Fd Chem. Toxic. Vol. 28, No. 5, pp. 339–349, 1990
Printed in Great Britain.

0278-6915/90 $3.00 + 0.00

SUMMARY OF SAFETY EVALUATION TOXICITY STUDIES OF GLUFOSINATE AMMONIUM*

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(*Received* 1 *September* 1989; *revisions received* 2 *January* 1990)

Abstract—This article reviews the results of toxicity studies to evaluate the safety of the herbicide glufosinate ammonium (GLA) and its formulation (200 g/litre) in laboratory animals. The data show that GLA and its formulation are slightly toxic following oral exposure. In addition, the formulation induced slight dermal toxicity and eye irritation. Testing for teratogenicity in rats and rabbits indicated no teratogenic potential, and numerous mutagenicity tests showed GLA to be non-genotoxic. Chronic toxicity testing in rats and dogs yielded no-observable-effect levels of 2 and 5 mg/kg body weight/day, respectively. Oncogenicity studies in rats and mice revealed no carcinogenic potential. On the basis of these toxicity data it is concluded that this herbicide is safe under conditions of recommended use.

INTRODUCTION

Glufosinate ammonium (GLA), the active ingredient of the new non-selective herbicide BASTA®, is a phosphinic acid analogue of glutamic acid. Registrations have been granted in many countries since 1984. The herbicidal properties of GLA were described by Hoechst AG in 1976 (Schwerdthe *et al.*, 1981). Its herbicidal action is related to the inhibition of glutamine synthetase (GS), an enzyme that plays an important role in ammonia detoxification and amino acid metabolism in plants. In view of the structural analogy between this compound and glutamic acid, numerous special examinations have been included in the toxicity studies. This paper reports the results of toxicological studies conducted to evaluate the safety of GLA for human health, both for users and consumers.

MATERIALS AND METHODS

All studies reported here were conducted, in accordance with Good Laboratory Practice regulations, at the Department of Toxicology, Hoechst AG, Frankfurt am Main, FRG, or in contract laboratories. References to the reports that give further details of these studies are cited in Results.

Test compound

The chemical structure of GLA [ammonium-DL-homoalanin-4-yl(methyl)phosphinate (IUPAC), CAS no. 77182–82–2] is given in Fig. 1. The physicochemical properties are as follows: mol wt, 198.2; appearance, white crystalline powder; sp. gr., 1.4 (20°C); m.p., 215°C (pyrolysis); vapour pressure, 3.5×10^{-6} mm Hg (20°C); solubility (water), 1370 g/litre (20°C).

All test materials were supplied by the Agricultural Division, Hoechst AG, and identified by analysis certificates for purity. None of the different technical lots had a purity <92.5%. The formulated product had a GLA content of 200 g/litre and contained as auxiliaries water, wetting agent (alkylether-sulphate), solvent (propylene glycol ether), defoamer and a blue dyestuff.

Analysis of GLA preparations

The concentration, homogeneity and stability of GLA in the diets were checked by high-performance liquid chromatography or gas chromatography. GLA proved stable in the diets for at least 3 wk at room temperature and the GLA concentrations corresponded closely to the nominal values.

In aqueous solutions GLA proved stable for at least 3 days.

Acute toxicity

Ten male and ten female Fischer 344 rats and ICR mice were given po, sc or ip various doses of GLA technical dissolved in physiological saline (ip, sc route) or deionized water. Dermal toxicity was tested in Wistar rats. In the studies with the formulation, five Wistar rats or NMRI mice per sex and dose group were treated using deionized water as vehicle. In the oral study with New Zealand White rabbits, three animals per sex and group were used. In all of the studies the animals were observed daily for 2 wk and time-to-death was recorded. The LD_{50} values were calculated by probit analysis [Litchfield and Wilcoxon (1949) or Finney (1971)].

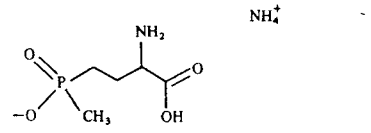

Fig. 1. Structural formula of glufosinate ammonium.

*This article is dedicated to Professor Dr rer. nat. Hansgeorg Gareis on the occasion of his 60th birthday.

Abbreviations: CNS = central nervous system; GLA = glufosinate ammonium; GS = glutamine synthetase; GSH/GSSG = glutathione reduced/oxidized; NOELs = no-observable-effect levels; 2-PAM = 2-pralidoxime; TOCP = triorthocresylphosphate.

Primary skin and eye irritation and skin sensitization

Skin and eye irritation were tested in female New Zealand White rabbits, weighing 2–3 kg, in compliance with OECD Guidelines nos 404 and 405 (OECD, 1981). Both GLA and the formulation were tested.

To assess contact sensitization in Pirbright White guinea-pigs the modified Buehler method (Ritz and Buehler, 1980) was used. As an induction phase, the guinea-pigs were exposed for 6 hr three times a week for 3 wk to a 50% aqueous dilution under an occluded patch. The animals were challenged with 50% solution in the third week after the final induction exposure. Ten control and 20 guinea-pigs treated with the test substance were used in these studies.

Subchronic oral toxicity

Subchronic (13-wk) feeding studies were carried out using rats, mice and dogs. Groups of 40 male and 40 female F344 rats were exposed to 0, 8, 64, 500 or 4000 mg GLA/kg diet and 25 rats in each group were killed after 13 wk exposure. The remaining 15 rats/group were maintained on the control diet for a further 4 wk and then killed. From each group five rats were used only for measurement of hepatic GS and serum ammonia at the end of the treatment and recovery periods.

Groups of ten male and ten female NMRI mice were fed GLA at dietary levels of 0, 80, 320 or 1280 mg/kg diet for 13 wk and groups of four male and four female beagle dogs were treated with 0, 4, 8, 16, 64 or 256 mg GLA/kg diet. Clinical observations, haematology, clinical biochemistry, urinalysis, gross necropsy and histopathology were conducted in compliance with OECD Guidelines nos 408 and 409 (OECD, 1981). In the rat study, GS activity in the liver was also determined, according to the modified method of Elliott (1955).

Chronic toxicity and oncogenicity

A combined chronic toxicity/oncogenicity study in Wistar rats was carried out in compliance with OECD Guideline no. 453 (OECD, 1981). The rats were exposed to 0, 40, 140 or 500 mg GLA/kg diet. The study included determinations of GS and NH_4 in kidneys, liver and brain and of glutathione reduced/glutathione oxidized (GSH/GSSG) in the liver and blood.

A 2-yr oncogenicity study was carried out in NMRI mice according to OECD Guideline no. 451 (OECD, 1981). GLA was fed at concentrations of 0, 20, 80, 160 (males only) or 320 (females only) mg/kg diet to groups of 60 male and 60 female mice. Ten mice/sex/group were allocated for assessment of chronic toxicity and were killed after 52 wk.

In a 12-month feeding study, GLA was administered to groups of eight male and eight female beagle dogs at dietary concentrations that provided doses of 0, 2, 5 or 8.5 mg GLA/kg body weight/day. Four males and four females from the 0-, 2- and 5-mg/kg groups and three males and three females from the high-dose group were killed after 6 months. The examinations carried out complied with OECD Guideline no. 452 (OECD, 1981).

Reproductive toxicity

Two-generation study. A two-generation reproduction study in Wistar rats was carried out according to OECD Guideline no. 416 (OECD, 1983). GLA was fed in the diet to groups of 30 male and 30 female rats of the F_0 generation in concentrations of 0, 40, 120 or 360 ppm. The rats were given the test material for 80 days before mating (F_{1a}) and throughout gestation and lactation. The animals were rested and then mated again to produce the F_{1b} generation. When weaning the F_{1b} generation, 26 male and 26 female pups from each group were selected to parent the F_{2a} and F_{2b} generations. Diet containing GLA was fed to all animals of all generations.

Embryotoxicity. In two studies GLA was administered by gavage on days 6–15 of gestation to groups of 20 Wistar rats at doses of 0, 10, 50 or 250 mg/kg/day (Main Study) or 0, 0.5, 2.24 or 10 mg/kg body weight/day (Supplementary Study). The rats were killed on day 21 of gestation and the pups were delivered by caesarian section.

GLA was administered by oral gavage to groups of 15 Himalayan rabbits on days 6–18 of gestation at dose levels of 0, 2, 6.3 or 20 mg/kg body weight/day. The rabbits were killed on day 29 of gestation and delivered by caesarian section.

About 50% of the foetuses in each study were examined for soft-tissue abnormalities according to the method of Wilson (1965). The remaining foetuses were stained with Alizarin Red S (Dawson, 1926) for the examination of skeletal anomalies. The test design complied with OECD Guideline no. 414 (OECD, 1981).

Mutagenicity

For an assessment of the mutagenic potential of GLA, nine tests using different endpoints were performed.

Microbial genotoxicity. GLA was tested in the Salmonella/histidine reversion assay with five *his* strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) and in the *Escherichia coli* WP2 reverse mutation assay at doses of 0, 1, 5, 10, 50, 100, 500 or 1000 μg/plate both in the presence and absence of metabolic activation (S-9) as described by Ames *et al.* (1975). A Rec-assay was also performed in which the normal (H17, *rec*$^+$) and recombination deficient (M45, *rec*$^-$) strains of *Bacillus subtilis* were tested at doses of 0, 50, 100, 500, 1000, 5000 or 10,000 μg/well according to the method described by Kada *et al.* (1972). After overnight incubation at 37°C, the length of the inhibitory zones were measured.

Yeast genotoxicity. GLA was tested for gene conversion/DNA repair in *Saccharomyces cerevisiae* D4 in the presence and absence of metabolic activation as described by Zimmermann (1971). Concentrations of 0, 1000, 2500, 5000 and 10,000 μg/ml were tested. A forward mutation assay was carried out in *Schizosaccharomyces pombe* P1 both with and without S-9 as described by Loprieno *et al.* (1974). GLA was tested at doses of 0, 125, 250, 500 and 1000 μg/ml.

Mammalian genotoxicity in vitro. An assay for unscheduled DNA synthesis was carried out in

non-induced primary rat hepatocytes from an adult male Fischer 344 rat according to Williams (1977). Concentrations of 0, 26.2, 52.4, 105, 262, 524, 1050, 2620 and 5240 μg GLA/ml were tested.

In the mouse lymphoma forward mutation assay in L5178Y *TK+/−* cells, GLA was tested at concentrations of 500–5000 μg/ml without metabolic activation and at 300–5000 μg/ml with activation, as recommended by Clive *et al.* (1979).

An assay for chromosomal aberrations in cultured human lymphocytes was performed using GLA concentrations of 0, 1, 10, 100 or 1000 μg/ml, according to the methods described by Buckton and Evans (1979). A single culture was prepared for each dose and exposed at 37°C for 3 hr both in the presence and absence of metabolic activation. One hundred metaphases from each dose were examined.

Mammalian genotoxicity in vivo. A micronucleus test was carried out using NMRI mice. Five male and five female mice were given by gavage 0, 100, 200 or 350 mg GLA/kg body weight or 50 mg Endoxan/kg body weight (positive control). The mice were killed 24, 48 or 72 hr later and examined for micronuclei as described by Salamone *et al.* (1980).

Metabolism and pharmacokinetics in rats

Studies of the absorption, excretion and metabolism of ^{14}C-labelled GLA were carried out in rats.

Groups of five male and five female rats were given GLA at doses of 2 or 30 mg/kg body weight by single or repeated (15 consecutive) oral intubation. A further group were given single iv doses of 2 mg/kg body weight. The rats were killed 7 days after radioactive dosing and examined for the quantities of label in the tissues and organs, urine, faeces and expired air during the observation period. Furthermore, the major metabolites were identified in urine and faeces. These examinations were carried out in accordance with EPA Guideline no. 85-1 (EPA, 1984).

Special examinations

An acute delayed neurotoxicity study was carried out in hens. The hens were treated twice with 4000 mg GLA/kg body weight (12 hens) or 10 ml water (vehicle)/kg body weight (six hens) with an interval of 21 days between each treatment, or once with 500 mg TOCP/kg body weight (six hens) as a positive control. They were assessed daily for the development of ataxia. They were killed 21 days after the last dosing and the brain, spinal cord and peripheral nerves were examined histologically.

In order to test for possible therapeutic measures in the event of acute poisoning, GLA was administered to three groups of ten male and ten female Wistar rats in lethal doses of 3200 (male) or 2200 (female) mg/kg body weight. Up to 3 days after administration of the GLA, individual ip injections of phenobarbital (20 mg/kg body weight), or atropine sulphate (10 mg/kg) with or without 2-pralidoxime (2-PAM) iodide (75 mg/kg) were given. The rats were observed for 14 days.

RESULTS AND DISCUSSION

Acute toxicity

The results obtained with GLA and its formulation are given in Tables 1 and 2, respectively. No sex-specific differences were observed by any route of administration. The predominant signs of intoxication after treatment with lethal and/or high sublethal doses were passivity, disequilibrium, hypersensitivity, generalized body tremors and convulsive movements, salivation and lacrimation, indicating the central nervous system as target organ. These symptoms occurred with a delay of several hours following treatment (Inoue and Okamura, 1982a,b,c; Ohtaka *et al.*, 1981a,b,c).

Table 1. Acute toxicity of glufosinate ammonium (GLA), technical, following oral, sc, ip and dermal exposure in rats and mice

Species	Sex	LD_{50} (mg/kg body weight) Oral	Sc	Ip	Dermal
Rat	M	1660	73	96	>4000
	F	1510	61	83	4000
Mouse	M	436	88	103	ND
	F	464	104	82	ND

ND = not done

There were differences between mice and rats in the acute oral toxicity of GLA. These were evidently due to differences in kinetics and bioavailability. Comparison of enteral and parenteral toxicity indicated that the enteral absorption rate was low in the rat and relatively higher in the mouse.

However, the oral LD_{50} values for the formulation were practically identical in rats (Markert and Leist, 1985a), mice (Diehl and Leist, 1987) and rabbits (Ebert and Leist, 1988), ranging between about 1500 and 2000 mg/kg body weight. In the rat, the enteral absorption rate was increased to 25% by the formulation agents; in the mouse, with its already relatively high absorption rate of 30%, there was no further enhancement.

The dermal toxicity of the formulation in rats was higher than that of the technical substance. It was shown that the low dermal absorption rate of GLA (Mayer and Weigand, 1982a) can be markedly increased by the formulating agents (Markert and Leist, 1985b).

Primary skin and eye irritation and skin sensitization

GLA was non-irritating to the skin and eyes of rabbits (Mayer and Weigand, 1982b). The formulation was shown to be slightly irritating to the skin and moderately irritating to the eyes due to the irritant properties of the formulating agents (Ebert and Leist, 1985; Markert and Leist, 1985c).

Both GLA technical and its formulation proved to be non-sensitizing in the modified Buehler test (Rupprich and Weigand, 1983; Ullmann and Sachsse, 1985).

Subchronic oral toxicity

The results of these studies are summarized in Tables 3–5. In the tested dose range GLA caused

Table 2. Acute oral and dermal toxicity of the glufosinate ammonium (GLA) formulation (200 g/litre) in different species

Species	Sex	Route of administration	LD_{50} (mg/kg body weight)
Rat	M/F	Oral	2170/1910
Mouse	M/F	Oral	1420/1570
Rabbit	M + F	Oral	1550
Rat	M/F	Dermal	1400/1380

Table 3. Changes induced in F344 rats fed glufosinate ammonium (GLA) for 13 wk

Parameter	No. of rats examined	Dose (mg/kg diet)...	Values for: Males 0	8	64	500	4000	Females 0	8	64	500	4000
Main study												
GLA intake (mg/kg body weight/day)	30		0	0.52	4.1	32	263	0	0.63	4.8	39	311
Body-weight gain and food consumption	30		—	NS	NS	NS	Dec in wk 1, 2, 3	—	NS	NS	NS	Dec in wk 1, 2, 3
Clinical signs, haematology and clinical biochemistry	30 10 10		—	NS	NS	NS	NS	—	NS	NS	NS	NS
Urinalysis:												
pH	10		7.5	7.6	7.4	7.5	6.0	7.6	7.2	7.3	7.2	7.0
Specific gravity	10		1.052	1.053	1.055	1.055	1.055	1.041	1.045	1.045	1.045	1.052*
Relative kidney weight (% of body weight):												
13 wk	20		0.62	0.63	0.64*	0.66**	0.71**	0.69	0.69	0.69	0.70	0.74**
4-wk recovery	10		0.60	0.60	0.60	0.63*	0.64**	0.68	0.68	0.65*	0.67	0.69
Histopathology	20		—	NS	NS	NS	NS	—	NS	NS	NS	NS
Satellite study												
Hepatic GS activity (μmol/mg/20 min $\times 10^{-1}$):												
13 wk	5		1.36	1.13	0.99	0.97	0.54*	1.53	1.54	1.28	0.94*	0.63*
4-wk recovery	5		1.40	1.33	1.22	1.39	1.26	1.30	1.34	1.41	1.36	1.50
Serum ammonia (μg/100 ml)	5		176	171	175	171	179	161	170	170	160	197

NS = not significantly different from control Dec = decrease

Values are means for the numbers of rats shown and are for 13 wk unless otherwise indicated.

Values marked with asterisks differ significantly (Student's t-test) from the corresponding control values (*$P < 0.05$; **$P < 0.01$).

Table 4. Changes induced in NMRI mice by feeding glufosinate ammonium (GLA) for 13 wk

Parameter / Dose (mg/kg diet) ...	Values for: Males 0	80	320	1280	Females 0	80	320	1280
GLA intake (mg/kg body weight/day)	0	16.6	67.1	277.7	0	19.4	86.6	288.2
Body-weight gain, food consumption, clinical signs and haematology	—	NS	NS	NS	—	NS	NS	NS
Clinical biochemistry:								
ASAT (μkat/litre serum)	1.08	1.25	1.01	1.45*	1.25	2.21*	1.64	1.52
AP (mmol/litre serum)	2.61	2.74	2.58	2.52	3.43	3.79	3.80	4.72*
K (mmol/litre serum)	4.35	4.40	5.16*	5.39*	4.14	4.18	4.01	4.62
Relative organ weights (%):								
Liver	4.5	4.5	4.7	4.9*	4.7	4.7	4.8	4.7
Kidneys	1.6	1.6	1.6	1.7	1.5	1.5	1.5	1.5
Histopathology	—	NS	NS	NS	—	NS	NS	NS

ASAT = aspartate aminotransferase AP = alkaline phosphatase
NS = not significantly different from control
Values are means for groups of ten mice and those marked with an asterisk differ significantly (Student's t-test) from the corresponding control value ($^*P < 0.05$).

slight effects that indicated that the renal system was a possible target organ in rats and mice and also that the liver might be affected in mice. Hepatic GS activity in rats was significantly inhibited at doses of $\geqslant$500 mg/kg diet, the inhibition being reversed after the 4-wk withdrawal period. However, this effect, which was to be expected in view of the structural analogy between GLA and glutamic acid (the substrate of the GS-catalysed reaction, glutamate + $NH_3 \rightarrow$ glutamine) did not correlate with measurable changes in hepatic function or the serum ammonia level. In the dog only a slight effect on food consumption and body weight was established at the highest dose level (8 mg/kg/day). On the basis of these findings, the following no-observable-effect levels (NOELs) were established: rat, 64 ppm = 4.1 mg/kg body weight/day (Ohtaka *et al.*, 1981d); mouse, 80 ppm = 17 mg/kg/day (Suter and Sachsse, 1984); dog, >64 ppm = >2 mg/kg/day (Til and de Groot, 1982).

Chronic toxicity and oncogenicity

The results of the study in rats are shown in Table 6. Mortality among the females treated with 140 or 500 ppm GLA in the diet for 130 wk was slightly higher. There were also slight increases in kidney weights after 130 wk, but no correlating signs of renal toxicity. Additionally, treatment at 500 ppm caused slight anaemia in both sexes that was observed only at 52 wk. Measurement of GS revealed slight inhibition in the liver after 52 wk and 104 wk at the 140- and 500-ppm doses and slight to moderate increases in kidneys after 52 and 104 wk at all doses. The glutathione level (GSH and GSH + GSSG) in liver and blood was slightly lower at the 140- and 500-ppm dose in comparison with the control. This effect appeared to be more marked in females. No changes were observed in any other parameters. Histopathology revealed no GLA-related non-neoplastic or neoplastic lesions. Thus, a NOEL of 40 ppm (2.1 mg/kg body weight/day) was established (Suter and Sachsse, 1986a).

In the oncogenicity study in mice, the survival rate of the males fed 160 ppm was decreased and the GSH level in the serum was slightly lower (Table 7). Body-weight gain, food consumption, mortality and general health were unaffected by GLA. The decrease in body weight during wk 3–33 among the mice in the highest dose group scheduled for the interim kill after 52 wk was considered to be incidental and related to the small group size. Haematology and histopathology indicated no GLA-related changes. The types and incidences of the neoplastic lesions observed were within the normal spectrum for mice of this age and strain and there was no evidence of an oncogenic potential of GLA in mice. On the basis of these results the NOEL was 80 ppm, equivalent to 11 mg/kg body weight/day (Suter and Sachsse, 1986b).

Table 5. Changes induced in beagle dogs by feeding glufosinate ammonium (GLA) for 13 wk

Parameter / Dose (mg/kg diet) ...	Values for: Males 0	4	8	16	64	256	Females 0	4	8	16	64	256
GLA intake (mg/kg body weight/day)	0	0.13	0.26	0.57	2.06	7.98	0	0.13	0.25	0.49	1.97	7.89
Body-weight gain (kg)	1.8	1.7	1.5	1.4	1.5	1.6	1.8	1.4	0.7**	1.0	0.9*	0.7**
Body-weight at 13 wk (kg)	14.3	14.1	14.2	13.6	13.8	13.6	13.1	13.1	11.7**	12.5	12.2*	11.8**
Clinical signs, haematology and clinical biochemistry	—	NS	NS	NS	NS	NS	—	NS	NS	NS	NS	NS
Relative organ weights (%)	—	NS	NS	NS	NS	NS	—	NS	NS	NS	NS	NS
Histopathology	—	NS	NS	NS	NS	NS	—	NS	NS	NS	NS	NS

NS = not significantly different from control
Values are means for groups of four dogs, and those marked with asterisks differ significantly (Dunnett's test, two-sided) from the corresponding control value ($^*P < 0.05$; $^{**}P < 0.01$).

Table 6. Changes induced by glufosinate ammonium (GLA) in the combined study on chronic toxicity and oncogenicity in Wistar rats

Parameter	Dose (mg/kg diet) ...	Values for: Males 0	40	140	500	Females 0	40	140	500
No. of rats									
Chronic toxicity—52 wk		10	10	10	10	10	10	10	10
—104 wk		20	20	20	20	20	20	20	20
Oncogenicity—130 wk		50	50	50	50	50	50	50	50
GLA intake (mg/kg body weight/day):									
104 wk		0	2.1	7.6	26.7	0	2.5	8.9	31.5
130 wk		0	1.9	6.8	24.4	0	2.4	8.2	28.7
Mortality at 130 wk (%)		50	54	54	42	30	46	54†	58†
Haematological data (52 wk):									
Erythrocyte count (T/litre)		9.4	9.7	9.6	8.8	8.5	8.2	8.5	7.9*
Haemoglobin concentration (mmol/litre)		9.3	9.5	9.3	8.7*	9.0	8.9	9.1	8.7*
Haematocrit (litre/litre)		0.42	0.43	0.43	0.39*	0.42	0.40*	0.42	0.39*
Glutamine synthetase activity‡:									
In liver—52 wk		2.29	2.07	1.80*	1.71*	2.85	2.67	2.38*	2.28*
—104 wk		2.33	2.39	2.21	2.35	2.92	3.28	3.56*	3.27
In kidney—52 wk		1.28	1.41	1.51*	1.72*	0.79	1.19*	1.27*	1.52*
—104 wk		1.47	2.03	2.15*	2.35*	1.19	1.61*	2.01*	2.28*
In brain—52 wk		2.48	2.48	2.52	2.34	2.35	2.37	2.19	2.14
—104 wk		—	—	2.34	2.19	2.53	2.32*	2.44	2.24*
Glutathione at 130 wk:									
In liver (μmol/g)									
—GSH		5.49	5.37	5.59	6.02	4.98	4.50	3.10*	3.87*
—GSSG		1.53	1.43	1.19*	0.98*	1.32	1.39	1.26	1.30
—GSH + GSSG		7.02	6.80	6.78	6.99	6.30	5.89	4.36*	5.17*
In whole blood (μmol/ml)									
—GSH		0.82	0.76	0.71	0.54*	0.98	0.79	0.66*	0.64*
—GSSG		0.20	0.19	0.24	0.32*	0.25	0.24	0.25	0.31
—GSH + GSSG		1.02	0.95	0.95	0.85	1.23	1.03	0.90*	0.95

‡μmol γ-glutamyl-hydroxamate found/ml reaction mixture/20 min at 37°C.

Values are means for the number of rats shown except for glutamine synthetase activity (liver, n = 10; kidneys, n = 5; brain, n = 5) and glutathione in liver and blood, n = 10). Values marked with superscripts differ significantly from the corresponding control value: *Dunnett's test based on pooled variance or Steel's test significant at 5% level; †significant in Fisher's exact test.

In the 12-month feeding study in beagle dogs, one male and one female from the high-dose group died during wk 2, showing signs of acute CNS intoxication. (During this phase the intake of GLA ranged from 11 to 14 mg/kg body weight/day in the males and from 15 to 16 mg/kg body weight/day in the females.) All of the other animals survived to the end of the study. No effects on food consumption were observed in any of the treated groups. A slight temporary retardation of body-weight gain was observed only in the high-dose males during the first 6 months. No toxic effects were detected by auditory function testing, eye examinations, electrocardiography, haematological or serum biochemical determinations or urinalysis. None of the organ weights showed changes attributable to treatment, and no histological changes were induced by GLA. On the basis of these results, the NOEL was 5 mg/kg body weight/day (Bathe and Sachsse, 1984).

Reproductive toxicity

Two-generation study. There were no treatment-related deaths during the two-generation study in rats. Body-weight gains were not affected in any of the parent or pup groups. No changes that could be related to treatment were observed in any of the reproductive parameters with the exception of the lower number of live pups at birth in the 360-ppm group (Table 8). This effect was caused by pre-implantation and early post-implantation losses in the pregnant females as proved by the results of a preliminary study. Pup survival was not impaired. Histological examination of a variety of organs, including the reproductive organs, revealed no treatment-related effects. The NOEL for fertility and reproductive performance was 120 ppm, equivalent to an average substance intake of 12 mg/kg body weight/day for the dams during pregnancy and lactation (Becker and Sachsse, 1986).

Embryotoxicity. The results of the embryotoxicity test in rats are shown in Table 9. Clinical signs, enlarged adrenals and smaller spleens indicated maternal toxicity in the groups given 50 or 250 mg/kg/day. Vaginal haemorrhages and an increase in prenatal mortality also occurred in connection with maternal toxicity. Embryotoxicity was indicated by more frequent distension of the renal pelvis and ureter in the foetuses of dams given 50 or 250 mg/kg/day and by retarded ossification in the foetuses of the highest dose group. However, there was no indication of teratogenic potential of GLA. On the basis of these findings, the maximum NOEL for maternal and embryonic/foetal toxicity was determined to be 10 mg/kg body weight/day (Baeder and Kramer, 1985).

In rabbits given the highest dose of GLA (20 mg/kg body weight), maternal toxicity was indicated by clinical signs of intoxication and by a reduction in food and water consumption and body-weight gain. There was also an increase in the incidence of premature deliveries and abortions. The foetuses of the highest dose group, like those of all the other dose groups, were normally developed and exhibited no increased incidence of anomalies. There was no indication of teratogenic potential (Table 10). On the basis of these findings, the NOEL for maternal and embryonic/foetal toxicity was determined to be

Table 7. Changes induced in NMRI mice after feeding glufosinate ammonium (GLA) for 2 yr

Parameter	Dose (mg/kg diet)...	Values for: Males 0	Males 20	Males 80	Males 160	Females 0	Females 20	Females 80	Females 320
No. of mice:									
Chronic toxicity study, 52 wk		10	10	10	10	10	10	10	10
Oncogenicity study, 104 wk		50	50	50	50	50	50	50	50
GLA intake (mg/kg body weight/day):									
52 wk		0	3.2	13	25	0	4.4	17.9	69.2
104 wk		0	2.8	10.8	22.6	0	4.2	16.2	64.0
Mortality rate total (%)		43	48	52	65†	72	67	58	72
Body-weight gain:									
52 wk		—	NS	NS	Dec wk 3–33		NS	NS	Dec wk 7–31
104 wk		—	NS	NS	NS		NS	NS	NS
Glucose in serum (mmol/litre):									
52 wk		8.65	10.20	9.78	14.46*	8.66	8.68	6.87	11.98*
ASAT in serum (μkat/litre):									
52 wk		1.35	1.21	1.64	1.51	1.36	1.52	1.41	3.28*
Glutathione in blood at 104 wk (μmol/ml):									
GSH		0.72	0.77	0.57	0.42*	ND	ND	ND	ND
GSSG		0.18	0.18	0.27*	0.20	ND	ND	ND	ND
GSH + GSSG		0.90	0.95	0.84	0.62	ND	ND	ND	ND

NS = not significantly different from controls Dec = decrease ND = not done
ASAT = aspartate aminotransferase

Values are means for the numbers of mice indicated and those marked with superscripts differ significantly from the corresponding control values: *Dunnett's test significant at 5% level; †significant in Fisher's exact test.

Table 8. Effect of feeding glufosinate ammonium (GLA) on the litter size of two generations of Wistar rats

Generation	Litter size† (no. of pups/dam after parturition) in rats given GLA doses (mg/kg diet) of: 0 (control)	40	120	360
F_{1a}	11.2 (27)	11.6 (28)	10.6 (29)	8.8* (29)
F_{1b}	11.7 (29)	11.6 (27)	11.3 (29)	7.4* (30)
F_{2a}	10.8 (25)	10.9 (25)	10.7 (26)	9.6 (25)
F_{2b}	11.2 (25)	11.8 (25)	11.9 (25)	8.2* (26)

†In brackets, no. of dams.
Values are means for the number of dams indicated and those marked with an asterisk differ significantly from the corresponding control value [$*P < 0.05$; Dunnett's test (normal distribution) or Steel's test (non-normal distribution)].

6.3 mg/kg body weight/day (Baeder and Kramer, 1984).

Mutagenicity

Microbial genotoxicity. In the Salmonella/histidine reversion assay and the *E. coli* WP2 reverse mutation assay GLA proved cytotoxic at 500 and 1000 μg/plate in both the presence and absence of S-9. No statistically significant induction of revertants attributable to GLA treatment and no dose–response relationship were observed. In the Rec assay no growth inhibition was observed in either the *rec*$^+$ or the *rec*$^-$ strain of *B. subtilis* at GLA doses of 50 to 10,000 μg/disc (Othaki *et al.*, 1981).

Yeast genotoxicity. In the assay for gene conversion/DNA repair in *S. cerevisiae* D4, GLA proved cytotoxic at 10,000 μg/ml in the presence and absence of S-9 mix. In the forward mutation assay in *Sch. pombe* P1, at 1000 μg GLA/ml, survival rates in the presence and absence of S-9 were 86 and 91%, respectively. There were no significant differences between the negative control and GLA incubations in the convertant/mutation frequencies in either of these studies (Mellano and Milone, 1984a,b).

Mammalian genotoxicity in vitro. In the assay for unscheduled DNA synthesis using non-induced primary rat hepatocytes, GLA proved non-cytotoxic and non-mutagenic (Cifone and Myhr, 1984). GLA was also non-cytotoxic and non-mutagenic in the mouse lymphoma forward mutation assay (Cifone and Myhr, 1985).

In the assay for structural chromosomal aberrations in cultured human lymphocytes, analysis of metaphases indicated no increase in aberrant cells (Pirovano and Milone, 1985).

Mammalian genotoxicity in vivo. In the micronucleus test no increase in the number of micronucleated polychromatic erythrocytes was found in the groups treated with GLA, although an increase was

Table 9. Maternal and developmental effects in Wistar rats administered glufosinate ammonium (GLA) on days 6–15 of gestation

Parameter	Dose (mg/kg body weight)...	Values† for rats in: Supplementary study 0	0.5	2.24	10	Main study 0	10	50	250
No. of pregnant dams		*20*	*20*	*20*	*20*	*20*	*20*	*20*	*20*
Maternal and near-term foetal effects									
Hyperactivity		—	—	—	—	—	2	7	5
Bristled fur		—	—	—	—	—	—	3	9
Blood around the mouth		—	—	—	—	—	—	—	3
Spleen small		—	—	—	—	—	—	1‡	2‡
Adrenals large		—	—	—	—	—	—	1‡	5§
Lethality		—	—	—	—	—	—	—	1
Vaginal haemorrhages		—	—	—	—	—	—	4	8
Early resorption/abortion		—	—	—	—	—	—	3	8
Dead implantations only		—	—	—	—	—	—	—	1
Premature delivery		—	—	—	—	—	—	1	—
Dams with viable foetuses		20	20	20	20	20	20	16	10
Developmental effects									
Soft-tissue examination									
Anomalies:									
No. of foetuses examined		118	118	112	110	109	108	91	57
No. of litters examined		20	20	20	20	19	19	16	10
Renal pelvis distended		2/2	1/1	1/1	—	10/6	17/10	19/10	8/5
Renal pelvis and ureter distended		—	—	—	—	1/1	3/3	4/3	9*/5*
Ureter distended		—	1/1	—	—	—	—	—	1/1
Variations		—	NS	NS	NS	—	NS	NS	NS
Necropsy and skeletal examination									
No. of foetuses examined		123	128	126	117	117	119	119‖	86¶
No. of litters examined		20	20	20	20	20	20	17‖	12¶
Anomalies		—	NS	NS	NS	—			
Variations		—	NS	NS	NS	—			
Retardations:									
Non-ossification of metacarpal 5		40/13	42/14	45/14	28/9	41/15	39/14	43/12	38*/8

NS = not significantly different from control
†Maternal and near-term foetal effects: no. of rats affected; developmental effects: no. of foetuses affected/no. of litters affected.
‡Dams killed prematurely.
§Four dams killed prematurely.
‖Of which 12 foetuses of one litter.
¶Of which 24 foetuses of two litters.
Values marked with an asterisk differ significantly (Fisher's Exact Test) from the corresponding control value ($*P < 0.05$).

Table 10. Maternal and near-term foetal effects in Himalayan rabbits administered glufosinate ammonium (GLA) on days 6–18 of gestation

Parameter	Values for rabbits given GLA at a dose (mg/kg body weight/day) of: 0 (control)	2	6.3	20
No. of pregnant dams ...	*15*	*15*	*15*	*15*
Group mean maternal body weight (g) on gestation day:				
0	2559	2603	2539	2510
7	2626	2652	2570	2573
14	2676	2655	2571	2468
20	2717	2706	2649	2551*
29	2887	2849	2827	2716*
Group mean maternal food consumption (g/100 g body weight) on gestation days:				
0–7	3.49	3.63	3.48	3.74
7–14	3.25	2.50	2.06	1.28
14–20	2.96	2.64	2.52*	1.82*
20–29	2.64	2.56	2.53	3.15
Maternal and near-term foetal effects (no. of dams affected):				
Spasm	—	—	—	1
Premature delivery	—	—	1	1
Early resorption (abortion)	—	—	—	2
Dams with viable foetuses	15	15	15	15
Developmental effects:				
Soft-tissue examination (no. of foetuses examined)	48	45	43	29
Skeletal examination (no. of foetuses examined)	53	54	55	62
Anomalies, variations, retardations	—	NS	NS	NS

NS = not significantly different from control

Values marked with an asterisk differ significantly (Dunnett's test) from the corresponding control value ($*P < 0.05$). The area under the curves was used for the evaluation of days 7–20.

observed in the positive control treated with Endoxan (Jung and Mayer, 1986).

Since none of the above tests yielded positive results, it is concluded that GLA possesses no genotoxic potential. The positive controls used in these studies yielded the expected positive responses, thus indicating the sensitivity of the test systems.

Metabolism and pharmacokinetics in rats

The absorption, distribution, excretion and metabolism of ^{14}C-labelled GLA were investigated in rats in relation to sex, dose level and number of oral doses. Maximum blood levels were attained 1 hr after administration. Elimination in the blood took place with a half-life of less than 4 hr. GLA, or its metabolites, was rapidly eliminated in the urine (about 90%) and faeces (about 10%). Seven days after treatment only 0.1–1.3% of the radioactivity was found incorporated, particularly in the liver and kidneys. The metabolic profile in the faeces and urine indicates that GLA undergoes oxidative deamination which results in the principal metabolite 3-methylphosphinico propionic acid (Fig. 2).

Special examinations

In view of the phosphinico group that forms part of GLA, an acute delayed neurotoxicity study in hens was performed. There was no indication of a neurotoxic effect of GLA, whereas the TOCP (positive control) group showed the typical clinical and neuromorphological signs of neurotoxicity (Leist and Weigand, 1979).

A test for possible therapeutic measures in the event of acute poisoning was also carried out. In view of the acute intoxication profile, phenobarbital and atropine plus 2-PAM were selected as possible therapeutic agents. In this experiment, ip administration of 20 mg phenobarbital/kg body weight proved highly effective, completely preventing both convulsions and death. Atropine, either alone or with 2-PAM, did not

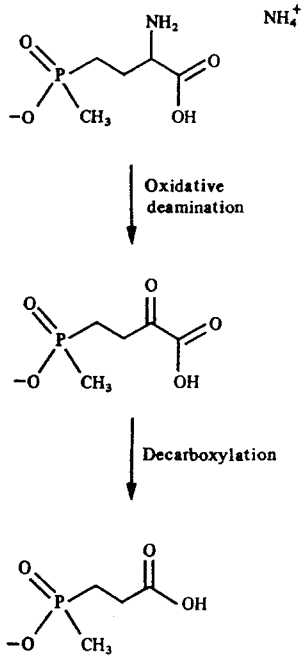

Fig. 2. Metabolic pathway of glufosinate ammonium in the rat.

show any therapeutic effect (Ebert and Weigand, 1983).

More recent special studies of the mode of action (paper in preparation) point to the conclusion that GLA inhibits GS in mammals. The only clear-cut biological consequence of this enzyme inhibition was a reduction in glutamine levels in the tissues. The decrease in GS activity was largely reversible after less than 7 days. Neurobehavioural effects related to stimulation of the central nervous system (CNS) were observed only at very high dose levels and proved to be reversible. This effect could not be explained by higher cerebral levels of glutamate or ammonia, or by changes in the levels of cerebral neurotransmitters. This conclusion is supported by the fact that GLA showed no effect in receptor binding assays (γ-aminobutyric acid, norepinephrine, dopamine, 5-hydroxytryptamine, calcium channel, benzodiazepine site). GLA does not interfere with enzyme systems that have glutamate as a substrate (e.g. aspartate amino transferase, alanine amino transferase, glycerophosphate dehydrogenase). No effects were observed on the metabolism of amino acids or carbohydrates, or on the Krebs cycle, gluconeogenesis, lipogenesis or oxidative phosphorylation.

Conclusion

The results of numerous toxicological studies show that the herbicide GLA and its formulation are safe for users and consumers under the conditions of recommended use.

According to the criteria for classification in Directive 83/467/EEC, GLA must be classified as harmful on the basis of acute oral toxicity testing. It causes no primary skin or eye irritation and possesses no sensitizing properties.

The 200-g/litre formulation must be classified as harmful following oral and dermal exposure, and as irritating in view of its irritant effects on the eyes.

The toxicological profile of GLA after exposure to high sublethal and lethal doses was characterized by CNS symptoms which occurred with a delay of several hours. In view of its structural similarity to glutamate, it is most likely that GLA itself acts as a convulsant by temporary interference with the neurotransmitter function of glutamate after a sufficient concentration has been reached in the brain. An interference with glutamate-related pathways through inhibition of GS, which is involved in the synthesis of neurotransmitter amino acids from the precursor glutamine, is also possible. Therefore a significant inhibition of cerebral GS is assumed to be toxicologically relevant. A special study showed that the lowest effect level for inhibition of cerebral GS was 8 mg GLA/kg body weight/day in the dog after subchronic oral exposure (Sachsse, 1986). Inhibition of hepatic GS in a slight to moderate degree, as was observed in the subchronic and chronic rat studies (Ohtaka *et al.*, 1981d; Suter and Sachsse, 1986a), was not related to changes in hepatic function. However, the slight reduction in the glutathione level observed after chronic exposure (Suter and Sachsse, 1986a) is possibly related to interference by GLA in the biosynthesis of glutathione, and therefore a toxicological significance cannot be excluded. The slightly to moderately increased GS activity observed in the rat kidney after chronic exposure (Suter and Sachsse, 1986a) appears to be an adaptation of renal function without toxicological significance.

No increased levels of ammonia in tissues as a consequence of GS inhibition were observed in any of the toxicological studies, and therefore it appears that ammonia is not involved in the toxicity of GLA in mammals and that marked differences exist in ammonia detoxification between mammalian and plant or microbial systems.

In summary, it can be stated that the lowest NOEL of 2 mg GLA/kg body weight/day, established in a chronic (24-month) feeding study in rats, has been verified by special investigations into the mode of action of GLA. Since the toxicological data for GLA indicated no genotoxic, carcinogenic or teratogenic potential or other special toxicological hazards, a safety factor of 100 seems to be sufficiently conservative for establishing an Acceptable Daily Intake (ADI) value. For these reasons an ADI of 0.02 mg/kg body weight/day is proposed for GLA.

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