in the USA in 1992 (Oppenhuizen, 1993). SL 41% formulation.

Location	Application <sup>1</sup>			PHI, days	Residues, mg/kg					
	No.	kg ai/ha	kg ai/hl		Glyphosate		AMPA		Total <sup>2</sup>	
					Grain	Fodder	Grain	Fodder	Grain	Fodder
Arkansas	1	1.7	0.90	8	<u>1.7</u>	<u>16</u>	<u>0.09</u>	<u>0.29</u>	<u>1.8</u>	<u>16</u>
Kansas (Chautauqua)	1	1.7	0.94	6	<u>5.3</u>	<u>33</u>	<u>0.09</u>	<u>0.33</u>	<u>5.4</u>	<u>34</u>
Kansas (Pratt)	1	1.7	1.0	8	<u>13</u>	<u>29</u>	<u>0.09</u>	<u>0.27</u>	<u>13</u>	<u>29</u>
Missouri	1	1.7	0.90	7	<u>6.0</u>	<u>28</u>	<u>0.11</u>	<u>0.22</u>	<u>6.2</u>	<u>28</u>
Nebraska	1	1.7	1.8	8	<u>1.8</u>	<u>7.0</u>	<u>&lt;0.05</u>	<u>0.09</u>	<u>1.9</u>	<u>7.1</u>
Oklahoma	1	1.7	1.2	7	<u>6.3</u>	<u>29</u>	<u>0.22</u>	<u>0.41</u>	<u>6.6</u>	<u>30</u>
South Dakota	1	1.7	0.95	7	<u>13</u>	<u>2.9</u>	<u>0.08</u>	<u>&lt;0.05</u>	<u>13</u>	<u>3.0</u>
Texas	1	1.7	1.4	8	<u>1.4</u>	<u>8.2</u>	<u>0.10</u>	<u>0.16</u>	<u>1.6</u>	<u>8.4</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine

<sup>2</sup>Glyphosate + AMPA expressed as glyphosate

Table 15. Residues of glyphosate and AMPA in susceptible sorghum hay and fodder from supervised trials in the USA in 1992 (Oppenhuizen, 1993). SL 41% formulation, single application.

Location	Application <sup>1</sup>		PHI, days	Residues, mg/kg		
	kg ai/ha	kg ai/hl		glyphosate	AMPA	Total <sup>2</sup>
Arkansas	1.7	0.90	12	<u>18</u>	<u>0.36</u>	<u>19</u>
Kansas (Chautauqua)	1.7	0.94	11	<u>15</u>	<u>0.22</u>	<u>15</u>
Kansas (Pratt)	1.7	1.0	11	<u>37</u>	<u>0.31</u>	<u>37</u>
Missouri	1.7	0.90	15	<u>15</u>	<u>0.15</u>	<u>15</u>
Nebraska	1.7	1.8	12	<u>4.3</u>	<u>0.08</u>	<u>4.4</u>
Oklahoma	1.7	1.2	14	<u>36</u>	<u>0.45</u>	<u>37</u>
South Dakota	1.7	0.95	10	<u>6.4</u>	<u>&lt;0.05</u>	<u>6.5</u>
Texas	1.7	1.4	11	<u>3.1</u>	<u>&lt;0.05</u>	<u>3.2</u>

<sup>1</sup>Expressed as glyphosate acid, *N*-(phosphonomethyl)glycine

<sup>2</sup>Glyphosate + AMPA expressed as glyphosate



## FATE OF RESIDUES IN STORAGE AND PROCESSING

### Storage

No information.

### Processing

Cotton seed. One processing trial was reported to the Meeting. Cotton which was genetically modified to be susceptible to glyphosate was treated five times with glyphosate at a total application rate of 8.4 kg/ha. Six days after the final treatment, cotton seed was harvested and about 30 kg was processed in a batch operation that simulated industrial practice.

The raw cotton seed was saw-delinted to reduce the amount of lint remaining on the seed from 11-15% to about 3%. The hulls were then mechanically cracked and separated to produce the hull and kernel fractions. The kernels were flaked and extruded, and the extruded material was extracted three times with hot hexane to remove the oil. After removal of the solvent, the extracted material was further dried by passing warm air through it to produce solvent-extracted meal.

A portion of the mesicella (crude oil and hexane mixture) was taken and the hexane evaporated off to supply the crude oil fraction. The remaining mesicella was refined by adjusting the ratio of crude oil to hexane to 60:40, adding sodium hydroxide, and centrifuging to separate the soapstock from the refined oil and hexane. The hexane was removed by evaporation under vacuum and the refined oil bleached by heating with activated bleaching earth. The bleached oil was deodorized by heating under vacuum to yield bleached-deodorized refined oil.

The results are shown in Table 16.

Table 16. Residues of glyphosate and AMPA in processed fractions of susceptible cotton seed, USA (Texas) 1994 (Oppenhuizen, 1995a).

Application, kg ai/ha, and timing	Sample	Residues, mg/kg			Processing factor <sup>2</sup>
		glyphosate	AMPA	Total <sup>1</sup>	
3.36 pre-emergence 0.84 3-4 leaf 1.26 5-6 leaf 1-26 post-directed 1-68 pre-harvest	Raw cotton seed	3.7	<0.05	3.8	1.0
	Delinted seed	0.63	<0.05	0.71	0.19
	Kernels	0.24	<0.05	0.32	0.084
	Hulls	1.2	<0.05	1.3	0.34
	Meal	0.39	<0.05	0.47	0.12
	Crude oil	<0.05	<0.05	<0.13	<0.034
	Soapstock	<0.05	<0.05	<0.13	<0.034
	Refined oil	<0.05	<0.05	<0.13	<0.034
	Bleached-refined deodorized oil	<0.05	<0.05	<0.13	<0.034

<sup>1</sup>Glyphosate + AMPA expressed as glyphosate

<sup>2</sup>Based on total residue

Maize. Two processing trials in the USA were reported, in both of which glyphosate-resistant maize plants were treated with glyphosate pre-harvest at an application rate of 2.5 kg/ha. The maize was harvested six or seven days after treatment and 130-200 kg batches were processed by both dry and wet milling in operations that simulated industrial practice.

**Dry milling.** The whole maize grain was dried and cleaned by aspiration and screening. The light impurities from the aspiration were classified as chaff and grain dust and were subdivided by sieving. The cleaned whole grain was moisture-conditioned to 20-22%, allowed to temper for 2-2.5 hours, and impact-milled in a Ripple mill. After milling, the cornstock was dried at 54-71°C for 30 minutes, allowed to cool to approximately 32°C after removal from the oven, and passed over a 1/8 inch shaker screen. The material held by the screen was further processed into large grits, germ and hull (bran). That which passed through the screen was separated into medium and small grits, coarse meal, meal, and flour.

The germ was moisture-conditioned to 12%, heated to 88-104°C, flaked and pressed. The resulting fractions were expelled crude oil and presscake with residual crude oil. The presscake was extracted 3 times with hexane at 50-60°C and the mesicella (crude oil and hexane) passed through a separation unit to separate the crude oil and hexane. The crude oil was heated to 72-90°C to remove hexane. The extracted presscake was dried in a current of warm air, and the crude oil-refined according to American Oil Chemists Society Method Ca9a52.

**Wet milling.** The cleaned whole grain was steeped in water at 50-54°C containing 0.1-0.2% sulfur dioxide for 22-48 hours, then passed through a Bauer mill with devil-toothed plates and the ground product (cornstock) floated in salt water to remove the germ. The cornstock was again ground to separate the hull (bran) and further processed to produce gluten and starch.

The germ was processed as in dry milling to crude and refined oil. The results are shown in Table 17.

Table 17. Residues of glyphosate and AMPA in processed fractions of resistant maize grain, USA, 1993 (Oppenhuizen, 1995c).

Application, Location	Sample	Residues, mg/kg			Processing factor <sup>2</sup>
		Glyphosate	AMPA	Total <sup>1</sup>	
	Raw grain	<0.05	0.08	0.17	1.0
	Cleaned grain	0.05	0.10	0.20	1.2
	Dry-milled crude oil	<0.05	<0.05	<0.13	<0.76
2.5 kg/ha, 6 days PHI	Dry-milled refined oil	<0.05	<0.05	<0.13	<0.76
	Flour	<0.05	0.07	0.16	0.94
Illinois	Grain dust	8.3	1.6	11	65
	Grain dust screenings	14	1.7	17	100
	Grits	<0.05	<0.05	<0.13	<0.76
	Meal	<0.05	0.07	0.16	0.94
	Starch	<0.05	<0.05	<0.13	<0.76
	Wet-milled crude oil	<0.05	<0.05	<0.13	<0.76
	Wet-milled refined oil	<0.05	<0.05	<0.13	<0.76
	Raw grain	<0.05	0.12	0.23	1.00
	Clean grain	<0.05	0.09	0.19	0.83
	Dry-milled crude oil	<0.05	<0.05	<0.13	<0.57
2.5 kg/ha, 7 days PHI	Dry-milled refined oil	<0.05	<0.05	<0.13	<0.57
	Flour	<0.05	<0.05	<0.13	<0.57
Iowa	Grain dust	0.64	<0.05	0.72	3.1
	Grain dust screenings	0.40	<0.05	0.48	2.1
	Grits	<0.05	0.05	0.13	0.57
	Meal	0.05	<0.05	0.13	0.57
	Starch	<0.05	<0.05	<0.13	<0.57
	Wet-milled crude oil	<0.05	<0.05	<0.13	<0.57

		Residues, mg/kg			
	Wet-milled refined oil	<0.05	<0.05	<0.13	<0.57

<sup>1</sup>Glyphosate + AMPA expressed as glyphosate

<sup>2</sup>Based on total residue

Sorghum. Two processing trials in the USA were reported. In both trials, sorghum was treated with glyphosate by pre-harvest application at a rate of 1.7 kg/ha. It was harvested six or eight days after and processed by both dry and wet milling in 70 kg batches according to industrial practice.

The grain was dried and cleaned by aspiration and screening. The light impurities from the aspiration were classified as grain dust. The cleaned grain was then processed by dry and wet milling.

In the dry process the grain was abrasively milled to remove most of the bran and grits, and the seed was ground into flour.

In wet milling, the cleaned grain was steeped in water and then milled to recover germ, hull, coarse gluten-starch, gluten and starch.

The results are shown in Table 18.

Table 18. Residues of glyphosate and AMPA in processed fractions of susceptible sorghum grain, USA, 1992 (Oppenhuizen, 1994).

Application, location	Sample	Residues, mg/kg			Processing factor <sup>2</sup>
		glyphosate	AMPA	Total <sup>1</sup>	
1.7 kg/ha 6 days PHI Kansas	Raw sorghum	4.5	0.05	4.6	1.0
	Bran	18	0.22	18	3.9
	Clean grain	5.9	0.10	6.1	1.3
	Flour	1.5	<0.05	1.6	0.35
	Germ	<0.05	<0.05	<0.13	<0.028
	Grain dust	28	0.29	28	6.1
	Grits, medium	2.7	<0.05	2.8	0.61
	Starch	<0.05	<0.05	<0.13	<0.028
	Steepwater	3.3	0.05	3.4	0.74
1.7 kg/ha 8 days PHI Texas	Raw sorghum	1.1	<0.05	1.2	1.0
	Bran	6.4	0.12	6.6	5.5
	Clean grain	1.1	<0.05	1.2	1.0
	Flour	0.35	<0.05	0.43	0.36
	Germ	<0.05	<0.05	<0.13	<0.11
	Grain dust	3.8	0.07	3.9	3.3
	Grits, medium	0.35	<0.05	0.43	0.36
	Starch	<0.05	<0.05	<0.13	<0.11
	Steepwater	0.39	<0.05	0.47	0.39

<sup>1</sup>Glyphosate + AMPA expressed as glyphosate

<sup>2</sup>Based on total residue

## NATIONAL MAXIMUM RESIDUE LIMITS

The only national MRLs reported were those in the USA which were amended recently. The residue is defined as glyphosate.

Commodity	US MRL, mg/kg
Cotton seed	15
Maize grain	1
Maize forage	1
Maize fodder	100
Sorghum grain	15
Sorghum fodder	40

## APPRAISAL

Glyphosate was first evaluated in 1986, and residue aspects were reviewed in 1987, 1988 and 1994. Maximum residue levels were estimated for kiwifruit and a range of vegetables, cereals, oilseeds and animal products.

The 1997 JMPR was requested to evaluate the new uses of glyphosate on cotton, maize and sorghum according to GAP. These new uses are (1) pre-harvest topical applications and (2) in-crop applications to cotton and maize crops which have been genetically modified to be resistant to glyphosate. Relevant data on metabolism and residue trials were submitted to the Meeting.

### Genetic modification of crops

Glyphosate binds to and blocks the activity of 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), an enzyme of the aromatic amino acid biosynthetic pathway. Glyphosate inhibition of EPSPS prevents the plant from synthesizing the aromatic amino acids essential for protein production. Glyphosate-resistant EPSPS is derived from *Agrobacterium sp.* strain CP4 (CP4 EPSPS), and has been used to develop glyphosate-resistant (i.e. glyphosate-resistant) crops.

While CP4 EPSPS has been successful in providing glyphosate resistance in cotton, its activity alone has been insufficient to ensure adequate resistance in other crops. In maize, a second mechanism has been developed to ensure sufficient levels of crop resistance to allow applications of glyphosate at rates necessary for effective weed control. The second mechanism is glyphosate inactivation, which effectively reduces cellular levels of glyphosate by converting it to aminomethylphosphonic acid (AMPA). The enzyme responsible for glyphosate inactivation is glyphosate oxidoreductase (*gox*). The gene encoding *gox* was isolated from a naturally-occurring bacterium, *Achromobacter sp.*, and has been modified to optimize its expression in plants.

### Plant metabolism

Numerous plant metabolism studies with vegetable, orchard tree, nut tree and pasture crops were reported to the 1986 JMPR. The 1986 Meeting concluded that glyphosate applied to the soil was absorbed very slightly or not at all by the crops examined and its conversion to AMPA, the primary metabolite, was not observed.

However, hydroponic administration allows sufficient uptake of glyphosate to elucidate its metabolism in plants. Metabolic studies with glyphosate in hydroponically-grown maize, wheat, cotton and soya beans have shown the conversion of glyphosate to AMPA and further degradation in plant tissues.

Metabolic studies in plants that have been genetically modified to be resistant to glyphosate show that the metabolism is the same as in susceptible plants. Glyphosate is metabolized to AMPA, which is either non-selectively bound to natural plant constituents, further degraded to one-carbon fragments that are incorporated into natural products, or conjugated with naturally-occurring organic acids to give trace-level metabolites. The metabolites are the same in resistant and susceptible crops but their relative distribution depends on the speed

and extent of conversion to AMPA.

#### Methods of residue analysis

Glyphosate and its major metabolite AMPA can be determined by GLC or HPLC after derivatization. In the GLC method evaluated by the 1986 JMPR, clean-up on anion exchange, cation exchange and carbon columns is followed by trifluoroacetylation and methylation. The limit of determination was 0.05 mg/kg in cotton seed and hay and recoveries of glyphosate and AMPA respectively at 0.05-0.4 mg/kg fortification levels were 66.3-89.4% and 66.0-84.9% in cotton hay, and 56.7-74.8% and 63.4-93.2% in cotton seed.

HPLC methods were discussed in the 1986 and 1994 monographs. The preferred method employs two-column switched HPLC with a post-column reactor. The limit of determination was 0.05 mg/kg in all commodities and mean recoveries were 77-88% for glyphosate and 78-90% for AMPA.

#### Residues of AMPA in or on crops and definition of the residue

The Meeting received data on supervised trials on maize into which the *gox* gene had been introduced, which showed that residue levels of AMPA were much higher than those in normal crops.

The Meeting agreed to recommend two MRLs for residues in maize, one as glyphosate to accommodate uses on glyphosate-susceptible crops and the other as AMPA to accommodate uses on glyphosate-resistant crops. A violation would occur if either MRL were exceeded.

The current definition of the residue is "glyphosate" because residues of AMPA in crops are usually very low or undetectable, except in soya beans.

The Meeting agreed that the definition of the residue for estimations of dietary intake should include AMPA but the definition for enforcement purposes for all commodities, including genetically modified crops, should remain as "glyphosate" for the following reasons.

1. Already many commodities have CXLs based on the residue defined as glyphosate. All existing CXLs would have to be reviewed if the definition of the residue were changed.
2. It is not thought appropriate to establish a separate definition of the residue for maize.
3. The existing definition of the residue has already been incorporated into many national regulations, and a change of the definition would be likely to cause difficulties in international harmonization.

The Meeting also noted the significant residue levels of AMPA that occurred in soya beans, and recommended that their significance should be evaluated in a future periodic review even though they are not believed to pose any risk to consumers.

#### Supervised trials

In the following text the sum of glyphosate + AMPA expressed as glyphosate is referred to as "total glyphosate". The total glyphosate residue was evaluated to estimate STMRs for the assessment of dietary intake.

Cotton. Twelve supervised trials were carried out on glyphosate-susceptible cotton in the USA with pre-harvest application at 3.4 kg ai/ha. US GAP allows pre-emergence (crop) application (including pre-plant or at-planting applications), post-directed application (post-crop-emergence, directed at weeds), spot treatment and pre-harvest application at 4.2 kg ai/ha as the maximum for each treatment. The total application is restricted to 6.7 kg ai/ha per year.

Six of the trials were with pre-emergence and post-emergence applications before a pre-harvest application. The pre-emergence application rate (6.7 kg ai/ha) and the total applied (10-26 kg ai/ha) exceeded the GAP limits, but the Meeting concluded that these trials were comparable with GAP because the rate of the pre-harvest application (3.4 kg ai/ha), which should be most influential on the residue in the harvested crops, was

within the GAP rate of 4.2 kg ai/ha and the studies of plant metabolism indicated that the uptake of glyphosate from soil would be negligible. The other six trials with only one pre-harvest application at 3.4 kg ai/ha were according to GAP.

Sixteen supervised trials, with three different application patterns in each, were carried out on glyphosate-resistant cotton in the USA with 4 or 5 applications which included pre-emergent, post-emergent, post-directed and pre-harvest treatments. Eleven trials were with genotype 1445 cotton and five with genotype 1698 cotton but these have the same basic genetic structure and would be expected to show no differences in glyphosate metabolism.

All the application patterns slightly exceeded US GAP: post-emergence (trials: 0.84-1.26 kg ai/ha, GAP: 0.84 kg ai/ha), post-directed (trials: 1.26 kg ai/ha, GAP: 0.84 kg ai/ha), and total application (trials 7.56-8.8 kg ai/ha, GAP: 6.7 kg ai/ha), but the Meeting again concluded that the trials complied with GAP because the most influential final applications were compatible with GAP and earlier applications would be unlikely to have much effect on the residues.

In susceptible cotton seed the residues of glyphosate were 0.54-5.9 mg/kg at 5-9 days and 0.15-3.6 mg/kg at 10-14 days, and those of AMPA were <0.05-0.20 mg/kg at 5-14 days. The residues of total glyphosate were 0.62-6.0 mg/kg at 5-9 days and 0.23-3.7 mg/kg at 10-14 days, and of total glyphosate after maximum GAP treatments 0.62, 0.71, 2.4, 2.8, 3.0 and 6.0 mg/kg.

In resistant cotton seed the residues of glyphosate were 0.13-5.0 mg/kg at 6-9 days and 0.30-0.50 mg/kg at 17 days, and those of AMPA were <0.05-0.21 mg/kg at 7-9 days. The residues of total glyphosate were 0.21-5.2 mg/kg at 6-9 days and 0.38-0.58 mg/kg at 17 days. Those of total glyphosate after maximum GAP treatments were 0.21, 0.30, 0.42, 0.49, 0.51 (2), 0.52, 0.54, 0.55, 0.66, 0.68, 0.73, 0.75, 0.77 (2), 1.1 (2), 1.3, 1.4, 1.5, 1.8, 1.9, 2.1 (2), 2.2, 2.3, 2.5, 2.6 (3), 2.8, 2.9 (2), 3.2, 3.5, 3.7, 3.8, 4.2 (2), 4.4, 4.7 and 5.2 mg/kg.

Since the differences between both the median and maximum total glyphosate residues in resistant and susceptible crops were not significant, the Meeting based the STMR on the combined residues from the two sets of trials.

The total glyphosate residues from the 48 individual trials which complied with GAP (six on susceptible cotton and 42 on resistant cotton) in rank order (median underlined) were 0.21, 0.30, 0.42, 0.49, 0.51 (2), 0.52, 0.54, 0.55, 0.62, 0.66, 0.68, 0.71, 0.73, 0.75, 0.77 (2), 1.1 (2), 1.3, 1.4, 1.5, 1.8, 1.9, 2.1 (2), 2.2, 2.3, 2.4, 2.5, 2.6 (3), 2.8 (2), 2.9 (2), 3.0, 3.2, 3.5, 3.7, 3.8, 4.2 (2), 4.4, 4.7, 5.2 and 6.0 mg/kg.

The Meeting estimated an STMR level of 2.0 mg/kg total glyphosate. Taking into account the residues of glyphosate alone in susceptible (0.54-5.9 mg/kg) and resistant (0.13-5.0 mg/kg) crops, the Meeting estimated a maximum residue level of 10 mg/kg glyphosate and recommended the withdrawal of the CXL of 0.5 mg/kg.

The residues of glyphosate in the hay from susceptible cotton were 3.8-84 mg/kg at 5-9 days and 6.3-84 mg/kg at 10-14 days, and those of AMPA were 0.10-0.46 mg/kg at 5-14 days. The residues of total glyphosate were 4.1-33 mg/kg at 5-9 days and 6.4-85 mg/kg at 10-14 days.

The glyphosate residues (3.8-84 mg/kg) were below the existing CXL for the straw and fodder (dry) of cereal grains (100 mg/kg), although cotton hay is not classified within this group of commodities. The Meeting agreed not to recommend an MRL for cotton hay in view of its insignificance in international trade.

The residues of glyphosate in the gin by-product from resistant cotton were 3.7-84 mg/kg at 6-9 days and 0.79-2.2 mg/kg at 17 days, and those of AMPA were <0.05-0.84 mg/kg at 6-9 days and <0.05 mg/kg at 17 days. The residues of total glyphosate were 3.8-85 mg/kg at 6-9 days and 0.87-2.3 mg/kg at 17 days.

The Meeting did not recommend an MRL because the commodity does not figure in international trade.

Maize. Twelve supervised trials on susceptible maize and 66 on resistant maize were carried out in the USA.

The 12 trials were with one pre-harvest application (2.5 kg ai/ha). US GAP allows pre-emergence application (0.32-4.2 kg ai/ha), spot treatment (0.32-4.2 kg ai/ha) and pre-harvest application (2.5 kg ai/ha for ground, 0.84 kg ai/ha for aerial) but the Meeting considered that the trials were effectively compatible with the maximum GAP application because the residue from pre-emergence application would be expected to be negligible and spot treatment should not affect crops if carried out according to GAP.

The 66 trials on resistant maize were with 2 to 4 applications which included pre-emergent, post-emergent and pre-harvest applications; 22 of the trials were according to maximum GAP.

Grain. The residues of glyphosate, AMPA and total glyphosate in the susceptible maize were <0.05-0.54 mg/kg, <0.05-0.13 mg/kg and <0.13-0.62 mg/kg respectively at 6-7 days. The residues of total glyphosate after maximum GAP treatments were <0.13 (5), 0.13, 0.14, 0.19, 0.23, 0.25, 0.27 and 0.62 mg/kg.

The residues of glyphosate, AMPA and total glyphosate in the resistant maize were <0.05-0.34 mg/kg, <0.05-1.4 mg/kg and <0.13-2.2 mg/kg respectively at 6-8 days. The residues of total glyphosate after maximum GAP treatments were <0.13 (2), 0.22 (2), 0.23, 0.26, 0.37, 0.38 (2), 0.41, 0.42, 0.51 (2), 0.52, 0.54 (2), 0.60, 0.67, 0.78, 1.0, 1.6 and 2.2 mg/kg.

Since the total glyphosate residues in the susceptible and resistant maize clearly belonged to difference populations, the Meeting estimated an STMR of 0.47 mg/kg total glyphosate, based on the residues in the resistant maize.

On the basis of the residues of glyphosate in susceptible (<0.05-0.54 mg/kg) and resistant (<0.05-0.34 mg/kg) maize, the Meeting recommended an MRL of 1 mg/kg for glyphosate to replace the existing CXL (0.1\* mg/kg). The Meeting also estimated a maximum residue level of 2 mg/kg for AMPA in maize on the basis of the residues of AMPA found in resistant maize (<0.05-1.4 mg/kg).

Fodder. The residues of glyphosate, AMPA and total glyphosate in the susceptible maize fodder were 3.7-92 mg/kg, 0.09-0.81 mg/kg and 3.8-93 mg/kg respectively at 6-7 days. The corresponding residues in the fodder from resistant maize were 1.8-41 mg/kg, <0.05-4.7 mg/kg and 2.0-48 mg/kg respectively at 6-8 days. The residues in both susceptible and resistant maize fodder were below the existing CXL for the straw and fodder (dry) of cereal grains (100 mg/kg).

The Meeting estimated a maximum residue level of 5 mg/kg for AMPA in maize fodder from the residues in fodder from resistant maize (<0.05-4.7 mg/kg).

Forage. According to GAP, the forage of susceptible crops should be cut before the pre-harvest application of glyphosate, whereas the forage of resistant crops can be cut after the application before harvest. Trials to determine residues in forage were therefore restricted to resistant maize.

The residues of glyphosate, AMPA and total glyphosate in the maize forage were <0.05-0.52 mg/kg, 0.06-1.1 mg/kg and 0.18-1.9 mg/kg respectively after 48-65 days. Those of total glyphosate from maximum GAP treatments were 0.18, 0.23, 0.26, 0.35, 0.55, 0.61, 0.64, 0.81, 0.86, 0.92, 1.0 (2), 1.1, 1.8 and 1.9 mg/kg.

The Meeting estimated maximum residue levels of 1 mg/kg glyphosate and 2 mg/kg AMPA, which are recommended for use as MRLs, and an STMR of 0.81 mg/kg total glyphosate.

Sorghum (pre-harvest applications to susceptible plants). Eight supervised trials were carried out in the USA with one pre-harvest application at 1.7 kg ai/ha. US GAP allows pre-emergence application at 0.32-4.2 kg ai/ha, spot treatment at 0.32-4.2 kg ai/ha and pre-harvest application at 1.7 kg ai/ha. For the reasons given above, the Meeting considered the trials to be compatible with maximum GAP.

Grain. The residues of glyphosate, AMPA and total glyphosate were 1.4-13, <0.05-0.22 and 1.6-13 mg/kg respectively after 6-8 days. Those of total glyphosate in rank order were 1.6, 1.8, 1.9, 5.4, 6.2, 6.6 and 13(2) mg/kg.

The Meeting recommended an MRL of 20 mg/kg for glyphosate to replace the existing CXL (0.1\* mg/kg), and an STMR of 5.8 mg/kg for total glyphosate.

Fodder and hay. Residue data said to be on sorghum hay were submitted, but the Meeting concluded that the commodity analysed in the trial should be classified as sorghum fodder.

The residues of glyphosate, AMPA and total glyphosate in fodder were 2.9-33, <0.05-0.41 and 3.0-34 mg/kg respectively at 6-8 days. The corresponding residues in "hay" were 3.1-37, <0.05-0.45 and 3.2-37 mg/kg at 10-15 days.

The glyphosate residues in both fodder (2.9-33 mg/kg) and hay (3.1-37 mg/kg) were below the existing CXL for the straw and fodder (dry) of cereal grains (100 mg/kg).

### Processing

Cotton. Although only one study was available the Meeting agreed to calculate STMR-Ps because the processing adequately simulated industrial practice.

Processing factors from cotton seed to delinted cotton seed, cotton kernels, cotton hulls and cotton meal were 0.19, 0.084, 0.34 and 0.12 respectively. They were <0.034 for processing to crude cotton seed oil, cotton soapstock, refined cotton seed oil and bleached-deodorized cotton seed oil.

The Meeting estimated maximum residue levels of 0.05\* mg/kg for crude and edible cotton seed oil, and STMR-Ps of 0.38, 0.17, 0.68 and 0.24 mg/kg for delinted cotton seed, cotton kernels, cotton hulls and cotton meal respectively, by calculation from the cotton seed STMR of 2.0 mg/kg.

Maize. Residues of glyphosate and AMPA were determined in the processed commodities but the residue of glyphosate in the raw grain was below the LOD, although AMPA was detected. Information on the conversion of glyphosate to AMPA during the processing was not available. The Meeting could not use the data to estimate STMR-Ps.

Sorghum. The mean processing factors were 4.7, 1.2, 0.36, 4.7 and 0.49 from sorghum to bran, clean grain, flour, grain dust and grits (medium) respectively and <0.028 or <0.11 for processing to germ and starch.

The Meeting estimated STMR-Ps of 0 for sorghum germ and starch because they contained negligible residues of glyphosate and AMPA individually, and 27, 7.0, 2.1, 27 and 2.8 mg/kg for bran, clean grain, flour, grain dust and grits (medium) respectively, by calculation from the sorghum STMR (5.8 mg/kg).

## **RECOMMENDATIONS**

### **Glyphosate**

On the basis of the data on residues from supervised trials, the meeting concluded that the residues listed below are suitable for establishing MRLs and STMR to replace previous recommendation for glyphosate.

Definition of the residue for compliance with MRL: glyphosate

Definition of the residue for estimation of dietary intake: sum of glyphosate and aminomethylphosphonic acid (AMPA) expressed as glyphosate.



Commodity		Recommended MRL, mg/kg		PHI, days	Estimated STMR, mg/kg	Estimated STMR-P, mg/kg
CCN	Name	New	Previous			
SO 0691	Cotton seed	10	0.5	7	2.0	
OC 0691	Cotton seed oil, crude	0.05*				0.0
OR 0691	Cotton seed oil, edible	0.05*				0.0
	Delinted cotton seed					0.38
	Cotton seed kernels					0.17
	Cotton seed hulls					0.68
	Cotton seed meal					0.24
GC 0645	Maize	1	0.1*	7	0.47	
AF 0645	Maize forage	1		50	0.81	
GC 0651	Sorghum	20	0.1*	7	5.8	
	Sorghum, cleaned					7.0
	Sorghum bran					27
	Sorghum flour					2.1
	Sorghum grain dust					27
	Sorghum grits (medium)					2.8
	Sorghum germ					0.0
	Sorghum starch					0.0

## AMPA

On the basis of the data on residues from supervised trials, the Meeting concluded that the maximum residue levels listed below are suitable for establishing MRLs.

Definition of the residue for compliance with MRLs: aminomethylphosphonic acid (AMPA)

Commodity		Maximum residue level, mg/kg		PHI, days
CCN	Name	New	Previous	
GC 0645	Maize	2	-	7
AS 0645	Maize fodder	5	-	7
AF 0645	Maize forage	2	-	50

## FURTHER WORK OR INFORMATION

### Desirable

Processing studies with both susceptible and resistant maize in which the raw grain contains measurable residues of both glyphosate and AMPA.

## REFERENCES

Baszis, S. R. 1980. Glyphosate Residues in Cotton Following Topical Treatment with Roundup® Herbicide. Monsanto Company, USA. Report MSL-1283. Unpublished.

Bleeke, M.S. 1997. Nature of Glyphosate Residues in Cotton Plants Tolerant to Roundup® Herbicide. Monsanto Company, USA. Report MSL-14113. Unpublished.

George, C. 1995. Nature of Glyphosate Residues in Corn Plants Which are Tolerant to Roundup® Herbicide. Monsanto Company, USA. Report MSL-14018. Unpublished.

Oppenhuizen, M.E. 1993. Magnitude of Glyphosate Residues Following Preharvest Use in Milo Raw Agricultural Commodities. Monsanto Company, USA. Report MSL-13037. Unpublished.

Oppenhuizen, M.E. 1994. Magnitude of Glyphosate Residues in Milo Processed Commodities Following Preharvest Use of Roundup® Herbicide. Monsanto Company, USA. Report MSL-13038. Unpublished.

Oppenhuizen, M.E. 1995a. Magnitude of Glyphosate Residues in Glyphosate-Tolerant Cotton Raw Agricultural and Processed Commodities. Monsanto Company, USA. Report MSL-13884. Unpublished.

Oppenhuizen, M.E. 1995b. Magnitude of Glyphosate Residues in Corn Raw Agricultural Commodities Following Preharvest Application of Roundup® Herbicide. Monsanto Company, USA. Report MSL-13654. Unpublished.

Oppenhuizen, M.E. 1995c. Magnitude of Glyphosate Residues in Corn Processed Commodities Following Preharvest Application of Roundup® Herbicide. Monsanto Company, USA. Report MSL-13655. Unpublished.

Oppenhuizen, M.E. 1995d. Magnitude of Glyphosate Residues in Glyphosate-Tolerant Corn Raw Agricultural Commodities. Monsanto Company, USA. Report MSL-13882. Unpublished.

Rueppel, M.L. 1973. The metabolism of CP 67573 in soybeans, cotton, wheat and corn. Monsanto Company, USA. Report 304. Unpublished.