GLUFOSINATE-AMMONIUM

First draft prepared by
G. Wolterink[1], C.M. Mahieu[1] and L. Davies[2]

[1] Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[2] Australian Pesticides and Veterinary Medicines Authority, Kingston, ACT, Australia

Explanation

Glufosinate-ammonium is the International Organization for Standardization (ISO)–approved name for ammonium-DL-homoalanin-4-yl(methyl)phosphinate (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service number 77182-82-2. Glufosinate-ammonium is used as a non-selective herbicide for total vegetation control and as a desiccant to aid in crop harvesting. Glufosinate-ammonium, a racemic mixture of the D- and L-isomers, is a phosphinic acid analogue of glutamic acid. Its herbicidal action is related to the inhibition of glutamine synthetase, an enzyme that plays an important role in ammonia detoxification, amino acid metabolism and protein and nucleotide biosynthesis in plants.

Glufosinate-ammonium was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1991 and 1999. In 1999, the Meeting established a group acceptable daily intake (ADI) of 0–0.02 mg/kg body weight (bw) for glufosinate-ammonium, 3-[hydroxy(methyl) phosphinoyl]propionic acid (MPP) and *N*-acetyl-glufosinate (NAG) (alone or in combination) on the basis of a no-observed-adverse-effect level (NOAEL) of 2 mg/kg bw per day in a long-term study in rats given technical-grade glufosinate-ammonium.

Glufosinate-ammonium was re-evaluated by the present Meeting as part of the periodic review programme at the request of the Codex Committee on Pesticide Residues. The present Meeting evaluated studies on glufosinate as well as studies on 3-methylphosphinico-propionic acid (= MPP), NAG and 2-methylphosphinico-acetic acid (MPA), three metabolites that are found in plants, soil and livestock. Both the new data and previously submitted studies were considered by the present Meeting.

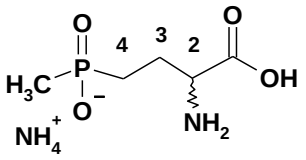
All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

A. GLUFOSINATE-AMMONIUM

The structure of glufosinate-ammonium is shown in Figure 1.

Figure 1. Structure of glufosinate-ammonium



D,L-glufosinate-ammonium

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

[^{14}C]Glufosinate-ammonium (radiochemical purity 97–98%), labelled at the 2 position, was administered either intravenously or orally by gavage at a single dose of about 2 mg/kg bw to groups of 10 male and 10 female SPF Wistar rats. The vehicle was polyethylene glycol 400 for intravenous administration or a 2% starch suspension for oral administration. Radioactivity was determined in blood samples taken from the retrobulbar venous plexus at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 24, 32, 48, 72, 96, 120, 144 and 168 hours post-dosing for all animals (five of each sex). For animals dosed intravenously, an additional sample was taken at 0.083 hour. Radioactivity in the expired air was measured in two animals 24 hours after administration. Furthermore, radioactivity was measured on a daily basis in faeces and urine. At day 7 after dosing, the orally dosed rats were killed, and levels of radioactivity in spleen, kidneys, liver, gonads, heart, lung, skeletal muscles, subcutaneous and retroperitoneal fat, brain, bones, blood and plasma were determined.

Total recovery of radioactivity was 95–96%. During the first 24 hours after oral dosing, no radioactivity was detected in the air. The blood levels after oral administration were low and measurable only in males up to 3 hours and in females up to 8 hours. Maximum concentrations (0.008 µg/ml in males, 0.029 µg/ml in females) were reached 0.5–1 hour after administration. The half-life in blood was 3.7 hours. After oral administration, 89% and 81% of the radiolabel were excreted in faeces in males and females, respectively, whereas 7% and 14% were excreted in urine of males and females, respectively. Radiolabel (< 0.2 µg equivalent [Eq] per gram tissue) was found only in kidneys, testes and liver, and at low levels. After intravenous administration, 85% and 93% of the dose were excreted in urine of males and females, respectively, whereas 18% and 8% were excreted in faeces of males and females, respectively. Plasma levels were 4.5 and 5 µg Eq/ml in males and females, respectively. After intravenous administration, renal elimination was biphasic, with half-lives of 7 and 52 hours in males and 8 and 64 hours in females (Kellner & Eckert, 1983).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99%), labelled at the 3 and 4 positions of the aminobutyric acid group and dissolved in a saline solution, was intravenously administered into the tail vein of 10 male Wistar rats at a single dose of approximately 2.3 mg/kg bw. Groups of five rats were killed 2 or 24 hours after dosing. Urine and faeces of the second group were collected separately. Each animal was exsanguinated, and kidney, liver, brain and blood were sampled

individually. All samples were radioassayed by liquid scintillation counting (LSC). Metabolite characterization was performed in faeces, urine, liver, kidney and brain and is described in section 1.2 in the evaluation of the study of Lauck-Birkel & Strunk (1999a). Statements of adherence to quality assurance (QA) and GLP were included.

Twenty-four hours after intravenous dosing, 84.5% of the administered dose was recovered, predominantly in urine (80.5% of the dose, including 2.7% in cage wash), with 2.4% excreted in the faeces. Total radioactivity levels in kidneys, liver, brain and blood amounted to 7.3% and 1.7% of the administered dose at 2 and 24 hours after administration, respectively (Maas & Braun, 1999a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) was administered by gavage to five male and five female Wistar rats via stomach tube at a dose of 31.5–31.6 mg/kg bw. Urine and faeces were collected at 0–2, 2–4, 4–8 and 8–24 hours and at daily intervals up to 7 days thereafter. At day 7, the rats were killed, and blood, spleen, kidneys, liver, gonads, heart, lung, skeletal muscle, subcutaneous and renal fat, brain and bones were sampled. All excreta and organ/tissue samples were radioassayed by LSC. Statements of adherence to QA and GLP were included.

In total, 95–96% of the administered radioactivity was recovered. Faecal excretion accounted for 88% and 84% of the administered radiolabel in males and females, respectively, whereas 7% and 11% were recovered from urine in males and females, respectively. More than 70% was excreted in the 0- to 24-hour collection interval in both sexes. The renal and faecal excretion patterns suggested a biphasic excretion rate. The half-lives of the renal excretion were 6–7 and 36 hours for both sexes. The faecal excretion half-lives were 5–6 and 38 hours. After 7 days, 0.06–0.07% of the administered dose was detected in organs and tissues of the rats (Kellner & Eckert, 1985a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) was orally administered to groups of three male and three female Wistar rats by gavage at 800 mg/kg bw. Urine and faeces were collected 0–6 and 0–24 hours after dosing. At the end of the collection period, the rats were killed, and kidneys, liver, spleen and brain were dissected. The excreta and organ samples were radioassayed by LSC and analysed by radio–high-performance liquid chromatography (radio-HPLC) and co-elution with reference standards. Metabolite characterization is described in section 1.2. Statements of adherence to QA and GLP were included.

Twenty-four hours after dosing, 21–24% of the dose was excreted with the faeces, and approximately 4% with the urine. The excretion pattern was independent of sex. In total, less than 0.20% of the radioactivity administered was present in the selected organs and tissues. At 6 and 24 hours after dosing, the highest levels of radiolabel were detected in kidneys (15–77 µg Eq/g). Lower radioactivity levels were found in liver (6–16 µg Eq/g) and spleen (9–18 µg Eq/g), whereas still lower levels were found in the brain (0.5–1.9 µg Eq/g) (Schwalbe-Fehlet al., 1985).

Following administration of ^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) by gavage at a dose of 800 mg/kg bw to three male and three female rats, blood samples (0.1 ml) were taken from the retrobulbar venous plexus and radioassayed at 0.25, 0.5, 1, 2, 4, 6, 7.5 and 24 hours after administration, and radioactivity levels were measured. Statements of adherence to QA and GLP were included.

Blood levels reached more than 70% of the maxima at the first sampling interval 0.25 hour after dosing in the animals of both sexes, indicating a rapid absorption. The maximum blood levels (3.18 and 3.32 µg Eq/ml in males and females, respectively) were reached 0.5–1.0 hour after dosing. The elimination of the residues from the blood occurred in an initial rapid (half-life 4–5 hours) and a subsequent slower phase. No sex differences were observed (Kellner & Eckert, 1985b).

A group of five male and five female Wistar rats (WISKf (SPF 71)) were gavaged with [^{14}C]glufosinate-ammonium (radiochemical purity 98%; labelled at the 3 and 4 positions of the aminobutyric acid group) at a dose of 2.5 mg/kg bw for males and 2.9 mg/kg bw for females. The test substance was dissolved in water. Faeces, urine and cage wash were collected once daily over 4 days, and each group of samples was pooled for each sex. At 96 hours, the animals were killed, liver, kidney, fat, brain, spleen and blood were sampled and each group of samples was pooled for each sex. Radioactivity levels in urine, faeces and organ samples were measured. Metabolite profiles in urine and faeces are described under section 1.2. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 98–100%. Within 24 hours, 88% and 81% were excreted in faeces of males and females, respectively, and 6% was excreted in urine of both sexes. The highest residues were detected in kidneys (0.098 and 0.217 mg Eq/kg in males and females, respectively) and liver (0.038 and 0.027 mg Eq/kg in males and females, respectively). Fat of male rats contained residue levels of 0.038 mg Eq/kg. Other residue levels were less than or equal to 0.014 mg Eq/kg (Wink, 1986a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity > 99%) was orally administered to 10 male and female Wistar rats via gavage after 14 doses of non-labelled glufosinate-ammonium (purity 98.6%) at a dose of 2 mg/kg bw per day. Urine and faeces were collected on a daily basis and each pooled per sex and collection interval. All rats were sacrificed 96 hours after the radioactive dose, and liver, kidney, fat, brain, spleen and blood were sampled. All samples were radioassayed. Statements of adherence to QA and GLP were included.

After 96 hours, the total excretion in faeces was 83.0% and 81% and in urine was 5% and 6% of the dose in male and female rats, respectively. The majority was excreted within the first 24 hours. At 96 hours after dosing, the kidneys and livers of males contained 0.276 and 0.055 µg Eq/g, whereas in females, 0.108 and 0.033 µg Eq/g were found. Blood, brain and spleen contained maximally 0.003 µg Eq/g in both sexes. The route and rate of excretion after repeated administration and the residue level in selected organs and tissues were similar to those after a single oral dose of about 2 mg/kg bw (see above) (Wink, 1986b).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 98%; labelled at the 3 and 4 positions of the aminobutyric acid group) was administered to groups of five male and five female Wistar rats via gavage at 500 mg/kg bw. Urine and faeces were collected on a daily basis. The groups were killed 24 or 96 hours after administration, at which time samples of spleen, kidneys, liver, brain, blood and plasma were collected. Extra single male and female rats also received an oral dose of 500 mg/kg bw, but were killed 2 and 6 hours after dosing. Metabolite characterization is described in section 1.2. Statements of adherence to QA and GLP were included.

Most (87–96%) of the dose was recovered in urine (8–11%) and faeces (75–89%) after 96 hours. Tissue radioactivity levels were highest 2 hours after dosing in kidney (76–81 µg Eq/g), spleen (12–41 µg Eq/g), blood (0.8–1 µg Eq/g) and plasma (3 µg Eq/g) and 6 hours after dosing in liver (12–18 µg Eq/g) and brain (0.6–1.1 µg Eq/g). No sex differences were observed (Lauck-Birkel, 1995a).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99%; labelled at the 3 and 4 positions of the aminobutyric acid group) dissolved in a saline solution was administered by gavage to groups of five male Wistar rats at a single dose of 20 mg/kg bw. The groups were killed 1, 6 or 24 hours after dosing. Urine and faeces of the 24-hour group were collected separately. Each animal was exsanguinated, and kidney, liver, brain, blood and plasma were sampled. Metabolite characterization performed in faeces, urine, liver and kidney is described in section 1.2 in the evaluation of the study of Lauck-Birkel & Strunk (1999b). Statements of adherence to QA and GLP were included.

Twenty-four hours after dosing, 92% of the dose was excreted with the faeces and 4.9% with the urine, and 0.2% of the dose was detected in the dissected organs and blood. Peak levels of radioactivity were detected in kidney (3.4 µg Eq/g) and blood (0.2 µg Eq/g) 1 hour after dosing, in liver (0.72 µg Eq/g) 6 hours after dosing and in brain (0.034 µg Eq/g) 24 hours after dosing (Maas & Braun, 1999b).

^{14}C-labelled glufosinate-ammonium (radiochemical purity > 97%) was orally administered to five male and five female Wistar rats via gavage after 14 doses of non-labelled glufosinate-ammonium (purity not reported) at a dose rate of 2 mg/kg bw per day. One male and one female rat served as control. Urine and faeces, collected at 2, 4, 8 and 24 hours and on a daily basis thereafter, were radioassayed. All animals were sacrificed 7 days after administration of the radioactive dose, and the organs and tissues were dissected and radioassayed. Statements of adherence to QA and GLP were included.

About 89–91% and 8–9% of the radiolabel were excreted in faeces and urine, respectively. About 90% of the total radioactivity was excreted within 24 hours. Both the renal excretion and the faecal excretion were biphasic. The half-lives for renal excretion were calculated to be 5–6 hours for the first phase and approximately 1.5 days for the second phase. For faecal excretion, the half-lives were 5 hours for the first phase and 1.3–2 days for the second phase. No sex-related differences were observed. In comparison with single-dose studies (Wink, 1986a; Stumpf, 1993a), the repeated dosing did not change the excretion pattern. Seven days after dosing, radioactive residues in the kidneys, testes, liver and carcass of males amounted to 0.138, 0.078, 0.028 and 0.029 µg Eq/g, respectively. At this time in females, radioactive residues in the kidneys, liver and spleen accounted for 0.047, 0.023 and 0.008 µg Eq/g, respectively. Mean levels of radioactivity in other organs and tissues were below the limit of detection (Kellner & Eckert, 1985c).

Glufosinate-ammonium was administered orally by gavage to groups of three female Wistar rats (WISKf, SPF 71) at about 12 or 109 mg/kg bw once a day for 10 days; on days 1, 8, 9 and 10, the animals received a dose of [^{14}C]glufosinate-ammonium (purity 99%), whereas on days 2–7, they were given non-labelled glufosinate-ammonium (purity 99.5%) at the same two doses. Radioactivity in blood was determined on days 1, 8, 9 and 10 at 1, 2, 4, 6, 8 and 24 hours after dosing. Statements of adherence to QA and GLP were included.

Maximum blood levels were observed 1–2 hours after oral administration of [^{14}C]glufosinate-ammonium, ranging from 0.11 to 0.25 µg Eq/ml at 12 mg/kg bw and from 1.25 to 1.65 µg Eq/ml at the 109 mg/kg bw dose. Blood levels increased slightly after two successive radioactive doses, but did not further increase after three successive radioactive doses at both dose levels. The elimination half-lives in the blood were 2.3–5.3 hours after both doses (Kellner & Eckert, 1986).

Five male and five female Wistar rats received seven daily doses of [^{14}C]glufosinate-ammonium (radiochemical purity ≥ 98%) at 2 mg/kg bw per day by gavage. Urine and faeces were collected on a daily basis until 4 days after the last dose and each pooled per sampling interval and sex. The animals were killed and dissected 96 hours after the last dose, and a wide range of organs and tissues was sampled and radioassayed. Statements of adherence to QA and GLP were included.

At 96 hours after the last dose of radiolabelled glufosinate-ammonium, faecal excretion was 87.4% and 88.2% of the cumulative dose in males and females, respectively, and urinary excretion was 6.5% and 5.9% of the cumulative dose in males and females, respectively (see Table 1). At this time, less than about 0.3% of the cumulative dose remained in the dissected organs and tissues of both sexes. The excretion patterns after multiple doses in this study were similar to those of single-dose studies (see above) (Gutierrez, 2002).

Table 1. Cumulative excretion of radiolabel in male and female rats receiving seven oral doses of ^{14}C-labelled glufosinate-ammonium at 2 mg/kg bw per day

Number of oral doses	Collection period (h)	% of administered radioactivity			
		Males[a]		Females[a]	
		Urine	Faeces	Urine	Faeces
1	0–6	3.08	—	2.19	—
	0–24	5.76	82.19	5.19	82.61
2	0–48	5.92	85.65	4.93	84.24
3	0–72	5.60	88.52	4.94	87.32
4	0–96	5.72	88.44	5.34	88.26
5	0–120	6.15	87.76	5.45	86.64
6	0–144	6.32	86.46	5.67	84.93
7	0–168	6.29	86.80	5.70	87.48
End of administration					
	0–192	6.42	87.37	5.83	88.15
	0–216	6.47	87.40	5.88	88.20
	0–240	6.50	87.40	5.90	88.21

From Gutierrez (2002)
[a] Mean of five animals of each sex.

Groups of three pregnant female Wistar rats at gestation day (GD) 6, 14 or 18 were given a single intravenous injection of ^{14}C-labelled glufosinate-ammonium (purity > 98%) at 2.4 mg/kg bw. Quantitative whole-body autoradiography was performed on one dam from each group 5 minutes, 30 minutes or 1 hour after administration. Whole-body sections from dams, uteri and fetuses were prepared separately. Statements of adherence to QA and GLP were included.

A similar distribution pattern was observed in all stages of pregnancy. At 5 minutes after administration, radioactivity was distributed ubiquitously in the body. The highest concentrations were found in the kidney, lacrimal gland and thymus. Up to 1 hour after administration, the concentration in most of the analysed regions decreased to approximately 10% of the initial values. In kidney, adrenal gland, lacrimal gland, salivary gland, pancreas, thyroid and thymus, the concentration was unchanged or increased slightly.

On GD 6, owing to the small size of the placenta and fetuses, no fetuses could be observed or analysed in the whole-body sections or in the separately prepared uterus. On GD 14 and GD 18, the distribution of the radioactivity was similar. At 5 minutes after administration, the concentration in the blood was about 2 times higher than in the placenta and 20–40 times higher than in the fetus, whereas at 30 minutes after dosing, the concentrations in blood and placenta were similar, but 12–13 times higher than in the fetus. The concentrations in the fetuses were the lowest of all organs and tissues of the pregnant rats, with the exception of the central nervous system. At 1 hour after administration, the radioactivity concentrations decreased considerably in the maternal blood, placenta and fetus.

The distribution of the compound in the body was ubiquitous, with poor penetration of the placental and blood–brain barrier (Maas & Zimmer, 2000).

Rabbits

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99.7%) was administered by gavage as a single oral dose to three female New Zealand White rabbits at 6 mg/kg bw. Urine and faeces were collected on a daily basis for a total period of 10 days. The excreta samples were radioassayed and analysed by radio-HPLC. Statements of adherence to QA and GLP were included.

Total recovery of administered radioactivity was 97.9%. Faecal excretion and urinary excretion were 70.2% and 27.7%, respectively. The cumulative excretion data (Table 2) indicate that excretion in female rabbits was relatively slow compared with that of rats (see above) and dogs (see below) (Koester, 2004).

Table 2. Cumulative excretion of radioactive residues by female rabbits following a single oral dose of [^{14}C]glufosinate-ammonium of 6 mg/kg bw

Time after dosing (h)	Cumulative % of administered radioactivity		
	Urine	Faeces	Urine + faeces
24	1.11	13.88	14.99
48	9.88	25.26	35.14
72	15.57	34.60	50.17
96	19.61	45.01	64.62
120	22.63	53.26	75.89
144	24.52	60.12	84.64
168	25.72	64.23	89.95
192	26.65	66.59	93.24
216	27.25	68.78	96.03
240	27.67	70.24	97.91

From Koester (2004)

Dogs

[^{14}C]Glufosinate-ammonium (radiochemical purity > 98%) was orally administered by gelatine capsule to two male and two female Beagle dogs at a dose of 8 mg/kg bw. Excreta were collected at 6 and 24 hours. Blood was sampled at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. One male and one female dog were killed 6 hours after dosing, whereas the other two dogs were killed 24 hours after dosing. Levels of radioactivity were determined in heart, liver, kidneys, several parts of the brain and spinal cord. Statements of adherence to QA and GLP were included.

Peak blood levels of radioactivity were detected 2–4 hours after dosing. Radiolabel was almost completely excreted in faeces (82–83% of the dose) and urine (9–10% of the dose) within 24 hours of dosing. In males, the highest residue levels were found in the kidneys (1.2–1.6 µg Eq/g) and liver (0.4–1.3 µg Eq/g) at 6–24 hours after dosing. In females, the highest residue levels were found in the kidneys (2.3–2.4 µg Eq/g) and liver (0.4–1.2 µg Eq/g) at 6–24 hours after dosing. Radioactivity levels in heart and brain were less than or equal to 0.07 µg Eq/g 6 and 24 hours after dosing. No sex differences in excretion or distribution of radioactivity were observed (Ellgehausen, 1986a).

Groups of male and female dogs (six animals per group) were orally administered glufosinate-ammonium (purity unknown) via gelatine capsules; 18 non-labelled daily doses of 1 or 8 mg/kg bw were followed by 10 daily doses of [^{14}C]glufosinate-ammonium (radiochemical purity > 99.7%) at 1 or 8 mg/kg bw. Urine and faeces were collected at 24 intervals from the 1st day of administration of the radiolabelled glufosinate-ammonium and at 6, 12, 24, 48, 72 and 96 hours after the last dosing. Blood was sampled 1 hour after each radiolabelled dose and 2, 4, 6, 24, 48, 72 and 96 hours after the last dose. One male and one female dog of both dose groups were sacrificed 6 hours after the first administration of [^{14}C]glufosinate-ammonium and 6, 24, 48 and 96 hours after the last (10th) administration of [^{14}C]glufosinate-ammonium. The dogs were exsanguinated and dissected, and samples of heart, liver, kidneys and different parts of the cerebral tissue were radioassayed by LSC. The faeces and organ extracts as well as the urine samples were analysed by radio–thin-layer

chromatography (radio-TLC) together with radiolabelled reference substances. Statements of adherence to QA and GLP were included.

The total renal excretion was 14–17% of the total ^{14}C dose at both dose levels and sexes. The faecal excretion accounted for 79–83.5% of the total [^{14}C]glufosinate-ammonium. In the high-dose group, the maximum blood and plasma levels (0.232 and 0.317 µg Eq/ml in male dogs and 0.260 and 0.351 µg Eq/ml in female dogs, respectively) were reached 6 hours after the last dose. Steady-state levels were reached at days 2–5 of the 10-day administration period. The elimination half-life was 46 hours for the whole blood and 16 hours for the plasma of male dogs. In female dogs, the elimination half-lives from blood and plasma were less than 18 hours. The highest residue levels were found 24–48 hours after the last ^{14}C dose in kidneys (1.111 and 0.500 µg Eq/g at the low dose and 6.437 and 5.140 µg Eq/g at the high dose for male and female dogs, respectively) and liver (0.638 and 0.422 µg Eq/g at the low dose and 3.659 and 3.267 µg Eq/g at the high dose for male and female dogs, respectively). At 1 mg/kg bw, the residue levels in the different regions of the brain and the heart were less than or equal to 0.1 µg Eq/g. At 8 mg/kg bw, the residue levels in the different regions of the brain and the heart were less than or equal to 0.6 µg Eq/g. No significant sex differences were observed. The study showed that glufosinate-ammonium has a low potential for accumulation in dogs (Ellgehausen, 1986b).

1.2 Biotransformation

Rats

A group of five male and five female Wistar rats (WISKf (SPF 71)) received by gavage [^{14}C]glufosinate-ammonium (radiochemical purity 93–95%) labelled at the 3 and 4 positions of the aminobutyric acid group at a dose level of 2.1 mg/kg bw. The test substance was dissolved in saline. Faeces and urine (without cage wash) were collected once daily over 4 days and each pooled for each sex. Metabolite profiles in urine and faeces were investigated by radio-HPLC or radio-TLC. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 101–104%. Levels of radioactivity and residue pattern in urine and faeces, expressed as a percentage of the administered dose, are presented in Table 3.

In urine, parent compound represented about 50% of residue, whereas 4-methylphosphinicobutanoic acid (MPB) and MPP each represented 8–22% of residue. Very low levels of MPA were found in urine. The study shows that following a single oral administration of [^{14}C]glufosinate-ammonium, the radiolabel is rapidly and virtually completely excreted in rats, with the faeces as the main excretion route. The major residue component was the parent compound. No relevant sex differences were observed (Stumpf, 1993a).

A group of three male Wistar (WISKf (SPF 71)) rats received by gavage [^{14}C]glufosinate-ammonium (radiochemical purity > 98%) labelled at the 3 and 4 positions of the aminobutyric acid group at a dose level of 2 mg/kg bw. The test substance was dissolved in saline. Faeces and urine (without cage wash) were collected once daily over 2 days, and samples of each were pooled. Potential microbial degradation of excreted residues during collection was prevented by cooling an addition of aqueous sodium azide. Metabolite profiles in urine and faeces were investigated by radio-HPLC or radio-TLC. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 96–100%. Levels of radioactivity and residue pattern in urine and faeces, expressed as a percentage of the administered dose, are presented in Table 4.

The study shows that following a single oral administration of [^{14}C]glufosinate-ammonium, the radiolabel is rapidly and virtually completely excreted in rats, with the faeces as the main excretion route. The major residue component was the parent compound (Lauck-Birkel, 1996).

Table 3. Residue pattern in urine and faeces of rats after a single oral dose of [^{14}C]glufosinate-ammonium of 2.1 mg/kg bw

	% of the administered dose							
	Males				Females			
	Urine		Faeces		Urine		Faeces	
	0–24 h	0–96 h	0–24 h	0–48 h	0–24 h	0–96 h	0–24 h	0–48 h
Total radioactivity	8.1	9.8	87.6	90.7	7.7	8.6	90.7	95.3
Identified	6.9	8.6	85.3	87.6	6.3	7.2	77.9	81.9
GA	4.1	5.1	73.5	75.3	3.9	4.5	66.0	68.5
MPB	1.5	1.6	< LOD[a]	< LOD	1.7	1.8	< LOD	< LOD
MPP	1.3	1.9	1.3	1.3	0.6	0.8	0.9	0.9
MHB	< LOD	< LOD	3.4	3.6	< LOD	< LOD	2.9	3.3
NAG	< LOD	< LOD	7.1	7.4	< LOD	< LOD	8.1	9.2
MPA	< LOD	< LOD	< LOD	< LOD	0.1	0.1	< LOD	< LOD
Unknown	1.2	1.3	0.6	0.6	1.4	1.5	0.3	0.3

From Stumpf (1993a)

GA, glufosinate-ammonium; LOD, limit of detection; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate

[a] Limit of detection is 0.05% of the dose.

Table 4. Residue pattern in urine and faeces of rats after a single oral dose of [^{14}C]glufosinate-ammonium of 2 mg/kg bw

	% of the administered dose			
	Urine		Faeces	
	0–24 h	24–48 h	0–24 h	24–48 h
Total radioactivity	5.3	0.6	90.9	2.8
Identified	5.1	0.6	88.0	2.7
GA	3.92	0.36	75.37	1.82
MPB[a]	0.23	< LOD[b]	0.34	0.09
MPP	0.61	0.17	1.21	0.05
MHB	0.11	0.03	4.07	0.21
NAG	0.08	< LOD	6.98	0.50
MPA	0.10	< LOD	< LOD	< LOD
M1	0.08	0.03	0.19	0.08
Unknown	0.13	< LOD	1.20	0.06

From Lauck-Birkel (1996)

GA, glufosinate-ammonium; LOD, limit of detection; M1, unknown polar metabolite eluted with the void volume of the column; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate

[a] MPB was an impurity of the test substance that amounted to 0.68% in the dosing solution.

[b] Limit of detection is 0.02% and 0.05% of the dose for urine and faeces, respectively.

A group of five male and five female Wistar (WISKf (SPF 71)) rats received by gavage [^{14}C]glufosinate-ammonium (radiochemical purity 98%) labelled at the 3 and 4 positions of the aminobutyric acid group at a dose level of 2.5 mg/kg bw for males and 2.9 mg/kg bw for females. The test substance was dissolved in water. Faeces, urine and cage wash were collected once daily over 4 days and each pooled for each sex. Metabolite profiles in urine and faeces were investigated by radio-HPLC or radio-TLC. The toxicokinetics are described under section 1.1. Statements of adherence to QA and GLP were included.

Total recoveries of radioactivity were 98–100%. Only parent compound was detected in urine (6% of the dose) and faeces (79% of the dose) by HPLC analysis. Eleven per cent of radioactivity in faeces was non-extractable (Wink, 1986a).

In the in vivo study of Maas & Braun (1999a) with intravenous dosing of glufosinate ammonium at 2.3 mg/kg bw (see study description in section 1.1), metabolites were analysed in pooled samples of faeces, urine, liver, kidney and brain by radio-HPLC. Blood and plasma were not analysed by radio-HPLC because the radioactivity level was too low. Statements of adherence to QA and GLP were included.

The main residue in all samples was the parent substance glufosinate-ammonium. MPP was also present as a minor metabolite in all samples (see Tables 5 and 6), representing about 10–20% of residue found in liver. In the faeces, additionally minor portions of NAG and 2-hydroxy-4-methylphosphinico-butanoic acid (MHB) could be detected. In 24-hour post-dosing samples, the glufosinate-ammonium/MPP ratio was slightly increased in kidneys but decreased in liver when compared with the corresponding 2-hour post-dosing samples (Lauck-Birkel & Strunk, 1999b; Maas & Braun, 1999a).

Table 5. Residue profile in the 0- to 24-hour excreta of male rats after intravenous administration of approximately 2 mg/kg bw of [^{14}C]glufosinate-ammonium

Compound	% of the administered dose	
	Urine	Faeces
GA	68.1	2.0
MPB	< LOD	< LOD
MPP	9.5	0.05
MHB	< LOD	0.11
NAG	< LOD	0.2
MPA	< LOD	< LOD
Unknown	0.4	< LOD
Identified	77.6	2.4

From Lauck-Birkel & Strunk (1999b); Maas & Braun (1999a)
GA, glufosinate-ammonium; LOD, limit of detection; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate

^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) was orally administered to groups of three male and three female Wistar rats via stomach tube at 800 mg/kg bw. Urine and faeces were collected 0–6 and 0–24 hours after dosing. At the end of the collection period, the rats were killed, and kidneys, liver, spleen and brain were dissected. The excreta and organ samples were radioassayed by LSC and analysed by radio-HPLC and co-elution with reference standards. An additional group of three male and three female rats was used for the investigation of blood kinetics

for 24 hours. The kinetics data are described in section 1.1. Statements of adherence to QA and GLP were included.

Table 6. Residue profile in kidneys, liver and brain of male rats after intravenous administration of approximately 2 mg/kg bw of [^{14}C]glufosinate-ammonium

	2 h post-dosing		24 h post-dosing	
	% of dose	mg Eq/kg	% of dose	mg Eq/kg
Kidneys				
TRR	5.47	15.08	0.52	1.39
Identified	5.47	15.07	0.52	1.40
GA	4.98	13.72	0.49	1.33
MPP	0.49	1.35	0.03	0.08
Liver				
TRR	1.63	0.76	1.15	0.56
Identified	1.62	0.75	1.14	0.56
GA	1.47	0.68	0.93	0.45
MPP	0.16	0.07	0.22	0.10
Unknown	0.01	0.01	0.01	< 0.01
Brain				
TRR	0.02	0.06	0.02	0.04
Identified	0.02	0.06	0.20	0.04
GA	0.02	0.04	0.01	0.03
MPP	< 0.01	< 0.01	< 0.01	0.01
Unknown	< 0.01	< 0.01	—	—

From Lauck-Birkel & Strunk (1999b); Maas & Braun (1999a)
GA, glufosinate-ammonium; MPP, 3-methylphosphinico-propionic acid; TRR, total radioactive residue

The major residue in urine was the parent compound, whereas low levels of the metabolites MPP and MPB were detected. In kidneys and liver, the major residue was also parent compound (73% and 48%, respectively), but MPP (12% and 29%, respectively) and MPB (16% and 23%, respectively) were also found in these organs. In faeces, only parent compound was detected. Because of the very low radioactivity concentrations in the spleen and brain extracts and the high sample background, only parent compound could be identified (Schwalbe-Fehl et al., 1985).

In the study of Maas & Braun (1999b), groups of five male rats were treated by gavage with a single dose of [^{14}C]glufosinate-ammonium at 20 mg/kg bw. The groups were killed 1, 6 or 24 hours after dosing, and samples of urine, faeces, kidney and liver were collected. The present study describes the metabolite characterization by radio-HPLC and co-elution with reference standards of the samples taken in the Maas & Braun (1999b) study. Statements of adherence to QA and GLP were included.

In samples of urine and faeces collected over 0–24 hours, the unchanged parent substance glufosinate-ammonium was the predominant residue component. A minor metabolite in both urine and faeces was identified as MPP (≤ 1%). MPB and MHB were detected in the urine only at trace levels, whereas NAG was detected in the faeces only as a minor metabolite (3% of the dose). In kidneys and liver, the parent substance glufosinate-ammonium was also generally the major residue component at all three sampling intervals. The level of glufosinate-ammonium decreased with time in the kidneys, whereas it slightly increased with time in the liver. MPP peaked at the first sampling

interval in the kidneys, followed by a decrease with time. In the liver, MPP peaked at the second sampling interval (6 hours after administration). MPB was detected at trace levels at all sampling intervals in both organs, with the maximum level at the second sampling interval. NAG could not be detected in the kidney and liver (Lauck-Birkel & Strunk, 1999a).

[^{14}C]Glufosinate-ammonium (radiochemical purity > 98%) labelled at the 3 and 4 positions of the aminobutyric acid group was orally administered to groups of five male and five female Wistar rats by gavage at 500 mg/kg bw. Urine and faeces were collected on a daily basis. The groups were killed 24 or 96 hours after administration, at which time samples of spleen, kidney, liver, brain, blood and plasma were collected. Extra single male and female rats also received an oral dose of 500 mg/kg bw, but were killed 2 and 6 hours after dosing. The samples were analysed for the metabolite profile by radio-HPLC and co-elution with authentic radiolabelled reference standards. Radio-TLC served for confirmation of the HPLC results. Statements of adherence to QA and GLP were included.

Of the radioactivity excreted with urine and faeces (0–96 hours), approximately 82% and 91% of the dose were identified in male and female rats, respectively. The unchanged parent substance glufosinate-ammonium proved to be the predominant residue component in urine and faeces (see Table 7). The metabolite MPP appeared as a minor metabolite, with proportions increasing with collection time in urine and faeces. MPB was found at a constant but low level in all samples of urine and faeces; it was assumed to be an impurity of the test substance. NAG was assumed to be exclusively formed in the intestine by action of the intestinal microflora, as only very small amounts could be detected in the 0- to 24-hour urine. The metabolite MHB was observed only in the faeces at low levels.

Table 7. Excretion profiles in urine and faeces of rats orally administered [^{14}C]glufosinate-ammonium at a dose of approximately 500 mg/kg bw

	% of administered dose							
	Males				Females			
	Urine		Faeces		Urine		Faeces	
	0–24 h	0–96 h	0–24 h	0–96 h	0–24 h	0–96 h	0–24 h	0–96 h
GA	2.52	5.92	37.51	72.11	2.22	4.27	49.30	84.0
MPB	0.19	0.22	0.22	0.29	0.17	0.17	0.11	0.11
MPP	0.46	1.20	0.31	0.58	0.24	0.51	0.31	0.44
MHB	< LOD[a]	< LOD	0.13	0.26	< LOD	< LOD	0.17	0.28
NAG	0.04	0.04	0.35	1.23	0.02	0.02	0.58	1.74
MPA	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Non-anionic unknown M1	0.15	0.19	< LOD	< LOD	0.13	0.20	< LOD	< LOD
Series of unknowns (≤ 5)	0.06	0.12	0.09	0.24	< LOD	< LOD	0.14	0.14
Identified	3.21	7.38	38.52	74.47	2.65	4.97	50.47	86.57

From Lauck-Birkel (1995a)

GA, glufosinate-ammonium; LOD, limit of detection; MHB, 2-hydroxy-4-methylphosphinico-butanoic acid; MPA, 2-methylphosphinico-acetic acid; MPB, 4-methylphosphinico-butanoic acid; MPP, 3-methylphosphinico-propionic acid; NAG, *N*-acetyl-glufosinate

[a] LOD (urine): 0.02% of the administered dose; LOD (faeces): 0.07–0.19% of the administered dose.

A comparison of the metabolite profile in urine and faeces with that observed in a low-dose (2 mg/kg bw) study indicated a very similar residue pattern independently from the dose, with one exception. The proportion of NAG in the faeces was relatively higher after low-dose administration (7–8% of the dose; Lauck-Birkel, 1996), whereas the proportion was only 1–2% of the dose after

high-dose administration (this study). This lower formation rate in the high-dose study suggested some saturation effects in the acetylating microbial enzyme systems (Lauck-Birkel, 1995a).

^{14}C-labelled glufosinate-ammonium (radiochemical purity > 99%) was orally administered to 10 male and 10 female Wistar rats via gavage after 14 doses of non-labelled glufosinate-ammonium (purity 98.6%) at a dose of 2 mg/kg bw per day. Urine and faeces were collected on a daily basis and each pooled per sex and collection interval. All rats were sacrificed 96 hours after the radioactive dose. Metabolic profiles in urine and extracts of the faeces were analysed by radio-HPLC and co-elution of authentic radiolabelled reference standards. Statements of adherence to QA and GLP were included.

Glufosinate-ammonium was the predominant residue component, accounting for 74% of the renally excreted and 80% of the faecally excreted radioactive residues. MPP appeared as a minor metabolite (≤ 0.7% of the dose in urine and ≤ 7.5% of the dose in the faeces of both sexes). A further metabolite, MPB, was found at low levels in both sexes (Wink, 1986b).

Glufosinate-ammonium was administered by gavage to groups of female Wistar (WISKf, SPF 71) rats at about 10 or 100 mg/kg bw once a day for 10 days. On days 1, 8, 9 and 10, the animals received radiolabelled [^{14}C]glufosinate-ammonium (purity 99%), and on days 2–7, non-labelled glufosinate-ammonium (purity 99.6%). Groups of females were killed either 24 hours after the first dose or 24 or 48 hours after the 10th dose. Radioactivity levels in urine, faeces, liver, spleen, kidneys and brain were determined, and the residues in urine and faeces were identified. Statements of adherence to QA and GLP were included.

Following oral administration of [^{14}C]glufosinate-ammonium, excretion was rapid, mainly through faeces (73–103%). Less than 5% was excreted in urine. The excretion pattern was not affected by the repeated dosing. Most (94%) of the excreted radiolabel consisted of parent compound. The metabolites MPP and MPB accounted for 5% and 1%, respectively. Liver, kidney, spleen and brain contained less than 0.1% of the administered radioactivity. No evidence of significant accumulation was observed by repeated dosing (Schwalbe-Fehl, 1986).

Five male and five female Wistar rats received seven daily doses of [^{14}C]glufosinate-ammonium (radiochemical purity ≥ 98%) at 2 mg/kg bw per day by gavage. Urine and faeces were collected on a daily basis until 4 days after the last dose and each pooled per sampling interval and sex. The animals were killed and dissected 96 hours after the last dose. Urine and water extracts of the faeces were profiled by radio-HPLC for the composition of radioactive residues. Statements of adherence to QA and GLP were included.

In urine, at least four different residue components could be detected. The major component, at 2–4.5% of the administered dose, was the parent substance glufosinate-ammonium. Minor metabolites detected were MPP (0.1–0.7% of the dose) and MPB (0–0.2% of the dose) and an unknown, polar peak (0.4–1% of the dose). In the faeces, at least seven different residues were present, with the parent substance as the main component (74–78% of the dose) throughout the study period. The metabolite NAG slightly increased with the number of administrations from 2% to 6% of the administered dose. This metabolite was not detected in urine, suggesting that it is formed only in the intestine or faeces. The metabolites MPB and MPP were observed at levels of about 2–3% of the dose throughout the study period. In addition, low levels of two unknown polar residues (< 1% of the dose) and a non-polar residue (< 0.5% of the dose) were found. No sex differences in the metabolic profile were observed. Furthermore, repeated dosing did not change the metabolite pattern in the urine (Gutierrez, 2002).

Rabbits

[^{14}C]Glufosinate-ammonium (radiochemical purity > 99.7%) was administered by gavage as a single oral dose to three female New Zealand White rabbits at 6 mg/kg bw. Urine and faeces were collected on a daily basis for a total period of 10 days. The excreta samples were radioassayed and analysed by radio-HPLC. Statements of adherence to QA and GLP were included.

HPLC analysis showed that in urine and faeces, mainly glufosinate-ammonium was identified. Two other (unknown) metabolites were also present in urine and faeces. Levels of metabolites were not quantified (Koester, 2004).

Dogs

[^{14}C]Glufosinate-ammonium was orally administered by gelatine capsule to two male and two female Beagle dogs at a dose of 8 mg/kg bw. Excreta were collected at 6 and 24 hours. Blood was sampled at 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours. One male and one female dog were killed 6 hours after dosing, whereas the other two dogs were killed 24 hours after dosing. The identity and levels of residues in faeces, urine, plasma, kidneys and liver were determined by radio-TLC. The toxicokinetics of glufosinate-ammonium in dogs is described under section 1.1. Statements of adherence to QA and GLP were included.

Urine contained predominately glufosinate-ammonium (8.0–8.5% of the dose) and low levels of MPP (1.2% of the dose). No sex difference in the metabolite profile in urine was observed. Only the parent compound could be identified in faeces, liver, kidneys and plasma (Ellgehausen, 1986a).

The metabolism of glufosinate-ammonium after repeated doses of 1 or 8 mg/kg bw per day in dogs was examined. The study design is described in section 1.1 (Ellgehausen, 1986b). Samples of heart, liver, kidneys and different parts of the cerebral tissue were radioassayed by LSC. The faeces, urine, liver and kidney samples were analysed by radio-TLC, together with radiolabelled reference substances. Statements of adherence to QA and GLP were included.

Glufosinate-ammonium was the major component in urine and faeces. MPP was observed as a minor metabolite, accounting for less than 1% of the total ^{14}C dose in urine and faeces. In liver and kidneys of the highly dosed dogs, MPP was the predominant residue (31–72% of total residue), except in the liver of the female animals 24 hours after the last dose, in which only glufosinate-ammonium was identified (74% of the total residue). In the other liver and kidney samples, glufosinate-ammonium amounted to 11–40% of the total residue. The excretion pattern and the residue levels in selected organs were comparable to those observed in rats dosed with [^{14}C]glufosinate-ammonium at a similar level (see Ellgehausen, 1986a) (Ellgehausen, 1986b).

The proposed metabolic pathway of glufosinate-ammonium is depicted in Figure 2.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with glufosinate-ammonium are summarized in Table 8.

Figure 2. Proposed metabolic pathway of glufosinate-ammonium in the rat

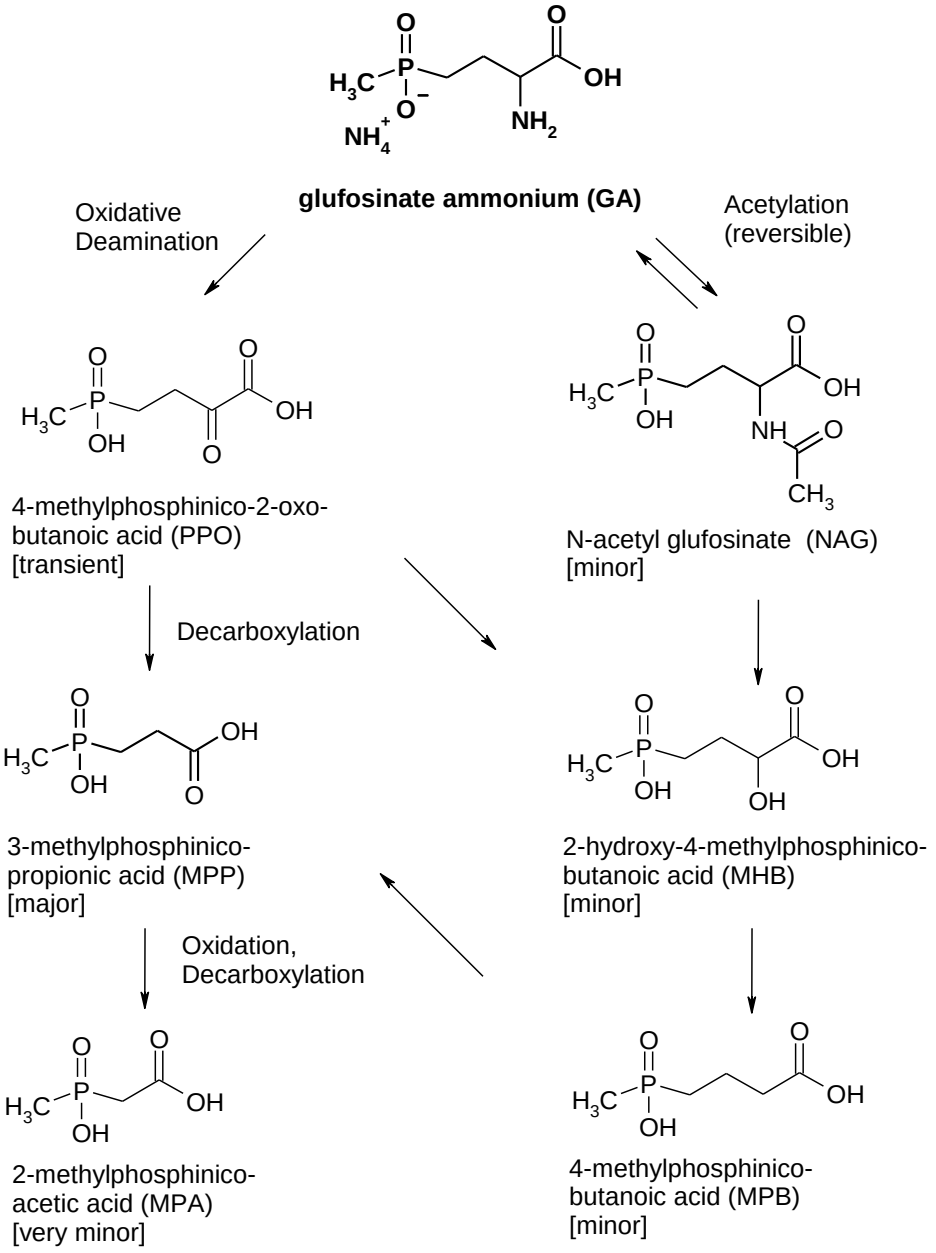


Table 8. Results of studies of acute toxicity with glufosinate-ammonium

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Reference
Mouse	NMRI	M	Oral	Deionized water	92.1	431	Mayer & Weigand (1980a)[a]
Mouse	NMRI	F	Oral	Deionized water	92.1	416	Mayer & Weigand (1980b)[a]
Mouse	ICR	M/F	Oral	Saline	92.1	436 (M) 464 (F)	Inoue (1982)[b]
Rat	Wistar	M	Oral	Deionized water	92.1	2000	Mayer & Weigand (1980c)[c]

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw) / LC_{50} (mg/l)	Reference
Rat	Wistar	F	Oral	Deionized water	92.1	1620	Mayer & Weigand (1980d)[c]
Rat	Fischer	M	Oral	Distilled water	92.1	1660	Ohtaka, Takahashi & Nakayoshi (1981a)[d]
Rat	Fischer	F	Oral	Distilled water	92.1	1510	Ohtaka, Takahashi & Nakayoshi (1981b)[d]
Rat	Wistar	M/F	Oral	Distilled water	50[e]	> 2000 (M) > 2000 (F)	Tavaszi (2011a)[f]
Dog	Beagle	M/F	Oral	Deionized water	92.1	200–400	Mayer & Kramer (1980)[g]
Rat	Wistar	M	Dermal	Deionized water	97.2	> 4000	Mayer & Weigand (1982a)[h]
Rat	Wistar	F	Dermal	Deionized water	97.2	> 4000	Mayer & Weigand (1982b)[h]
Rat	Wistar	M/F	Dermal	—	50[e]	> 2000	Zelenák (2011a)[i]
Rabbit	New Zealand White	M/F	Dermal	Distilled water	96.9	> 2000 (M) 1500–2000 (F)	Kynoch & Parcell (1986)[j]
Rat	Wistar	M/F	Inhalation	—	95.3	1.26 mg/l dust 2.60 mg/l dust	Hollander & Weigand (1985)[k]
Rat	Wistar	M/F	Inhalation	—	50[e]	> 5.0 mg/l	Grosz (2011)[l]

F, female; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; M, male

[a] Performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 401. Doses of 315, 500 and 800 mg/kg bw were used. Dose-dependent mortality was observed at all doses. The following clinical signs were observed: ataxia, bizarre movements, squatting, abdominal position, clonic convulsions, convulsive jumping and rolling spasms, "Straub" phenomenon and irregular, jerky respiration.

[b] Doses of 231–857 mg/kg bw were used. At all doses, males and females exhibited clinical signs. Observed clinical signs were decreased spontaneous motility, clonic convulsions, a ventral or abdominal posture, ataxic gait, piloerection and loss of coat lustre. Dose-dependent mortality was observed at doses of 300 mg/kg bw and higher.

[c] Performed according to OECD Test Guideline No. 401. Doses of 630–3150 mg/kg bw were used. Males at doses of 1000 mg/kg bw and higher and females at doses of 1600 mg/kg bw and higher exhibited hyperreflexia, Dalrymple's sign, exophthalmus, squatting, straddled legs and retracted abdomen or flank, hyporeflexia, bristled hair, poor general condition, ataxia, lateral position, blood crusted eyes and snouts, decreased and irregular respiratory rate, passiveness, disequilibrium, high-legged posture, abdominal and lateral position, trembling, convulsions, clonic convulsions and rolling spasms. Dose-dependent mortality was observed from 1000 mg/kg bw in males and from 1600 mg/kg bw in females. No symptoms of intoxication were seen in males or females from the 630 mg/kg bw dose group or females dosed at 1000 mg/kg bw.

[d] Performed according to OECD Test Guideline No. 401. Doses of 1000–3000 mg/kg bw were used. Every dose group exhibited occurrences of quietness, hypersensitivity to touch, salivation, lacrimation, ventral position and piloerection. At doses of 1600 mg/kg bw and higher in males and 2190 mg/kg bw and higher in females, diarrhoea and hypersensitivity to touch manifested as jumping and convulsions were seen. Dose-dependent mortality was observed from 1170 mg/kg bw in both sexes.

[e] Fifty per cent technical concentrate.

[f] Performed according to OECD Test Guideline No. 423. Doses of 300 and 2000 mg/kg bw were used. No clinical signs or mortality were observed.

Table 8 (continued)

[g] Performed according to OECD Test Guideline No. 401. Doses of 200 and 400 mg/kg bw were used. At 200 mg/kg bw, dogs showed squatting, benumbedness, trembling, diarrhoea and disequilibrium, abdominal position, retracted flank, hyporeflexia, increased lacrimation, salivation and rhinorrhoea, paraesis or paralysis of the hindlegs and discoloration of the tongue (bluish) and ocular and oral mucosae (distinctly reddened). During irregular intervals, both animals showed quick turning of the body on the hindlegs (rotary or arena motions). In addition, periodic extension spasms and marked dyspnoea accompanied by sounds in the form of loud cries were observed. During the following 8 days, all symptoms of intoxication were regressive and completely reversible by the termination of the experiment. At 400 mg/kg bw, both dogs died 2–3 days after treatment. The symptoms of intoxication in the male dog largely corresponded to those observed in the 200 mg/kg bw group, with additional symptoms of ataxia, emesis and noisy, jerky respiration. The female animal proved to be distinctly more sensitive and showed ataxia, abdominal position, tonoclonic convulsions, orthotonus, opisthotonus, miosis and noisy, jerky respiration. The body temperature of both animals was within the normal range 24 hours after dosing.

[h] Performed according to OECD Test Guideline No. 402. No symptoms of intoxication were observed in male rats in the 2000 mg/kg bw group. Males dosed at 4000 mg/kg bw showed hyperactivity, convulsions, retracted abdomen and sides, Dalrymple's sign, increased salivation and aggressivity. In females, the following symptoms were observed: hyperactivity, passiveness, lethargy, loss of equilibrium, squatting, abnormal posture, abdominal position, trembling, convulsions, retracted abdomen and sides, convulsive jumping, "Straub" phenomenon, bristled hair, Dalrymple's sign or blepharophimosis, increased salivation, blood-coloured urine, aggressivity, masticator movements, emaciation and poor general condition.

[i] Performed according to OECD Test Guideline No. 402 with glufosinate-ammonium technical, a liquid. No clinical signs or dermal signs were observed.

[j] Performed according to United States Environmental Protection Agency (USEPA) Guideline 81-2 (resembles OECD Test Guideline No. 402). Clinical signs of intoxication were piloerection, unsteady gait, lethargy, ataxia and prostration. There were no dermal reactions at the site of application in any of the treated animals.

[k] Performed according to USEPA Guideline 81-3 (resembles OECD Test Guideline No. 403). Clinical signs of intoxication were observed at all dose levels and included narrowed eyes, periodic tremors and clonic convulsions, hyperactivity, piloerection, increased salivation and passivity.

[l] Performed according to OECD Test Guideline No. 403 At 5.0 mg/l, slightly to moderately laboured respiration and slightly increased respiratory rate were the main clinical signs observed following the exposure on day 0. On the following days (days 1–4), toxicologically relevant clinical signs were slightly laboured respiration (all animals), weak body condition (all animals), slightly to severely decreased activity (3/5 males and 3/5 females), increased irritability (5/5 males and 3/5 females) and slight to moderate ataxia (1/5 males and 3/5 females). These signs were no longer observed after day 4, except for a single male and female. In the male rat, the clinical signs ceased on day 5, whereas weak body condition was observed up to day 13. In the female rat, the majority of clinical signs persisted up to death (day 8).

(b) Dermal irritation

In an acute dermal irritation study using six New Zealand White rabbits (sex not specified), the intact skin as well as the abraded skin were exposed for 24 hours under semi-occlusion to 0.5 g glufosinate-ammonium (purity not reported) moistened with 0.1 ml of 0.9% saline. Dermal irritation was scored according to the Draize system at 24, 48 and 72 hours after patch removal.

One animal exhibited erythema of the intact skin and four animals exhibited erythema of the abraded skin immediately after patch removal. No oedema was observed in any animal at any time point. Glufosinate-ammonium was non-irritating to rabbit skin (Mayer & Weigand, 1982c).

In an acute dermal irritation study, performed in accordance with Organisation for Economic Co-operation and Development (OECD) Test Guideline No. 404, the intact skin of three male New Zealand White rabbits was exposed for 4 hours under semi-occlusion to 0.5 ml of glufosinate-ammonium 50% technical concentrate. Dermal irritation was scored according to the Draize system at 1, 24, 48 and 72 hours after patch removal. Statements of adherence to QA and GLP were included.

Very slight oedema was observed in two animals at 1 and 24 hours after patch removal. Erythema and oedema were also recorded in one animal at 48 hours after patch removal. Glufosinate-ammonium 50% technical concentrate was non-irritating to rabbit skin (Zelenák, 2011b).

(c) Ocular irritation

In an acute eye irritation study, performed according to OECD Test Guideline No. 405, 0.0690 g (0.1 ml weight equivalent) of glufosinate-ammonium (purity 95.2%) was instilled into the conjunctival sac of the right eye of four male and two female New Zealand White rabbits. The untreated left eye served as a control. The eyes were macroscopically examined for signs of irritation according to the Draize system at 1, 24, 48 and 72 hours (all animals) and at day 7 (five animals) and day 10 (two animals) post-instillation. Statements of adherence to QA and GLP were included.

The test article produced iritis in five of six test eyes at the 1-hour scoring interval. The iridial irritation was completely reversed by the 24-hour scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in all (6/6) test eyes at the 1-hour scoring interval. Conjunctival irritation diminished over the remainder of the study and was no longer evident in any animals by study day 10. Under the conditions of this study, glufosinate-ammonium was not irritating to the eyes of rabbits (Merriman, 1995).

In an acute eye irritation study, 0.1 g of glufosinate-ammonium (purity not reported) premixed with one drop of 0.9% sodium chloride solution was placed into the conjunctival sac of the left eye of nine New Zealand rabbits (sex not specified). One minute after the application of the test substance, the eyes of three animals were flushed with physiological saline. The eyes of the remaining six animals were flushed after 24 hours. The eyes were macroscopically examined for signs of irritation at 1, 24, 48 and 72 hours after application, using a scale similar to the system of Draize.

In the rabbits whose eyes were rinsed 24 hours after application, slight signs of irritation of the iris (at 1 hour), redness of the conjunctivae (at 1–48 hours), swelling of lids and nictitating membranes (at 1–7 hours) and discharge with moistening of the lids (at 1–7 hours) were observed. All animals had recovered by day 3. Irritation of the iris and conjunctivae was also observed when the eye was rinsed 1 minute after application of the substance, although to a lesser degree. Under the conditions of this study, glufosinate-ammonium was not irritating to the eyes of rabbits (Mayer & Weigand, 1982c).

In an acute eye irritation study, 0.1 ml of glufosinate 50% technical material was placed into the conjunctival sac of the left eye of three male New Zealand rabbits. The eyes were macroscopically examined for signs of irritation according to the Draize system at 1, 24, 48 and 72 hours after application. Statements of adherence to QA and GLP were included.

One hour after the application, conjunctival redness (score 1, two animals), conjunctival discharge (score 2, two animals) and conjunctival chemosis (score 1, 1 animal) were observed. At 24 hours after treatment, conjunctival redness (score 1, two animals) and conjunctival discharge (score 1, one animal) were observed. At 48 hours after treatment, one animal displayed conjunctival redness. At 72 hours after treatment, no clinical signs were observed. Under the conditions of this study, glufosinate-ammonium was not irritating to the eyes of rabbits (Tavaszi, 2011b).

(d) Dermal sensitization

A dermal sensitization study (local lymph node assay) was performed according to OECD Test Guideline No. 429. CBA/J female mice (4–5 per group) were treated for 3 consecutive days with glufosinate-ammonium 50% technical concentrate at a concentration of 10%, 25% or 50% in vehicle (1% Pluronic Acid L92® in water). The test substance was applied in a volume of 25 µl to the external surface of each ear. A positive control group (four animals) received 30% α-hexylcinnamaldehyde in vehicle. The draining auricular lymph nodes were examined for T-lymphocyte proliferation, as measured by incorporation of tritiated thymidine. Statements of adherence to QA and GLP were included.

Four out of five animals treated with glufosinate-ammonium at 50% and one out of five animals treated with glufosinate-ammonium at 25% were found dead on day 3. No clinical signs were observed in these animals. No mortality and no clinical signs were observed in the other study groups. The isotope incorporation was less than 3-fold at treatment concentrations of 10% and 25% compared with the vehicle control. Treatment with the positive control, α-hexylcinnamaldehyde, resulted in a 19-fold isotope incorporation. Under the conditions of this study, glufosinate-ammonium was not sensitizing (Repetto, 2011).

A dermal sensitization study (Buehler test) with glufosinate-ammonium (purity 95.3%) was performed according to United States Environmental Protection Agency (USEPA) Guideline 81-6. In a preliminary study, 0.5 ml of test substance dissolved in 0.9% saline at a concentration of 50%, 5% or 0.5% was applied to the shaven flanks of female Pirbright-White guinea-pigs (two animals per dose). After 24 hours, no skin irritancy was observed; therefore, the highest concentration was chosen for the definitive study.

In the main study, the shaven left flanks of 20 female guinea-pigs were induced by 6-hour occluded topical applications of 50% glufosinate-ammonium in saline on days 1, 3, 5, 8, 10, 12, 15, 17 and 19. A control group of 10 animals remained untreated during the induction phase. On day 37, all test and control animals were challenged by 6-hour occluded topical applications of 50% glufosinate-ammonium to the shaven right flank. Dermal responses were assessed approximately 24 and 48 hours after application. A positive control was not included. Statements of adherence to QA and GLP were included.

No irritant effects on the treated sites were noted during the induction phase. Challenge treatment on day 37 produced no irritant effects on the treated skin sites at 24 and 48 hours after treatment. Under the conditions of this study, glufosinate-ammonium was not a skin sensitizer (Rupprich & Weigand, 1983).

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline No. 406, glufosinate-ammonium (purity 95.2%) was tested in 20 female Pirbright-White guinea-pigs. A preliminary study established 1% and 50% test substance concentrations in saline as suitable for the intradermal induction and dermal induction and challenge phases, respectively. The control group consisted of 10 animals. In the first induction phase, the animals were subjected to two intradermal injections of 50% Freund's Complete Adjuvant, 1% glufosinate-ammonium in isotonic saline and 1% glufosinate-ammonium in 50% Freund's Complete Adjuvant. Seven days later, the same area of skin was treated by topical application of 0.5 ml of a 50% solution of glufosinate-ammonium in saline and the test site covered with an occlusive dressing for 48 hours. The same induction procedure was carried out on control groups with vehicles only. On day 22, all animals were challenged by a 24-hour occluded topical application of 50% glufosinate-ammonium in saline. The test sites were assessed 24 and 48 hours after removal of the occlusive bandages. Benzocaine was used as a positive control. Statements of adherence to QA and GLP were included.

The intradermal injections with Freund's Complete Adjuvant (with and without test substance) caused severe erythema and oedema as well as indurations and encrustations. The application sites treated with the test substance showed slight erythema. Because of these strong irritation reactions of the skin, a local irritant such as sodium dodecylsulfate was not applied at day 7.

Following dermal induction, removal of the patches at day 10 revealed severe erythema and oedema with indurated and encrusted skin at the sites previously treated with Freund's Complete Adjuvant. The application sites treated with the test substance showed slight erythema and oedema. The vehicle alone showed no signs of irritation.

After the dermal challenge treatment on day 22, no signs of irritation were observed in the control and the treated groups 24 and 48 hours after the removal of the occlusive bandage. None of

the 20 animals in the treated group showed a positive skin response after the challenge procedure. Under the conditions of this study, glufosinate-ammonium was not a skin sensitizer (Hammerl, 1996).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 90-day dietary range-finding study, groups of 10 male and 10 female NMRI mice were administered glufosinate-ammonium (purity 95.5%) at 0, 1750, 3500 or 7000 ppm. The low and middle doses were equal to 274 and 561 mg/kg bw per day for males and 356 and 644 mg/kg bw per day for females, respectively. The mice of the high-dose group died within 8 days. Animals were observed daily for mortality and clinical signs. Feed consumption and body weights were recorded weekly. At the end of treatment, blood samples were collected (five animals of each sex per group for haematology and five other animals of each sex per group for clinical biochemistry). All surviving animals were necropsied, adrenals, kidneys, liver and testes were weighed and a wide range of organs and tissues was histologically examined. Statements of adherence to QA and GLP were included.

At 7000 ppm, all the animals died between days 4 and 8 of treatment. At 3500 ppm, four males and five females died between days 6 and 11 of treatment, whereas another male died at day 45. At 1750 ppm, one female died on day 8 of treatment. The death of a 1750 ppm male occurred after blood sampling and was not considered to be treatment related. At 3500 and 7000 ppm, ruffled fur, sedation, ventral recumbence or hunched posture, and emaciation were mainly noted. At 1750 ppm, ruffled fur was evident in all animals, and sedation and emaciation were seen in some females. Apart from ruffled fur, the clinical signs were observed only during the first part of the study. A body weight reduction was observed in mice of the low-dose (4–8%) and mid-dose groups (14–20%) during the 1st week of treatment. Statistically significant reduced body weights were observed in the low-dose males during the first 4 weeks of treatment, mid-dose males throughout the treatment period and mid-dose females during the first 2 weeks of treatment. Feed consumption showed a pattern similar to body weight gain. At 1750 and 3500 ppm, no toxicologically relevant effects on haematology or clinical chemistry were found. Necropsy revealed no toxicologically relevant gross findings, effects on organ weights or histopathological changes (Dotti, Luetkemeier & Biedermann, 1994).

Technical glufosinate-ammonium was administered via the diet to groups of 10 male and 10 female NMRI mice for 13 weeks at a dietary level of 0, 80, 320 or 1280 ppm (equal to 0, 17, 67 and 278 mg/kg bw per day for males and 0, 19, 87 and 288 mg/kg bw per day for females, respectively). Animals were examined daily for mortality and clinical signs. A detailed clinical examination was performed weekly. Feed consumption and body weights were recorded weekly. Ophthalmological examinations and hearing tests were performed prior to and at the end of treatment. Haematology and clinical chemistry were performed on five animals of each sex per dose at the end of the treatment period. A complete necropsy was performed on all mice. Tissues and organs were examined macroscopically. Selected organs were weighed and examined microscopically. Statements of adherence to QA and GLP were included.

No treatment-related effects on mortality, clinical signs, body weight changes, feed consumption or ophthalmology were noted. At the high dose, slightly higher aspartate aminotransferase (AST) activities (34%) in males and alkaline phosphatase (ALP) activities (38%) in females were noted. As no histopathological changes were observed in the liver, these increases are considered not adverse. A slight, but statistically significant, dose-related increased potassium concentration was noted in the males at 320 ppm (19%) and 1280 ppm (24%). Haematological investigations showed statistically significant reductions of total leukocyte count (white blood cells) for males at 320 ppm (44% decrease) and 1280 ppm (41% decrease) and of erythrocyte count (red blood cells) (5–6% decrease) and haematocrit (4% decrease) in females at 320 and 1280 ppm. A

statistically significant reduction (33–44%) of segmented neutrophils was noted for all treated females. As no corresponding histological changes were observed and as no haematological effects were observed in a study using higher doses or a study of longer duration, these findings are considered not toxicologically relevant. Although statistically significant, a slightly increased relative liver weight (8%) in high-dose males was not considered adverse. No treatment-related macroscopic or histopathological changes were found.

The NOAEL was 1280 ppm (equal to 278 mg/kg bw per day), the highest dose tested (Suter, 1984a).

Rats

In a dietary range-finding study, Wistar rats (five of each sex per group) were fed diets containing glufosinate-ammonium (purity 95.3%) for 4 weeks at a concentration of 0, 50, 500, 2500 or 5000 ppm (equal to 0, 5, 53, 276 and 534 mg/kg bw per day for males and 0, 6, 58, 271 and 557 mg/kg bw per day for females, respectively). Rats were examined daily for clinical signs of toxicity. Body weight and feed consumption were measured weekly during the study. Haematology, serum biochemical determinations and urine analyses were performed after 4 weeks. At the end of the treatment period, the rats were necropsied, liver and kidneys were weighed and kidneys were examined microscopically. Acetylcholinesterase activity was assessed in brain and erythrocytes. Statements of adherence to QA and GLP were included.

No deaths or clinical signs of systemic toxicity were observed. No toxicologically relevant effects were observed on body weight gain, feed consumption, haematology, clinical chemistry, urine analysis or pathology (Suter, 1984b).

In a 90-day dietary study, groups of 30 male and 30 female Fischer rats received glufosinate-ammonium (purity 92.1%) at a concentration of 0, 8, 64, 500 or 4000 ppm (equal to 0, 0.52, 4.1, 32 and 263 mg/kg bw per day for males and 0, 0.63, 4.8, 39 and 311 mg/kg bw per day for females, respectively). Twenty animals of each sex per group were sacrificed at the end of the 13-week study period, and 10 animals of each sex per group were maintained for a 4-week post-dosing recovery period. Animals were checked daily for clinical signs of toxicity. Body weights, feed consumption and water consumption were measured weekly. Ophthalmological examinations were carried out on high-dose rats before dosing, on day 47 and on the last day of dosing. Blood samples were taken from 10 animals of each sex per group and sampled for haematology and clinical biochemistry on days 47 and on the last day of dosing. Urine analysis was performed on samples taken from 10 animals of each sex per group on days 47 and 86 of dosing and on day 23 of the recovery period. At necropsy, selected organs were weighed and examined macroscopically and histopathologically.

No treatment-related mortality or clinical signs were seen. Reduced body weight gain (up to 11%) and feed consumption (up to 26%) were observed in both sexes in the 4000 ppm group for the first 2–3 weeks of treatment, in particular during the 1st week of treatment. The study authors attributed this to poor palatability of the test substance. Final body weights were not affected. No toxicologically relevant changes in ophthalmoscopy, haematology, clinical chemistry or histopathology were seen. Urine analysis demonstrated a lower pH in urine of high-dose rats. In treated males, statistically significant increases in absolute (all doses, 7–19%) and relative (at doses ≥ 64 ppm, 3–15%) kidney weights were observed. In females, statistically significant increases in absolute (11%) and relative (7%) kidney weights were observed at the high dose only. Such increases were still observed at the end of the recovery period, although to a lesser extent. As the increases in kidney weights were not accompanied by histopathological changes in the kidneys and were not observed in other studies, they are considered not related to treatment.

The NOAEL was 4000 ppm (equal to 263 mg/kg bw per day), the highest dose tested (Ohtaka, Takahashi & Nakayoshi, 1982).

In a 13-week dietary toxicity study, glufosinate-ammonium (purity 95.5%) was administered to groups of 10 male and 10 female SPF bred Hanover-derived Wistar rats at 0, 7500, 10 000 or 20 000 ppm (equal to 0, 521, 686 and 1351 mg/kg bw per day for males and 0, 574, 741 and 1443 mg/kg bw per day for females, respectively). Animals were checked daily for clinical signs of toxicity. Body weights, feed consumption and water consumption were measured weekly. The rats were tested in a functional observational battery before treatment and at weeks 1, 2, 3, 4, 8 and 13. Ophthalmological examinations were carried out before dosing and at week 13. Blood was sampled for haematology and clinical biochemistry at the end of the treatment period. All animals were necropsied, and all gross lesions and a wide range of tissues of rats of the control and high-dose groups and the brain, spinal cord, and left and right sciatic and tibia nerves from rats of the low- and mid-dose groups were examined microscopically. Statements of adherence to QA and GLP were included.

At 20 000 ppm, one female died on each of day 6 and day 8. These deaths were considered to be treatment related. In the first weeks of treatment in the high-dose group, sedation, lateral recumbency, hunched posture, dyspnoea, ruffled fur and emaciation were noted in both sexes. In addition, in a few females, spasms and lacrimation were noted. Ophthalmoscopic examinations revealed no treatment-related effects. For the first 2 weeks of treatment, reduced body weight gain was observed in males of the low-dose group (up to 15%), mid-dose group (up to 13%) and high-dose group (up to 24%) and in females of the high-dose group (up to 19%). In fact, high-dose males and females showed a 6–7 g body weight loss during the 1st week of treatment, while control rats gained 19–44 g over this period. Final body weights were not affected. Mean feed consumption over the entire test period was reduced in the males by about 10% in the low- and mid-dose groups and by about 15% at 20 000 ppm and in high-dose females by about 10%. The effects on feed consumption were mainly evident during the first half of treatment and in particular during weeks 1 and 2 of treatment.

Functional observational battery testing showed miosis and a slight decrease in exploratory activity, alertness and/or startle response, particularly in the early stages of treatment, in the low-dose rats. In addition, mid-dose rats showed increased body tone, increased pain response and fearfulness and occasionally rearing with convulsive twitches and perfuse salivation. In high-dose rats, signs of abnormal behaviour were similar to those seen in animals at 10 000 ppm, although more severe and persistent. In addition, diarrhoea, increased vocalization and apathy were recorded.

Haematology showed small, but statistically significant, reductions in erythrocyte count in low-dose males and mid- and high-dose males and females. Mean corpuscular volume was slightly increased (4%) in males at 10 000 and 20 000 ppm. Increases in high (140–160%) and middle fluorescent reticulocyte ratios (22–25%) were observed in mid- and high-dose males. Low fluorescent reticulocyte ratios were decreased (11–16%) in males of all treatment groups. Changes in haematocrit and reticulocyte count were not dose dependent.

Necropsy and histopathological examination did not reveal treatment-related findings.

The lowest-observed-adverse-effect level (LOAEL) was 7500 ppm (equal to 521 mg/kg bw per day), based on reduced body weight gain and feed consumption, reductions in erythrocyte count and low fluorescent reticulocyte ratios in males and miosis and a slight decrease in exploratory activity, alertness and/or startle response observed in the functional observational battery in both sexes (Dotti, Luetkemeier & Powell, 1993).

Dogs

In a 28-day oral range-finding study in Beagle dogs, the mode of action and toxicokinetics of glufosinate-ammonium were explored. Groups of six male and six female dogs received glufosinate-ammonium (purity 95.3%) in gelatine capsules at a dose of 0, 1 or 8 mg/kg bw per day for 28 days, followed by a 4-day recovery period. From days 19 to 28, the dogs received ^{14}C-labelled glufosinate-ammonium (radiochemical purity 98%) in distilled water. One dog of each sex per dose group was killed on day 18, 19 or 28 of the treatment period or on day 1, 2 or 4 post-treatment. Dogs were

observed daily for mortality and clinical signs. Feed consumption was measured daily and body weight weekly during the study. The dogs in the control and high-dose groups were subjected to neurobehavioural assessments before dosing and on days 1, 2, 3, 4, 8, 11, 15, 18, 22 and 25. Animals of the low-dose group were subjected to neurobehavioural assessments before dosing and on days 4, 11, 18 and 25. The dogs were subjected to ophthalmoscopy (pretest and on days 14 and 25), haematology, blood chemistry (including catecholamine measurements) and urine analysis (pretest, on days 11 and 28 of treatment and on day 4 post-treatment). At necropsy, liver, heart, kidney, spinal cord and four brain regions (cortex, midbrain, cerebellum and brain stem) were collected, weighed and examined. Glutamine synthetase activity and free amino acid levels in different tissues were measured. Statements of adherence to QA and GLP were included.

No animals died during the study. A slight increase in spontaneous motor activity in high-dose animals was observed. Detailed observations and neurological examinations showed no treatment-related effects. Ophthalmological examinations showed no treatment-related findings. The body weight gain and feed consumption of the high-dose males were reduced during the 1st week of treatment, and those of the corresponding females were reduced during the entire treatment phase. No treatment-related effects on haematology, clinical biochemistry, urine analysis, organ weights or macroscopy were observed. In high-dose animals, inhibition of glutamine synthetase activity in midbrain, cerebellum and spinal cord (males only) was observed. Reductions in glutamine synthetase activity in spinal cord of females and in cortex and liver of both sexes were not statistically significant (Table 9). Glutamine synthetase activity in kidney was not affected.

Table 9. Mean levels of glutamine synthetase activity in tissues

Sex	Tissue	0 mg/kg bw per day	1 mg/kg bw per day		8 mg/kg bw per day	
		Mean[a]	Mean	% of control	Mean	% of control
Males	Liver	6.41	6.06	95	3.98	62
	Midbrain	4.52	4.49	99	2.93*	65
	Cerebellum	3.64	3.58	98	1.72*	47
	Cortex	6.92	7.17	104	5.09	74
	Spinal cord	1.10	0.90	82	0.55*	50
Females	Liver	5.96	6.24	105	4.19	70
	Midbrain	4.44	4.88	110	3.20*	72
	Cerebellum	3.22	3.39	105	2.16*	67
	Cortex	6.37	6.78	106	5.87	92
	Spinal cord	1.01	1.05	104	0.70	69

From Sachsse (1986a)

* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

[a] One dog of each sex per dose group was killed on day 18, 19 or 28 of the treatment period or on day 1, 2 or 4 post-treatment. The data are the means of these six dogs of each sex per dose. Glutamine synthetase activity is expressed as micromoles of γ-glutamyl-hydroxamate formed per millilitre reaction mixture per 20 minutes.

In cerebellum of high-dose animals, an increased level of α-ketoglutarate was found. In cerebellum, the levels of taurine and phosphoethanolamine were significantly reduced at the highest dose in both sexes. Glutamate, aspartate, cysteine, γ-aminobutyric acid, glycine and β-alanine levels in neural tissue and brain cholinesterase activity were not affected by treatment.

The NOAEL was 1 mg/kg bw per day, based on the reductions in glutamine synthetase activity in the central nervous system, a slight increase in spontaneous motor activity and a reduction in body weight gain and feed consumption at 8 mg/kg bw per day (Sachsse, 1986a).

In a 90-day toxicity study, four male and four female Beagle dogs per dose group received feed containing glufosinate-ammonium (purity 92.1%) at a dietary level of 0, 4, 8, 16, 64 or 256 ppm (equal to 0, 0.13, 0.26, 0.53, 2.0 and 7.8 mg/kg bw per day, respectively). Animals were checked daily for viability and clinical signs. Feed and water consumption and body weights were measured weekly. Ophthalmological examinations were performed in week 12. Blood samples for haematology and clinical chemistry were collected pretest and in weeks 7 and 13. A liver function test (bromosulfophthalein [BSP] method) was carried out on all dogs of the two highest-dose groups and the control group during week 13; a kidney function test (phenolsulfonphthalein [PSP] method) was conducted on the two highest-dose groups and the control group during week 12. Urine analysis was performed pretest and in weeks 7 and 12. Control and 256 ppm dogs were subjected to electrocardiography (ECG) pretest and at week 13. All dogs were necropsied, and weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, ovaries, thymus, thyroid with parathyroid and lungs were recorded. Histology was performed on a large selection of organs from all dogs. Statements of adherence to QA and GLP were included.

No mortality or clinical signs of toxicity were observed. Feed consumption was 14% lower in high-dose (256 ppm) females. Water intake was similar in all groups. In high-dose (256 ppm) females, a statistically significant reduction in body weight gain (10%) was observed during the second half of the study. Ophthalmological and ECG examinations revealed no treatment-related effects.

No consistent effects on haematological or urine analysis parameters were observed. Slight reductions in plasma inorganic phosphate levels in some treatment groups at week 7 were not accompanied by changes in calcium concentrations or abnormalities in the histopathology of the parathyroid or kidneys and were attributed to the relatively high control values at week 7.

At week 13, direct plasma bilirubin levels were relatively low in males of all dose groups but showed no dose–response relationship. This finding was not accompanied by any changes in total bilirubin levels and was considered not to be of toxicological significance. Plasma PSP concentrations were relatively low in males as well as in females of the two highest-dose groups, but within the historical control range. As an impaired renal function is reflected by an increase rather than a decrease in PSP concentration and as no treatment-related pathological changes were observed in the kidneys, the reduction in PSP levels is considered not toxicologically relevant. Other isolated changes in clinical chemistry parameters were considered not to be of toxicological significance.

Microscopic and histopathological examination and organ weight measurements did not reveal effects related to glufosinate-ammonium treatment.

The NOAEL was 64 ppm (equal to 2.0 mg/kg bw per day), based on the reduction in body weight gain and feed consumption in females at 256 ppm (equal to 7.8 mg/kg bw per day) (Lina et al., 1982).

In a 1-year study, glufosinate-ammonium was administered to groups of eight male and eight female Beagle dogs via the diet at 0, 60, 150 or 250 ppm. The achieved intake levels were 0, 1.8, 4.5 and 8.4 mg/kg bw per day, respectively, except for the first 10–17 days of the study, when high-dose animals received 375 ppm, equal to 10.6–13.6 mg/kg bw per day for males and 15.4–16.0 mg/kg bw per day for females. As this was above the target dose of 8.5 mg/kg bw per day, the dietary concentration was lowered to 250 ppm from day 12 onward. Four dogs of each sex per dose group were assigned for interim kill after 6 months of treatment, and four animals of each sex per group were assigned for terminal kill after 1 year of treatment. Animals were examined for mortality and clinical signs. Feed consumption was recorded daily, and body weights were recorded weekly. Ophthalmoscopy and hearing tests and examinations of mucous membranes and teeth were performed pretest and after 3, 6, 9 and 12 months. ECG was performed pretest and at 6 or 12 months. Haematology, clinical chemistry and urine analysis were performed pretest and after 1, 3, 6 and 12 months. The BSP clearance test for measuring liver function and the PSP clearance test for measuring

kidney function were performed after 6 and 12 months of treatment. The dogs were necropsied, and weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, ovaries, thymus, thyroid with parathyroid and lungs were recorded. Histology was performed on a large selection of organs and tissues from all dogs. Bone marrow was examined histologically in control and high-dose animals only. Statements of adherence to QA and GLP were included.

One male and one female from the high-dose group were found dead on days 14 and 10, respectively. In these two animals and an additional high-dose female, trismus, salivation and hyperactivity followed by somnolence and hypoactivity were observed immediately after feed consumption, as well as stereotypic stiff gait, tremor, ataxia, whining, urinating, tonoclonic spasm, paddling movements, opisthotonus and lateral recumbence. These clinical signs were first seen after 9 days of treatment. The deaths of the two high-dose dogs were caused by heart and circulatory failure attributed to marked myocardial necrosis in one dog and to slight myocardial necrosis and severe necrotizing aspiration pneumonia in the other dog and were considered treatment related. In the other animals, no treatment-related effects on feed consumption, body weight gain, hearing, teeth, mucous membranes, ophthalmoscopic examination, ECG, haematology, clinical chemistry, macroscopy, histopathology including bone marrow and organ weights were observed.

The NOAEL was 150 ppm (equal to 4.5 mg/kg bw per day), based on clinical signs of toxicity and mortality observed at 375 ppm (equal to 10.6–13.6 mg/kg bw per day) during the first 2 weeks of the study. The study indicates that glufosinate-ammonium has a steep dose–response curve in dogs (Bathe, 1984).

(b) Dermal application

Groups of six male and six female Wistar rats were dermally exposed 6 hours/day, 5 days/week, to a 0%, 7.5%, 22.5% or 75% solution of technical glufosinate-ammonium (purity 95.3%) in deionized water, equal to doses of 0, 100, 300 and 1000 mg/kg bw per day. Twenty-one applications were given over a 30-day period. Additionally, two groups of five males and five females, one control and one dosed at 1000 mg/kg bw per day, were treated by a similar regimen followed by a 14-day recovery period. The behaviour and the general health and condition of the animals were observed daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Haematology, clinical chemistry and urine analysis were performed at the end of the study. At termination of the study, all animals were killed and necropsied. Weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, ovaries, thyroid and lungs were recorded. Histology was performed on a selection of organs and tissues. Statements of adherence to QA and GLP were included.

No dermal irritation was noted. At 300 mg/kg bw per day, one male showed aggressive behaviour, squatting position, piloerection and convulsive jumping and rolling spasms at the end of the treatment period. At the high dose, four male and two female animals showed either timid or aggressive behaviour, increased motor excitation (especially following tactile stimuli), piloerection or squatting position. One male refused its feed almost entirely from the beginning of the 2nd week of treatment and was removed in an emaciated condition on day 16. Body weight gain and feed and water consumption were not affected by treatment.

Occasional changes in haematology and clinical chemistry in the mid- and high-dose groups were not dose related and/or were within the range of normal variation and were considered not to be treatment related. Urine analysis was normal for all groups. Therefore, examination of haematological, clinical chemistry and urinary parameters was not performed in the recovery animals. Macroscopic, histopathological and organ weight examinations showed no toxicologically significant effects of glufosinate-ammonium treatment.

The NOAEL was 100 mg/kg bw per day, based on clinical signs observed in a male at 300 mg/kg bw per day (Ebert & Kramer, 1985a).

(c) Exposure by inhalation

Groups of 15 male and 15 female Wistar rats were exposed nose-only to dust particulate aerosol atmospheres of glufosinate-ammonium (purity 95.3%) at a concentration of 0, 0.012, 0.025 or 0.050 mg/l air, 6 hours/day, 5 days/week, over a 40-day period. The control animals received air only. About 82–85% of the total aerosol had a mass median aerodynamic diameter (MMAD) of less than 7 µm, and 46–49% had an MMAD of less than 3 µm. The behaviour and the general health and condition of the animals were observed daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Haematology and clinical chemistry were performed at the end of the study. One day after the last exposure, 10 males and 10 females from each group were killed and necropsied. The remaining rats were killed and necropsied after a 29-day recovery period. Weights of heart, spleen, brain, liver, pituitary, adrenals, kidneys, testes, seminal vesicles, ovaries, thyroid and lungs were recorded. Histology was performed on a large selection of organs and tissues. Statements of adherence to QA and GLP were included.

Two females of the high-dose group died during the 2nd exposure, and one male of the high-dose group died after the 2nd exposure and another after the 17th exposure. In the low-dose group, one rat showed squatting position and piloerection on 2 days. In the mid-dose and high-dose rats, staggering gait, squatting position, tremors, hyperactivity, aggressiveness, tonoclonic convulsions and haematuria were observed. In addition, in the high-dose rats, salivation, contracted flanks, narrowed eye openings and piloerection were observed. There were no behavioural abnormalities in rats in any of the groups during the recovery period.

A statistically significant increase in body weight gain was noted in males (9%) and females (15%) of the high-dose group and for females of the mid-dose group (13%). In the high-dose group, a slight increase in feed consumption was found. Relative water consumption was increased during certain periods in mid- and high-dose rats. Haematology and clinical chemistry revealed no treatment-related effects. A few changes in relative organ weights were considered to be incidental and not related to treatment. No treatment-related macroscopic or microscopic findings were noted in the rats surviving the duration of the study. One male that died showed aspiration pneumonia. The other three animals that died showed cell atrophy in thymus and bone marrow and contraction of the spleen. In addition, blood congestion and focal or single hyperkeratosis in the forestomach were noted in two of these animals (one male and one female).

The no-observed-adverse-effect concentration (NOAEC) was 0.012 mg/l air, based on clinical signs of toxicity (neurotoxic symptoms) and increased body weight gain (females only) at 0.025 mg/l air (Hollander & Kramer, 1985).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year dietary carcinogenicity study, technical glufosinate-ammonium (purity 95.3%) was administered to groups of 60 male and 60 female NMRI mice at 0, 20, 80 or 160 ppm (equal to 0, 2.8, 10.8 and 23 mg/kg bw per day, respectively) for males and 0, 20, 80 or 320 ppm (equal to 0, 4.2, 16 and 64 mg/kg bw per day, respectively) for females. Ten mice of each sex per group were designated for interim sacrifice after 52 weeks of treatment. The mice were checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Feed consumption and body weight were recorded weekly during months 1–3 and biweekly thereafter. Ophthalmoscopy and hearing tests were performed on 10 animals of each sex per group pretest and at 6, 12, 18 and 24 months of treatment. Haematology (in five non-fasted mice of each sex per group) and clinical chemistry (in five fasted mice of each sex per group) were performed at weeks 52 and 104. Glutathione levels in whole blood and liver tissue were measured in the remaining fasted animals at termination of the study. Blood smears for differential counts were prepared from all surviving animals at termination of the study. At termination at 52 or 104 weeks of treatment, all animals were necropsied, organ weights were recorded and histological examinations were performed. Statements of adherence to QA and GLP were included.

At termination, increased mortality was observed in high-dose males (65%) compared with controls (43%). No effect of treatment on clinical signs, ophthalmoscopic and hearing examinations, haematology or feed consumption were noted. A reduction in body weight gain was observed in high-dose males of the interim kill group (up to 16%) during weeks 3–33 and in high-dose females of the terminal kill group (up to 9%) during weeks 7–31 of treatment. Isolated reductions in body weight gain were also observed in high-dose males of the terminal kill group. Clinical chemistry indicated an increased glucose level (138–167% of control) for high-dose male and female mice at 52 weeks of treatment, an increased AST activity (241% of control) for high-dose female mice at 52 weeks of treatment and a decreased glutathione level (58% of control) in whole blood for high-dose male mice at 104 weeks of treatment (not investigated in females after 104 weeks).

Furthermore, slightly decreased albumin (84% of control) and total protein levels (88% of control) were noted for high-dose female mice at 52 weeks of treatment, but not at 104 weeks. All other statistical differences in biochemical parameters were within the normal range for this mouse strain or lacked a dose–response relationship and were considered not to be treatment related. Absolute and relative liver weights were decreased (66–72% and 63–66% of controls, respectively) in females of all treatment groups, but not in males. As the reductions in liver weights in females were not observed in males and were not correlated to histopathological changes, they are considered not toxicologically relevant. Histopathological examination revealed no treatment-related changes in non-neoplastic or neoplastic lesions.

The NOAEL was 80 ppm (equal to 10.8 mg/kg bw per day), based on increased mortality and reduced body weight gain in males and changes in clinical chemistry parameters in both sexes at the next higher dose (males: 160 ppm, equal to 23 mg/kg bw per day; females: 320 ppm, equal to 64 mg/kg bw per day) (Suter, 1986a).

Rats

A 130-week combined toxicity and carcinogenicity study was performed according to OECD Test Guideline No. 453 in Wistar KFM-Han rats. The control group consisted of 130 rats of each sex, and treatment groups consisted of 80 rats of each sex per dose. Ten rats of each sex per group were killed after 52 weeks of treatment, and 20 rats of each sex per group were killed after 104 weeks. The groups of rats were fed diet with glufosinate-ammonium (purity 95.3%) at 0, 40, 140 or 500 ppm (equal to 0, 2.1, 7.6 and 26.7 mg/kg bw per day for males and 0, 2.5, 8.9 and 31.5 mg/kg bw per day for females of the chronic toxicity groups [104 weeks], respectively; and equal to 0, 1.9, 6.8 and 24.4 mg/kg bw per day for males and 0, 2.4, 8.2 and 28.7 mg/kg bw per day for females of the carcinogenicity groups [130 weeks], respectively). The rats were examined daily for mortality and clinical signs. Additionally, a detailed clinical examination was performed weekly. Body weight and feed consumption were recorded weekly for the first 3 months and every 2 months thereafter. Ophthalmoscopic evaluations were performed on 10 rats of each sex per group pretest and at 12, 24 and 30 months. Hearing tests were performed on 10 rats of each sex per group pretest and at 6, 12, 18, 24 and 30 months. Haematology, clinical chemistry and urine analysis were performed at weeks 26, 52, 78 and 104. Weights of adrenals, brain, gonads, heart, kidneys, liver, lung, pituitary, thymus and thyroid were determined at scheduled necropsy. Macroscopic and microscopic examinations of a wide range of organs and tissues were performed on all animals. Glutamine synthetase and ammonia levels were assessed in kidneys, liver and brain after 52 and 104 weeks. Ammonia levels were determined in urine and plasma after 26–28, 52–53, 78 and 104 weeks. Glutathione (reduced [GSH]/oxidized [GSSG]) levels were determined in liver and blood after 130 weeks of treatment. Liver (BSP) and kidney (PSP) function tests were performed on 10 rats of each sex of the control and high-dose groups after 43 and 96 weeks of treatment. Statements of adherence to QA and GLP were included.

There were no significant differences in mortality between treatment and control groups of the chronic toxicity animals for the major duration of the study. Only after 125 weeks was mortality in mid- and high-dose females (54% and 58% mortality, respectively) increased, compared with controls (30% mortality). No treatment-related clinical signs of toxicity were noted. In some treatment groups, statistically significant increases in body weight gain and feed consumption were observed during certain treatment periods. However, the increases were small (generally < 10%) and not considered

toxicologically significant. Similarly, increases observed in the absolute weights of some organs in these animals are also considered to be related to their increased body weight. It is concluded that organ weights were not affected by treatment. Eye and hearing examinations and urine analysis revealed no treatment-related findings. Slight reductions in haemoglobin concentrations and haematocrit in high-dose males and females and in erythrocyte counts in high-dose females were observed at week 52 only (Table 10). Mean corpuscular haemoglobin concentration was consistently increased in high-dose females at weeks 52, 78 and 104 (Table 11).

Table 10. Haematology after 52 weeks in rats administered glufosinate-ammonium

Parameter	Sex	Mean value			
		0 ppm	40 ppm	140 ppm	500 ppm
Red blood cells ($\times 10^{12}$/l)	Males	9.4	9.7	9.6	8.8
	Females	8.5	8.2	8.5	7.9*
Haemoglobin (mmol/l)	Males	9.3	9.5	9.3	8.7*
	Females	9.0	8.9	9.1	8.7*
Haematocrit (l/l)	Males	0.42	0.43	0.43	0.39*
	Females	0.42	0.40*	0.42	0.39*
Mean corpuscular haemoglobin concentration (mmol/l)	Males	22.0	22.0	21.9	22.3
	Females	21.5	22.1*	21.9	22.4*

From Suter (1986b)
* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

Table 11. Mean corpuscular haemoglobin concentrations after 52, 78 and 104 weeks in female rats administered glufosinate-ammonium

Week	Mean corpuscular haemoglobin concentration (mmol/l)			
	0 ppm	40 ppm	140 ppm	500 ppm
52	21.5	22.1*	21.9	22.4*
78	21.8	22.1	22.5*	22.4*
104	21.6	22.1*	22.0	22.3*

From Suter (1986b)
* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

No consistent effects on clinical chemistry parameters were observed. The data from the special biochemical investigations are presented in Tables 12 and 13. The biochemical studies indicate that, particularly in high-dose animals, glufosinate-ammonium treatment results in inhibition of glutamine synthetase activity in liver (up to 25%) and brain (up to 11% in high-dose females), decreases glutathione levels and increases the GSH to GSSG ratio in liver and blood. These effects are considered related to the structural analogies between glufosinate-ammonium and the substrate of glutamine synthetase (i.e. glutamate). The increased renal glutamine synthetase activity is considered to reflect adaptation to chronic treatment. In the absence of any related changes in organ function and histopathological changes, these biochemical changes are considered not toxicologically relevant. Ammonia levels were unaffected by treatment. Histological examination showed no effect of glufosinate-ammonium treatment on the incidences of non-neoplastic or neoplastic lesions.

Table 12. Glutamine synthetase activity after 52 and 104 weeks in the liver, kidney and brain of rats administered glufosinate-ammonium

Organ	Week	Sex	Mean glutamine synthetase activity (µmol/ml[a])			
			0 ppm	40 ppm	140 ppm	500 ppm
Liver	52	Males	2.28	2.07	1.80*	1.71*
		Females	2.85	2.67	2.38*	2.28*
	104	Males	2.33	2.39	2.21	2.35
		Females	2.92	3.28	3.56*	3.27
Kidney	52	Males	1.28	1.41	1.51*	1.72*
		Females	0.79	1.19*	1.27*	1.52*
	104	Males	1.47	2.03	2.15*	2.35*
		Females	1.19	1.61*	2.01*	2.28*
Brain	52	Males	2.48	2.48	2.52	2.34
		Females	2.35	2.37	2.19	2.14
	104	Males	ND	ND	2.34	2.19
		Females	2.53	2.32*	2.44	2.24*

From Suter (1986b)
ND, no data; * $P < 0.05$ (Dunnett test based on pooled variance or Steel test)
[a] Expressed in the table as micromoles of γ-glutamyl-hydroxamate formed per millilitre reaction mixture per 20 minutes at 37 °C.

Table 13. Glutathione (GSH, GSSG and total) concentration in the liver and whole blood after 130 weeks in rats administered glufosinate-ammonium

Organ	Sex	Glutathione	Mean glutathione concentration (µmol/g)			
			0 ppm	40 ppm	140 ppm	500 ppm
Liver	Males	GSH	5.49	5.37	5.59	6.02
		GSSG	1.53	1.43	1.19*	0.98*
		Total	7.02	6.80	6.78	6.99
	Females	GSH	4.98	4.50	3.10*	3.87*
		GSSG	1.32	1.39	1.26	1.30
		Total	6.30	5.89	4.36*	5.17*
Whole blood	Males	GSH	0.82	0.76	0.71	0.54*
		GSSG	0.20	0.19	0.24	0.32*
		Total	1.02	0.95	0.95	0.85
	Females	GSH	0.98	0.79	0.66*	0.64*
		GSSG	0.25	0.24	0.25	0.31
		Total	1.23	1.03	0.90*	0.95

From Suter (1986b)
* $P < 0.05$ (Dunnett test based on pooled variance or Steel test)

The NOAEL was 140 ppm (equal to 7.6 mg/kg bw per day), based on effects on haematology, GSH and GSSG levels and reduction of brain glutamine synthetase activity at 500 ppm (equal to 26.7 mg/kg bw per day) (Suter, 1986b).

In a 2-year carcinogenicity study performed in accordance with OECD Test Guideline No. 451, groups of 60 Wistar rats of each sex received diets containing glufosinate-ammonium (purity

96%) at a concentration of 0, 1000, 5000 or 10 000 ppm (equal to 0, 45, 229 and 466 mg/kg bw per day for males and 0, 57, 282 and 579 mg/kg bw per day for females, respectively). The animals were observed daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Body weight and feed consumption were recorded weekly for the first 13 weeks and biweekly thereafter. Blood samples for differential blood count were prepared from controls and animals at the high dose in weeks 52, 78 and 104. At termination, the animals were necropsied, and weights of adrenals, brain, gonads, heart, kidneys, liver, spleen, pituitary and thyroid were determined. Histopathological examinations of a wide range of organs and tissues were performed on all animals. Statements of adherence to QA and GLP were included.

No effects of treatment on mortality, clinical signs, the incidence of nodules or masses or differential blood count were observed. During the first 3–4 weeks, reductions in body weight gain were observed at 5000 ppm (up to 9% in weeks 2–3) and 10 000 ppm (up to 12% in week 2). After that period, no remarkable changes in body weight gain were noted. A dose-related increase in kidney weight of 15–30% was observed in all treatment groups. However, no histopathological changes were found in the kidneys. Necropsy showed a decreased incidence of pituitary nodules in males of all treatment groups and an increased incidence of adrenal gland foci in males at the high dose. Histopathology revealed an increase in the incidence of retinal atrophy in females at 5000 ppm (19/59) and in males and females at 10 000 ppm (12/60 and 29/59, respectively) compared with control males and females (4/60 and 3/58, respectively). No effect of glufosinate-ammonium treatment on the incidences of neoplasms was observed.

The NOAEL was 1000 ppm (equal to 45 mg/kg bw per day), on the basis of the increased incidence of retinal atrophy (Schmid et al., 1998).

2.4 Genotoxicity

Glufosinate-ammonium was tested for genotoxicity in a range of guideline-compliant assays. No evidence for genotoxicity was observed in any test.

The results of the genotoxicity tests are summarized in Table 14.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a range-finding one-generation reproductive toxicity study, glufosinate-ammonium (purity 95.3%) was administered in the diet during a 3-week premating period and continuing throughout the mating, gestation and lactation periods to groups of 10 Wistar/HAN rats of each sex per group. Dietary concentrations were 0, 50, 500, 2500 and 5000 ppm (equal to 0, 4.3, 44, 206 and 396 mg/kg bw per day for males and 0, 4.3, 44, 207 and 394 mg/kg bw per day for females, respectively, during the premating period; equal to 0, 3.2, 33, 163 and 327 mg/kg bw per day for males and 0, 4.7, 45, 205 and 407 mg/kg bw per day for females, respectively, during the gestation period). During the lactation period, females of the 50 and 500 ppm groups received 9.3 and 74 mg test material per kilogram body weight per day, respectively. The rats were checked daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. In dams that littered, body weight and feed consumption were measured on lactation days (LDs) 1, 4, 7, 14 and 21. Litter size, sex of pups, number of stillbirths, number of live births and presence of gross anomalies were determined. Maternal rats were killed and necropsied at the end of lactation. The numbers of corpora lutea and implantation sites were recorded. Liver, spleen, kidneys and ovaries of all parental animals and two males and two females per litter were weighed. Statements of adherence to QA and GLP were included.

Table 14. Overview of genotoxicity tests with glufosinate-ammonium[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2 *uvrA*	1.6–5000 µg/plate (±S9)	99.2	Negative	Ballantyne (2001a)[b,c]
Forward mutation	*Schizosaccharomyces pombe* strain SP ade 6-60/rad 10-1998, h-	125–1000 µg/ml (±S9)	95.3	Negative	Mellano (1984a)[b,d]
Mitotic recombination	*Saccharomyces cerevisiae* D4	1000–10 000 µg/ml (±S9)	95.3	Negative	Mellano (1984b)[b,e]
Chromosomal aberrations	Human lymphocytes	1000– 4640 µg/ml (−S9) 2150–10 000 µg/ml (+S9)	97	Negative	Mosesso (1990)[f]
Gene mutation	Mouse lymphoma L5178Y *Tk* locus	50–5000 µg/ml (−S9), 300–5000 µg/ml (+S9)	95.3	Negative	Cifone (1985)[g]
Gene mutation	V79 Chinese hamster cells, HPRT test	625–10 000 µg/ml (−S9) 625–8000 µg/ml (+S9)	97	Negative	Seeberg (1989)[h]
Unscheduled DNA synthesis	Male Fischer 344 rat hepatocytes	26.2–5240 µg/ml	95.3	Negative	Cifone (1984)[i]
In vivo					
Chromosomal aberrations	NMRI mouse bone marrow	100, 200 or 350 mg/kg bw (gavage)	96.9	Negative	Jung & Weigand (1986)[j]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to QA and GLP were included.

[c] Batch 26880-125-M29. Performed in accordance with OECD Test Guideline No. 471. Toxicity was observed at and above 200 µg/plate.

[d] Batch Lfd 12027.

[e] Batch Lfd 12027. Test design resembles OECD Test Guideline No. 481, with some minor deviations.

[f] Batch 27/85. Performed in accordance with OECD Test Guideline No. 473. In the absence of S9, marked cytotoxicity was observed at 10 000 µg/ml.

[g] Batch Lfd 12027. Study design resembles OECD Test Guideline No. 476.

[h] Batch 27/85. Performed in accordance with OECD Test Guideline No. 476. In the presence of S9, marked cytotoxicity was observed at concentrations of 5000 µg/ml and higher.

[i] Batch Lfd 12027. Study design resembles OECD Test Guideline No. 482. Cytotoxicity was observed at doses of 2620 µg/ml and higher.

[j] Glufosinate-ammonium (batch 13999) was administered by gavage to groups of NMRI mice (15 of each sex). The mice were killed at 24, 48 or 72 hours after administration of the test compound and negative control and at 24 hours after administration of the positive control. The frequencies of micronuclei in bone marrow polychromatic erythrocytes were determined. At 350 mg/kg bw, increased spontaneous activity, aggressiveness, tactile hyperaesthesia, motor excitation, uncoordinated gait, narrowed palpebral fissures and clonic convulsions were observed. At this dose, two females died. The study design generally resembles OECD Test Guideline No. 474; however, only 1000 immature erythrocytes per animal were scored for the incidence of micronuclei.

No mortality or clinical signs were observed. No significant reductions in body weight gain were observed during the premating period. During the first 2 weeks of the premating period, feed consumption was reduced by up to 19% in males at 2500 ppm (up to 14%) and 5000 ppm (up to 19%) and by up to 21% in females at 5000 ppm. During the lactation period, feed consumption was reduced by 22% in females of the 500 ppm group. This was considered to be related to the reduced number of pups per dam at this dose. No effects on mating performance or pregnancy rate were observed. Duration of pregnancy was not affected at 50 or 500 ppm. The dams at 2500 and 5000 ppm delivered no pups. At 500 ppm, the number of pups per litter (6.4) was markedly reduced compared with controls (12.7). No treatment-related postnatal loss of pups was found. Preimplantation loss was increased at 2500 and 5000 ppm, whereas post-implantation loss was increased at 500, 2500 and 5000 ppm. No effects of treatment on external malformations, sex ratio, development and behaviour of the offspring, body weight gain, feed consumption or organ weights were observed at 50 and 500 ppm. Necropsy revealed no treatment-related effect on parental rats.

Because of the total post-implantation loss observed in females at 2500 and 5000 ppm, a supplementary study was performed to clarify whether the effects on fertility were caused by the parent males or by the parent females. The males that had been treated continuously for 9 weeks at 0, 500, 2500 and 5000 ppm were mated with untreated mature females. No effect of treatment of males with glufosinate-ammonium at dietary concentrations up to 5000 ppm on mating performance, pregnancy rate, number of corpora lutea, rate of implantation and preimplantation and post-implantation loss were found.

The NOAEL for parental toxicity was 500 ppm (equal to 44 mg/kg bw per day), based on the reduced feed consumption in males at 2500 ppm (equal to 206 mg/kg bw per day). The NOAEL for offspring toxicity was 500 ppm (equal to 44 mg/kg bw per day, based on maternal intake), the highest dose at which dams produced a litter. With respect to reproduction, the NOAEL was 50 ppm (equal to 4.3 mg/kg bw per day), based on the reduced litter sizes (number of pups per dam) at 500 ppm (equal to 44 mg/kg bw per day) (Becker, 1986a).

In a two-generation dietary reproduction study, Wistar/Han rats (30 of each sex per group for the F_0 generation, 26 of each sex per group for the F_1 generation) were fed technical glufosinate-ammonium (purity 95.3%) at a dietary concentration of 0, 40, 120 or 360 ppm. The corresponding glufosinate-ammonium intakes during the different phases of the study are presented in Table 15.

The F_0 and F_1 generations were mated 2 times. Clinical examination of parental rats was performed daily and body weight was recorded weekly during the premating and gestation periods and on LDs 1, 4, 7, 14 and 21 (females). Feed consumption was recorded at the same time as the body weights, until day 14 postpartum. Rats were mated after 80 days of treatment (F_0, first mating) and about 10 days after weaning of the F_{1a} and F_{2a} generations (F_0 and F_1, second mating). Pups were weighed on LDs 1, 4, 7, 14 and 21. Twenty-six pups of the F_{1b} generation were selected for producing the F_{2a} and F_{2b} generations. F_{1b} rats were mated 101 days after weaning. All litters were examined (number of pups, sex of pups, number of stillbirths, number of live births, presence of gross anomalies). Necropsy was performed on F_0 rats, on one male and one female F_{1a} and F_{1b} pup per litter at LD 21 and on parental F_{1b} rats. The uterus was examined for metrial glands. In the control and high-dose groups, a full range of organs and tissues of the F_{1b} parental rats and one male and one female pup per litter of the F_{2b} generation was examined histologically, and selected organs from these rats were weighed. Statements of adherence to QA and GLP were included.

No effect of treatment was observed on viability, behaviour, clinical signs and general appearance, body weight and body weight gain, precoital time, pregnancy rate, duration of gestation, fertility, parturition, lactation or nursing. A significantly reduced feed consumption (up to 20%) was noted in the F_0 and F_1 parent females in the 360 ppm group during the lactation periods for breeding the F_{1a}, F_{1b}, F_{2a} and F_{2b} litters. This is considered to be related to the smaller litter size at this dose. No effect was seen at other time periods at this dose level or at 40 or 120 ppm. No effects were seen in males. At 360 ppm, litter size was significantly reduced (average of F_{1a}, F_{1b}, F_{2a} and F_{2b} is 8.5

pups/litter versus 11.2 pups/litter in controls). This finding corresponds to the result generated in the preliminary study. The decrease in pup number per litter in the high-dose group is considered by the study authors to reflect an interference during the period of implantation due to a toxic effect on early embryonic development. Non-dose-dependent increases in absolute (up to 14%) and relative (up to 15%) kidney weights were found at 120 ppm in male rats and at 360 ppm in male and female rats. The increases in kidney weights were not accompanied by gross pathological or histopathological changes and were therefore considered not to be adverse. Similar changes were also regularly observed in subchronic toxicity studies and should be considered as related to glufosinate-ammonium administration. No treatment-related changes were found in the F_{1a}, F_{1b}, F_{2a} and F_{2b} pups with respect to viability, postnatal loss, breeding loss, rate of malformations and/or anomalies, necropsy findings and organ weights. Macroscopic and histopathological examinations of the F_{1b} and F_{2b} generations revealed no effect of treatment.

Table 15. Glufosinate-ammonium intake in parental rats during different phases of the multigeneration reproductive toxicity study

Generation	Sex	Intake (mg/kg bw per day)		
		40 ppm	120 ppm	360 ppm
F_0 premating	Male	3.3	10	30
F_0 post-mating—F_{1a}	Male	2.0	6.8	20
F_0 post-mating—F_{1b}	Male	2.0	6.2	19
F_0 premating	Female	3.6	11	33
F_0 gestation—F_{1a}	Female	3.0	9.3	28
F_0 lactation—F_{1a}	Female	6.3	19	51
F_0 gestation—F_{1b}	Female	3.3	9.7	30
F_0 lactation—F_{1b}	Female	6.3	20	49
F_1 premating	Male	3.0	9.4	28
F_1 post-mating—F_{2a}	Male	2.0	6.7	19
F_1 post-mating—F_{2b}	Male	2.0	6.0	18
F_1 premating	Female	3.5	10	31
F_1 gestation—F_{2a}	Female	3.0	8.7	27
F_1 lactation—F_{2a}	Female	6.3	18	47
F_1 gestation—F_{2b}	Female	3.0	8.7	25
F_1 lactation—F_{2b}	Female	5.7	19	45

From Becker (1986b)

The NOAEL for parental toxicity was 360 ppm (equal to 18 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 360 ppm (equal to 18 mg/kg bw per day), the highest dose tested. With respect to reproduction, the NOAEL was 120 ppm (equal to 8.7 mg/kg bw per day, based on maternal intake during gestation), based on the reduced litter sizes (number of pups per dam) noted in all litters of both generations at 360 ppm (equal to 18 mg/kg bw per day) (Becker, 1986b).

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated orally, by gavage, with glufosinate-ammonium (purity 97.7%) in distilled water at a dose level of 0, 10, 50 or 250 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were

detected in the vaginal smear). The doses were based on the results of a range-finding study. Clinical signs and feed consumption were recorded daily. Body weight was measured weekly. Feed consumption was measured over 2-day periods. All females were killed on day 21 of gestation. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea and implantations were counted. Body weight, crown–rump lengths and sex ratios of the fetuses as well as placental weight and diameter of fetuses undergoing resorption were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

Clinical signs, such as motorial unrest, hyperactivity, piloerection, flabbiness, squatting and arching of the spine, were noted in dams at 50 and 250 mg/kg bw per day. The clinical signs started between days 9 and 11 and persisted for 3–5 days. Two treated dams from the 10 mg/kg bw per day group also presented signs of hyperactivity. However, this finding was not clearly treatment related and could not be repeated in a supplementary study. Therefore, it was considered not to be significant. Feed consumption was significantly reduced (19%) during days 14–17 of treatment in the 250 mg/kg bw per day group. Body weight gain was slightly reduced (up to 6% at GD 17) at 250 mg/kg bw per day. Feed consumption and body weight gain were not affected at 10 or 50 mg/kg bw per day.

All dams in the 10 mg/kg bw per day and control groups, 16 dams in the 50 mg/kg bw per day group and 10 dams in the 250 mg/kg bw per day group carried live fetuses to full term. Macroscopic examination of dams killed at GD 21 and their fetuses revealed no treatment-related effects. At the high dose, eight dams were killed following vaginal haemorrhage, first observed after four treatments, and one dam died on study day 17. At 50 mg/kg bw per day, four dams were killed following vaginal haemorrhage, first observed after three treatments. The vaginal haemorrhages probably are the result of abortions, as indicated by vacant implantation sites and the presence of conceptuses in the birth canal of some dams. The numbers of corpora lutea and implantations were not affected by treatment. The litters of the dams that carried live fetuses to full term were of the same sizes as those in the control group. The live fetuses in these litters in the treated groups were normally developed, with normal body weights and body lengths, and the male/female ratio was balanced.

In the dams that were sacrificed prematurely in the 50 and 250 mg/kg bw per day groups as a result of vaginal haemorrhage or dams that had only dead implantations in the uterus, relatively small spleens (0.1–0.17 g versus 0.61 ± 0.1 g in controls) and relatively large adrenals (0.1–0.14 g versus 0.072 ± 0.009 g in controls) were frequently observed.

The dam in the 250 mg/kg bw per day group that died on day 17 had 13 normally developed conceptuses. In the dams that were killed due to vaginal haemorrhages, the following uterine findings were recorded. Three early implantations in the birth canal were found in one animal of the 50 mg/kg bw per day group and one in an animal from the 250 mg/kg bw per day group. There were nine other normally developed early implantations in the uterus of each of these animals. One animal in the 50 mg/kg bw per day group and three in the 250 mg/kg bw per day group had only implantation sites without embryonic tissue. In one dam from the 50 mg/kg bw per day group and three dams from the 250 mg/kg bw per day group, only embryonic resorptions were found. One dam in the 50 mg/kg bw per day group, which was killed on day 20 of gestation, had only stunted, live fetuses with weights up to 1.15 g (premature delivery) and a supernumerary implantation site without embryonic tissue. One dam in the 250 mg/kg bw per day group, which was killed on day 19 of gestation, showed stunted fetuses, both live and dead, with weights up to 0.62 g. No remnants of aborted fetuses from any of the dams with vaginal haemorrhages were found in the cage litter. One dam in the 250 mg/kg bw per day group without vaginal haemorrhages delivered only stunted, dead fetuses and a supernumerary implantation site without fetal tissue on day 21 of gestation.

The placentas of the live fetuses showed no macroscopic abnormalities, and their weights were within the range of previous control values. The placentas of the dead fetuses from the dam in the 250 mg/kg bw per day group were smaller than those of the live fetuses in the other litters. No malformations were observed in any of the fetuses.

In the fetuses, a dose-related incidence of distension of the renal pelvis and/or ureter was found in all treatment groups (10%, 18.5%, 25.3% and 31.6% at 0, 10, 50 and 250 mg/kg bw per day, respectively), reaching statistical significance at the high dose. The maximum incidence of historical controls was 13.2%. A slight retardation of skeletal ossification of os metacarpale 5 was observed in the 250 mg/kg bw per day group. Incidences of other variations were not related to treatment.

The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on clinical signs and abortions observed at 50 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on intrauterine deaths and increased incidence of distension of the renal pelvis and/or ureter at 50 mg/kg bw per day. No evidence of a teratogenic effect was observed (Baeder, Weigand & Kramer, 1985a).

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated orally, by gavage, with glufosinate-ammonium (purity 97.2%) in distilled water at a dose level of 0, 0.5, 2.24 or 10 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were detected in the vaginal smear). Clinical signs and feed consumption were recorded daily. Body weight and feed consumption were measured on days 0, 7, 14, 17 and 21. All females were killed on day 21 of gestation. All dams were necropsied and examined macroscopically for abnormalities, and selected organs were weighed. The uterus was examined, and the numbers of live and dead fetuses, corpora lutea and implantations were counted. Body weight, crown–rump lengths and sex ratios of the fetuses as well as placental weight and diameter of fetuses undergoing resorption were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

No mortality or clinical signs were observed. The feed consumption and body weight gains of the dams were not affected by treatment. At terminal necropsy, no gross findings or organ weight changes were seen in the treated groups. All dams in all groups carried live fetuses to full term. Treatment had no effect on the numbers of corpora lutea, implantations, embryonic resorptions or live and dead fetuses per dam, fetal development, body weights, body lengths or sex ratio. The placentas of the fetuses showed no macroscopic abnormalities, and they were normal in weight. No effect of treatment on external anomalies or anomalies of the internal organs and skeleton was revealed.

The NOAEL for both maternal and fetal toxicity was 10 mg/kg bw per day, the highest dose tested (Baeder, Weigand & Kramer, 1985b; Hoerlein & Gorbach, 1985).

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated orally, by gavage, with glufosinate-ammonium (purity 97.2%) in distilled water at a dose level of 0, 0.5, 2.24 or 10 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were detected in the vaginal smear). Clinical signs and feed consumption were recorded daily. Body weight and feed consumption were measured on days 0, 7, 14, 17 and 21. All dams were allowed to deliver normally and to rear their offspring for 21 days (up to weaning). During the 21-day lactation period, body weights of the dams were recorded on days 0, 7, 14 and 21 postpartum. The offspring were examined daily for viability and general behaviour, and their body weights were recorded on days 4, 7, 14 and 21 after delivery. Their physical development was evaluated by recording the time of pinna separation, start of coat growth, incisor eruption and eyelid opening. Dams and offspring were killed between days 21 and 23 postpartum, dissected and examined macroscopically. Selected organs were weighed. Offspring with externally visible anomalies were stained and examined for skeletal anomalies. Statements of adherence to QA and GLP were included.

One dam in each of the 0.50 and 2.24 mg/kg bw per day groups had not littered by GD 25 and were killed. Examination of these females revealed that one dam had only empty implantation sites and the other dam had only two implantation sites and one dead, normally developed fetus. No mortality or clinical signs were observed. The feed consumption, body weight gains and gestation duration of the dams were not affected by treatment. Treatment did not affect the number of live offspring per litter, sex ratio, body weight at birth, offspring mortality, physical development and

body weight gain, general behaviour of the offspring and survival rate of the offspring at 21 days. At necropsy, no treatment-related external anomalies or organ weight changes were observed in dams and offspring.

The NOAEL for maternal and offspring toxicity was 10 mg/kg bw per day, the highest dose tested (Pensler et al., 1986).

Rabbits

In a developmental toxicity study, groups of 15 pregnant Himalayan rabbits were treated orally, by gavage, with glufosinate-ammonium (purity 95.3%) in distilled water at a dose level of 0, 2, 6.3 or 20 mg/kg bw per day from days 7 through 19 of gestation (day 0 = day of mating). The doses were based on the results of a range-finding study. Clinical signs were recorded daily. Body weight and feed consumption were measured weekly for the first 3 weeks and once again 9 days later. All females were killed on day 29 of gestation. All does were necropsied and examined macroscopically for abnormalities, and selected organs were weighed. The uterus was examined, the numbers of live and dead fetuses, corpora lutea and implantations were counted, and placental weight and diameter of fetuses undergoing resorption were recorded. The fetuses were weighed and reared for 24 hours in an incubator to assess viability. After this, the crown–rump lengths and sex ratios were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

At the high dose, a 115 g reduction in body weight was observed during the 1st week of treatment (GDs 7–14), while the control does gained 51 g. The mid-dose females gained 1 g during this period (not statistically significantly different from controls). Body weight gains of all groups were similar from GD 14 onwards. At termination, the body weight of high-dose rabbits was 94% of that of controls. During the 1st week of treatment, feed consumption was reduced at the middle and high doses (2.06 and 1.28 g/100 g bw, respectively) compared with controls (3.25 g/100 g bw). During the 2nd week of treatment, reduced feed consumption was observed only at the high dose (1.82 g/100 g bw versus 2.96 g/100 g bw in controls). A reduction in the quantity of faeces at the high dose is considered to be related to the decreased feed consumption.

A slightly higher kidney weight (11%) was found at 20 mg/kg bw per day. Three does at the high dose were killed prematurely: one doe displaying severe clinical signs was killed at GD 17 for humane reasons (necropsy revealed seven conceptuses and a resorption site), one doe aborted on GD 20 (necropsy revealed six live fetuses and one dead fetus) and one doe delivered prematurely on GD 25 (six dead fetuses and placentas were found). At termination, one doe at 20 mg/kg bw per day had seven implantation sites and two conceptuses under resorption in the uterus, but no live fetuses. At 6.3 mg/kg bw per day, one doe died on GD 29 while giving premature birth (necropsy revealed five placentas and five living and two dead fetuses). The numbers of abortions and premature deliveries are within the historical control range. All does in the control and 2 mg/kg bw per day groups carried live fetuses to full term. There was no difference in the numbers of corpora lutea and implantations or in the litter sizes and sex ratios in the treatment groups compared with the controls. The live fetuses delivered on day 29 of gestation in the treated groups were normally developed, with normal body weights and body lengths. The aborted and prematurely delivered fetuses, as well as those from the doe that was killed intercurrently, were normally developed for the stage at which gestation had been interrupted. The placentas of the dead fetuses were in most cases smaller than those of the live fetuses and were also anaemic.

No effect of treatment on the external development or internal organs and skeleton was revealed.

The NOAEL for maternal toxicity was 6.3 mg/kg bw per day, on the basis of clinical signs, body weight loss and reduced feed consumption, increased number of abortions and dead fetuses and increased kidney weight at 20 mg/kg bw per day. The NOAEL for fetal toxicity was 6.3 mg/kg bw per day, on the basis of an increased number of dead fetuses at 20 mg/kg bw per day (Baeder, Weigand and Kramer, 1984; Baeder, Mayer & Langer, 1986; Debruyne, 2003).

2.6 Special studies

(a) Neurotoxicity

Mice and rats

A single dose of glufosinate-ammonium (purity 95.3%) was administered by gavage to groups of five female NMRI mice and five female Wistar rats. Mice received 0, 50 or 200 mg/kg bw, whereas rats were dosed with 0, 200 or 800 mg/kg bw. Four hours after treatment, the animals were killed, and glutamine synthetase activity and the ammonium level were determined in brain, liver, kidneys and heart. In the rat, the glutamine and glutamic acid levels in these organs were also determined. Statements of adherence to QA and GLP were included.

No clinical signs were present during the 4-hour observation period. The results of glutamine synthetase activity measurements in the brain, liver, kidneys and heart of rats and mice 4 hours after oral administration are presented in Table 16.

Table 16. Effect of glufosinate-ammonium on glutamine synthetase activity in mice and rats

Dose (mg/kg bw)	Glutamine synthetase activity (± standard deviation)[a]			
	Brain	Liver	Kidneys	Heart
Female mice				
0	36.2 (± 0.8)	32.2 (± 1.1)	16.5 (± 0.7)	0.8 (± 0.2)
50	35.0 (± 0.6)	31.5 (± 1.6)	8.2* (± 0.3)	1.4* (± 0.1)
200	34.2 (± 0.9)	30.9 (± 0.8)	6.0* (± 1.2)	1.3 (± 0.2)
Female rats				
0	13.9 (± 0.3)	9.3 (± 0.7)	2.5 (± 0.1)	0.4 (± 0.02)
200	13.8 (± 0.4)	6.8 (± 1.1)	0.8* (± 0.1)	0.5 (± 0.05)
800	14.6 (± 0.2)	2.8* (± 0.8)	1.0* (± 0.2)	0.5 (± 0.04)

From Ebert & Kramer (1985b)

* $P < 0.05$ (according to the Dunnett test)

[a] Expressed in the table as micromoles of γ-glutamyl-hydroxamate formed per millilitre reaction mixture per 20 minutes at 37 °C.

Glufosinate-ammonium had no effect on glutamine synthetase activity in either brain or heart, even at high doses. Glutamine synthetase activities in the kidneys of both species and in the rat liver were markedly reduced compared with the corresponding control values.

No change in ammonium levels was observed in the brain, kidneys or heart of treated rats or mice. In the liver, a slight (14%) but statistically significant increase in ammonium level was detected only in mice at 200 mg/kg bw. Glutamine and glutamic acid levels in rat brain, liver, kidneys and heart showed treatment-related changes (Ebert & Kramer, 1985b).

Rats

In an acute oral neurotoxicity study, Wistar rats (10 of each sex per dose) were treated by gavage with glufosinate-ammonium 50% aqueous technical concentrate (purity 50.2%) in bi-distilled water at a dose of 0, 10, 100 or 500 mg/kg bw. Mortality and clinical signs were observed daily up to 15 days post-dosing. Feed consumption and body weights were recorded pretest and weekly during the observation period. Functional observational battery, locomotor activity, body temperature, rearing, landing foot splay and grip strength were measured pretest and at 1, 7 and 14 days after application. All animals were killed on test day 15 and examined macroscopically. Statements of adherence to QA and GLP were included.

No mortality was observed. Hunched posture, tachypnoea, ruffled fur and emaciation were observed in one high-dose female on the 2nd and 3rd days after treatment. This animal showed ptosis, tachypnoea, hunched posture and piloerection in the functional observational battery test 1 day after application. No other treatment-related findings were noted during the functional observational battery tests. Feed consumption, body weights, locomotor activity, grip strength, body temperature, rearing and landing foot splay were unaffected by treatment.

The NOAEL was 100 mg/kg bw, based on clinical signs observed in the home cage and in the functional observational battery in one animal at 500 mg/kg bw (Hamann, 1999a).

In an acute oral neurotoxicity study, Wistar rats (10 of each sex per dose) were treated by gavage with glufosinate-ammonium 50% aqueous technical concentrate (purity 50.2%) in bi-distilled water at a dose of 0, 10, 100 or 500 mg/kg bw. Mortality and clinical signs were observed daily up to 15 days post-dosing. Feed consumption and body weights were recorded pretest and weekly during the observation period. Water maze tests evaluating learning, memory and relearning skills were performed with all animals pretest and at 1, 7 and 14 days after application. All animals were killed on test day 15 and examined macroscopically. Histological examinations were performed on the brain, the spinal cord and the sciatic tibial nerves from all animals after perfusion fixation. Statements of adherence to QA and GLP were included.

No mortality or clinical signs were noted. Body weight and feed consumption were not affected by treatment. No treatment-related neurotoxic effects were observed with the water maze test. Macroscopic and histopathological examinations revealed no effect of treatment with glufosinate-ammonium.

The NOAEL was 500 mg/kg bw, the highest dose tested (Hamann, 1999b).

Groups of 10 male and 10 female Wistar rats were given glufosinate-ammonium aqueous technical concentrate (purity 50.2%) at a dietary level of 0, 20, 200 or 2000 ppm (equal to 0, 1.5, 15 and 143 mg/kg bw per day for males and 0, 1.8, 17 and 162 mg/kg bw per day for females, respectively) for up to 38 days. An additional five rats of each sex were used at each dose level for determining glutamine synthetase activity in the liver, kidney and brain at the end of the treatment period. The animals were observed daily for clinical signs, whereas body weight and feed consumption were recorded weekly. A functional observational battery, locomotor activity, body temperature, landing foot splay distance and grip strength were measured pretest and after 2 and 4 weeks of treatment. A water maze test was performed pretest and in week 5. A single dose of midazolam (2 mg/kg bw) given intraperitoneally before or 10 minutes after the water maze test was used as a positive control. At termination, all the animals were killed and examined macroscopically. The brain, heart, liver and kidneys were weighed, and histopathological examination was performed on the brain, spinal cord, and sciatic and tibial nerves of all the control and high-dose animals. Statements of adherence to QA and GLP were included.

No treatment-related mortality or clinical signs were observed. Body weight gain, feed consumption, functional observational battery parameters, locomotor activity, rearing, grip strength, body temperature, landing foot splay, or learning and memory tests (water maze) were unaffected by treatment with glufosinate-ammonium.

In animals receiving glufosinate-ammonium, a statistically significant dose-related inhibition of glutamine synthetase activity was observed in the liver of both sexes and the kidney of males only at all dose levels. Inhibition of glutamine synthetase activity was also observed in the brain tissue of males at 200 ppm and in both sexes at 2000 ppm (see Table 17). A greater than 50% reduction of glutamine synthetase activity in the liver and/or a greater than 10% reduction of glutamine synthetase activity in the brain are considered to be adverse. In the absence of histopathological findings in the kidney, the reduction in glutamine synthetase activity was considered to be a non-adverse finding.

Table 17. Mean glutamine synthetase activity following 38 days of treatment with glufosinate-ammonium in rats

Organ	% of control activity					
	Males			Females		
	20 ppm	200 ppm	2000 ppm	20 ppm	200 ppm	2000 ppm
Liver	67**	44**	35**	77*	55**	37**
Kidney	75**	64**	65**	98	93	94
Brain	96	93*	75**	102	100	73**

From Hamann et al. (2000)
* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

Relative kidney weight was 15% increased in high-dose males. No treatment-related macroscopic or microscopic abnormalities were seen in any of the animals.

The NOAEL for glufosinate-ammonium was 20 ppm (equal to 1.5 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver in males at 200 ppm (equal to 15 mg/kg bw per day) (Hamann et al., 2000).

The effect of subchronic treatment with glufosinate-ammonium on the activity of glutamine synthetase was investigated in liver, kidneys and brain. Glufosinate-ammonium (supplied as an aqueous technical concentrate; concentration 50.2% weight per weight [w/w]) was administered in the diet to groups of 10 male Wistar rats for 6, 13, 20 or 90 days at a dose of 100 or 1000 ppm. Additional groups of 10 male Wistar rats received glufosinate ammonium at a dose of 100 or 1000 ppm for 91 days followed by a 30-day recovery period. Control groups of 10 male Wistar rats received the untreated diet for the same period of time. Achieved nominal intakes of glufosinate-ammonium were 6.2 and 63.6 mg/kg bw per day at 100 and 1000 ppm, respectively. Mortality and clinical signs were recorded daily, and feed consumption and body weights were measured weekly. All animals were necropsied at scheduled sacrifice. Organ weights (brain, liver and kidney) were measured, and all macroscopic findings were recorded. Samples of liver, kidney and brain were collected from all animals and processed for measurement of glutamine synthetase activity. Statements of adherence to QA and GLP were included.

No treatment-related effects were noted for clinical signs, feed consumption or body weight.

Glufosinate-ammonium induced a significant inhibition of glutamine synthetase activity in the liver and kidney (see Table 18). In the brain, only a slight inhibition was observed at the high dose of glufosinate-ammonium. No cumulative effect over time was observed. The effects were (almost) completely reversed after a 30-day recovery period.

There were no treatment-related macroscopic findings or effects on liver and brain weights. Necropsy at 6, 13 or 20 days showed an increase in absolute and relative kidney weights in rats treated with glufosinate-ammonium (up to 23%) that was fully reversible after 30 days of recovery.

The NOAEL for glufosinate-ammonium was 100 ppm (equal to 6.2 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver and a greater than 10% reduction in glutamine synthetase activity in the brain at 1000 ppm (equal to 63.6 mg/kg bw per day) (Schmid et al., 1999).

Table 18. Inhibition of glutamine synthetase activity in the rat by glufosinate-ammonium

Tissue	Sacrifice after day:	% inhibition	
		100 ppm	1000 ppm
Liver	6	45**	64**
	13	49**	70**
	20	49**	67**
	90	40**	60**
	Recovery	3	15*
Brain	6	0	11**
	13	0	9**
	20	0	15**
	90	0	18**
	Recovery	2	12**
Kidney	6	40**	42**
	13	39**	42**
	20	38**	47**
	90	33**	54**
	Recovery	10	3

From Schmid et al. (1999)
* $P < 0.05$; ** $P < 0.01$ (according to the Dunnett test based on pooled variance)

In a dietary developmental neurotoxicity study, glufosinate-ammonium (purity 50.8%) was administered to 25 mated female Crl:C (SD) IGS BR rats at 0, 200, 1000 or 4500 ppm (equal to 0, 14, 69 and 292 mg/kg bw per day during gestation and 0, 36, 176 and 756 mg/kg bw per day during lactation, respectively). All animals were observed daily for mortality and clinical signs. A detailed clinical examination of the dams was performed on GDs 6 and 13 and on postnatal days (PNDs) 10 and 21. Body weights and feed consumption were recorded daily during the treatment period. After parturition, the numbers of dead and live pups were recorded, and the pups were sexed and examined for malformations. Females that did not deliver or with total litter loss were killed and examined macroscopically. On PND 4, litters were culled to 10 pups per litter. Females with litters that consisted of fewer than eight pups or fewer than four pups of each sex were killed on LD 5 and examined macroscopically. All remaining females with viable pups were killed after PND 21 and examined macroscopically. Litters were examined daily for clinical signs. On PNDs 4, 11 and 21 and weekly thereafter, a detailed physical examination of the pups was performed until termination on PND 72. The 1st day of balanopreputial separation and vaginal patency was recorded for all males and females, respectively. Twenty selected pups of each sex per group (subset A) were subjected to a detailed clinical examination on PNDs 4, 11, 21, 35, 45 and 60, acoustic startle response test on PNDs 20 and 60, locomotor activity tests on PNDs 13, 17, 21 and 61 and learning and memory test (water maze) on PND 62. From this subset, 10 pups of each sex per group were selected for neuropathological, morphometric and brain weight examinations on PND 72. A second subset (B) of 20 pups of each sex per group was subjected to the learning and memory test on PND 22. A third subset (C) of 10 pups of each sex per group was selected for neuropathological, morphometric and brain weight examinations on PND 21. All non-selected pups were killed and examined macroscopically on PND 28. Statements of adherence to QA and GLP were included.

No mortality was observed. In the high-dose group, light-coloured faeces were observed in dams primarily between GD 8 and GD 13. In this group, statistically significant reductions in mean maternal body weight gains (8% on GD 20) and feed consumption (17% between GD 6 and GD 20) were noted. On the 1st day of treatment, all treatment groups showed a dose-dependent body weight

loss (1–6 g), while control animals gained 4 g. The reduction in body weight gain in the low- and mid-dose groups was observed during the first 4–5 days of treatment. Feed consumption was also dose-dependently decreased in the low- and mid-dose groups during the first 4–5 days of treatment and in the high-dose dams throughout the treatment period. The initial body weight and feed consumption effects were attributed to the decreased palatability of the test diet at the initiation of the diet administration and are not considered to be a systemic effect of glufosinate-ammonium. No treatment-related clinical observations were noted for dams on GDs 6 and 13 and PNDs 10 and 21. Mean gestation length, gestation index, mean number of pups born, mean live litter size and sex ratio per litter were unaffected by glufosinate-ammonium treatment. At scheduled termination, necropsy of the dams revealed no effects of treatment. The mean number of implantation sites, number of pups born and numbers of unaccounted sites recorded at scheduled necropsy were unaffected by treatment. No macroscopic findings were noted at the scheduled necropsy of F_0 females on LD 21.

No clinical signs of toxicity were observed in treated pups. Slight, but non-statistically significant, reductions in mean body weights of male and female offspring (7% and 6% for males and females, respectively) were noted in the 4500 ppm group on PND 1. Body weights were statistically significantly reduced by 18–19% on PND 21 at this dose. At 1000 ppm, body weights were about 9–10% lower than those of controls during the period up to weaning, occasionally reaching statistical significance. During the period up to PND 70, body weights in the high-dose group were 6–9% lower than those of controls. Total motor activity was increased in high-dose pups at PND 17 and in both sexes of the mid- and high-dose groups at PND 21. At PND 61, total motor activity of males and females was slightly increased at 1000 and 4500 ppm, either during part of the 60-minute locomotor activity test or over the total duration of the test. Patterns of habituation were comparable between treatment and control groups. No treatment-related effects were observed on acoustic startle or learning and memory tests. Histological examination of the brains of pups revealed a significant decrease in vertical height between the layers of pyramidal neurons in the hippocampal formation in males (11%) and in radial thickness of the cortex in females (8%) of the high-dose group. The decrease (7%) in vertical height between the layers of pyramidal neurons in the hippocampal formation in mid-dose males was not statistically significant.

The NOAEL for maternal toxicity was 1000 ppm (equal to 69 mg/kg bw per day), based on decreased body weight gain and feed consumption at 4500 ppm (equal to 292 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 14 mg/kg bw per day), based on reduced body weight gain during the preweaning period, effects on motor activity at PNDs 17, 21 and 62 and the decrease in vertical height between the layers of pyramidal neurons in the hippocampal formation in males at 1000 ppm (equal to 69 mg/kg bw per day) (Nemec, 2004).

Glufosinate-ammonium was administered once orally by gavage to groups of 5–10 female Wistar rats at 0, 200, 800 or 1600 mg/kg bw. After treatment, mortality rates and clinical signs were recorded for 7.5 days. The animals were also subjected regularly to a comprehensive observational assessment. The surviving animals were weighed and subsequently killed and dissected 24 hours or 3 or 7.5 days after dosing. Glutamine synthetase activity and glutamate and ammonium levels were measured in the brain, liver and kidneys. Acetylcholinesterase activity was also measured in the brain. Statements of adherence to QA and GLP were included.

In the high-dose group (1600 mg/kg bw), three animals died. Clinical signs in the 200 mg/kg bw group consisted of enhanced spontaneous activity and isolated piloerection on day 1 after treatment. At 800 mg/kg bw, frequent enhancement of spontaneous activity and, in one case, convulsions and Straub tail from 12 to 24 hours after treatment were observed. At 1600 mg/kg bw, diarrhoea, convulsions, enhancement of spontaneous activity and piloerection were observed, starting 6 hours after treatment. Additionally, 2–3 days after treatment, tonoclonic convulsions, squatting position, contracted flanks, lagophthalmos, drowsiness, reduced respiratory rate and blood-encrusted eyelids and mouth were observed. The comprehensive observational assessments of the animals in the 200 and 800 mg/kg bw groups revealed marginal impairment of behaviour in the form of reduced motivation and performance, which began to recede quite noticeably from day 5 onwards. In the high-

dose group, a tendency to convulsions and spasms followed by exhaustion was observed, starting 1.5 days after treatment All signs of intoxication began to disappear 3.5 days after treatment.

The effect of glufosinate-ammonium on organ glutamine synthetase activity is presented in Table 19.

Table 19. Effect of glufosinate-ammonium on glutamine synthetase activity in various organs

Dose (mg/kg bw)	Glutamine synthetase activity					
	1 day after treatment		3 days after treatment		7.5 days after treatment	
	Mean (± SD) (mg/g tissue in 20 min)	% change relative to control	Mean (± SD) (mg/g tissue in 20 min)	% change relative to control	Mean (± SD) (mg/g tissue in 20 min)	% change relative to control
Brain						
0	65 (± 4.1)	—	51.5 (± 2.9)	—	45 (± 2.6)	—
200	61 (± 9.8)	−6	49 (± 1.6)	−5	43.5 (± 5.3)	−3
800	56 (± 6.3)	−14	46 (± 3.3)	−11	46 (± 1.4)	−12
1600	44* (± 5.4)	−32	26.5^{+} (± 10.8)	−49	32^{+} (± 4.3)	−29
Liver						
0	38 (± 6.3)	—	25.5 (± 3.0)	—	52 (± 9.0)	—
200	10* (± 1.1)	−74	13.5* (± 1.5)	−47	29* (± 2.9)	−44
800	10.5* (± 1.7)	−72	9* (± 1.9)	−65	29* (± 4.4)	−44
1600	8* (± 1.2)	−79	7* (± 2.0)	−73	25* (± 2.6)	−52
Kidneys						
0	17 (± 1.5)	—	23 (± 0.7)	—	15 (± 1.5)	—
200	8* (± 1.2)	−53	13 (± 0.8)	−43	14 (± 0.4)	−7
800	6* (± 0.6)	−65	10.5^{+} (± 0.8)	−54	14 (± 1.3)	−7
1600	4.5* (± 0.4)	−74	9.5^{+} (± 1.0)	−59	16 (± 1.1)	+5

From Ebert et al. (1986a)
SD, standard deviation; * $P < 0.05$ (procedure of Dunnett); $^{+}$ $P < 0.05$ (procedure of Nemenyi/Dunnett)

Treatment with glufosinate-ammonium did not affect the levels of ammonium in liver, kidneys or brain.

The glutamate levels in brain were reduced in the mid-dose (11–18%) and high-dose groups (20–26%). Brain levels were lower throughout the test period. An increase in the liver glutamate level was observed in the high-dose group, especially on the 1st day after treatment. On days 1 and 3, kidney glutamate levels were slightly increased (up to 21%) at 800 and 1600 mg/kg bw. Brain acetylcholinesterase levels were not affected by glufosinate-ammonium treatment.

No NOAEL could be identified. The LOAEL was 200 mg/kg bw, the lowest dose tested, based on clinical signs (enhanced spontaneous activity and isolated piloerection on day 1 after treatment) and a greater than 50% reduction in glutamine synthetase activity in liver (Ebert et al., 1986a).

Groups of 40 male and 40 female Wistar rats were administered glufosinate-ammonium (batch Lfd 13143; purity 96.9%) via the diet over 28 days at a concentration of 0, 40, 200, 1000 or 5000 ppm (equal to 0, 3.7, 19, 93 and 443 mg/kg bw per day for males and 0, 3.6, 18, 89 and 424 mg/kg bw per day for females, respectively). At the end of 28 days, 10 male and 10 female rats per

dose group were allocated to each of four subgroups corresponding to recovery periods of 0, 3, 7 or 28 days. Animals were observed daily for mortality/viability and clinical signs. Detailed clinical examinations were performed weekly, including a comprehensive observational assessment for neurological disturbance. Males and females in the 0, 1000 and 5000 ppm groups were subjected to neurobehavioural assessments weekly through the treatment period and during the first 2 weeks of recovery. Body weight and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. At the end of each recovery period, all animals were killed, and livers, kidneys and brain (left and right hemispheres) were removed for examination and weighing. The catecholamine levels in the brain (five rats of each sex per group), glutamine synthetase activity (five rats of each sex per group), ammonium levels (five rats of each sex per group) and amino acid levels (five rats of each sex per group) in brain, liver and kidneys, and enzyme activity in the liver were investigated. Statements of adherence to QA and GLP were included.

During the 1st week of treatment, there was a temporary reduction in feed consumption in both sexes and a slight retardation of body weight gain in male rats in the highest-dose group. The highest-dose males showed a slight increase in water consumption throughout the whole treatment period. The comprehensive observational assessments revealed faint signs of central nervous system excitation together with reduced body temperatures (males) up to day 18 of treatment in the animals treated at 5000 ppm, but these subsided rapidly afterwards. Macroscopic examination revealed no abnormalities. A marginal increase in kidney weights at the end of treatment in the females of the highest-dose group was noted. Examination of the catecholamine transmitters revealed only a slight lowering of dopamine in females in the highest-dose group, which was no longer apparent after 3 days of recovery. There were no changes in the cerebral levels of glutamate or any other amino acids with excitatory or inhibitory neurotransmitter function. A statistically significant dose-related inhibition of glutamine synthetase activity was found in the liver (both sexes) and kidneys (males only) from 200 ppm onwards and in the brain at 5000 ppm (males only). In all cases, definite signs of reversibility were seen 7 days after termination of treatment, and there was no effect detectable at the end of the 4-week recovery period (Table 20). The inhibition of glutamine synthetase led to glutamine depletion in the affected organs, but this effect was no longer in evidence following a 3-day recovery period (Table 21).

No indications of increased glutamate or ammonium levels, a possible hypothetical consequence of glutamine inhibition, were found in any organ. Enzyme biochemistry in the liver indicated no effects on any parameters in connection with either energy and carbohydrate metabolism or glutathione metabolism (e.g. glutathione depletion). Glufosinate-ammonium did not affect alanine aminotransferase (ALT), AST or glutamate dehydrogenase or the levels of free glutamate, asparagine, aspartate or alanine in liver samples. The effects were largely reversible after 7 days of recovery and fully reversed to normal values after 28 days.

The NOAEL was 200 ppm (equal to 18 mg/kg bw per day), based on a greater than 50% inhibition of glutamine synthetase in the liver at 1000 ppm (equal to 89 mg/kg bw per day) (Ebert et al., 1986b).

Dogs

In an acute toxicity test, a single oral dose of glufosinate-ammonium (purity 95.3%) was administered by gavage to Beagle dogs (two of each sex per dose level) at 400 or 350 mg/kg bw (10% solution in a 4% carboxymethylcellulose vehicle). In addition to the standard examinations for such an acute toxicity study, a neurological examination was performed. Statements of adherence to QA and GLP were included.

All dogs, except one male, died. Glufosinate-ammonium caused clonic and tonic convulsions followed by post-epileptic inactivity. The convulsions had a delayed onset, with the maximum toxicity appearing approximately 24 hours following treatment. Short periods of stereotypic behaviour were also noted. Postural and phasic reflexes were attenuated rather than enhanced. Marked miosis preceded death in four of eight dogs. Anisocoria was observed for one female treated with 350 mg/kg bw. Death probably resulted from cardiovascular depression. Death from respiratory failure cannot be excluded (Sachsse, 1986b).

Table 20. Effect of glufosinate-ammonium on glutamine synthetase activity in the rat

Organ	Recovery (days)	Sex	Glutamine synthetase activity (mean ± standard deviation)[a]				
			0 ppm	40 ppm	200 ppm	1000 ppm	5000 ppm
Liver	0	Male	5.9 (± 0.47)	5.2 (± 0.36)	4.1*** (± 0.78)	2.9*** (± 0.31)	2.9*** (± 0.33)
		Female	5.2 (± 0.52)	5.4 (± 0.34)	3.2** (± 0.31)	2.1** (± 0.39)	1.8** (± 0.36)
	3	Male	5.9 (± 0.26)	5.7 (± 0.23)	4.2* (± 0.26)	3.4* (± 0.41)	2.6* (± 0.21)
		Female	5.8 (± 0.26)	5.3 (± 0.26)	3.3* (± 0.23)	2.3* (± 0.26)	2.6* (± 0.26)
	7	Male	5.7 (± 0.7)	5.7 (± 0.26)	4.7* (± 0.23)	4.4* (± 0.21)	4.1* (± 0.47)
		Female	5.4 (± 0.52)	6.0 (± 4.6)	4.6* (± 0.18)	4.0* (± 0.67)	3.8* (± 0.49)
	28	Male	6.1 (± 0.11)	—	5.3 (± 0.39)	5.3 (± 0.34)	5.9 (± 0.52)
		Female	5.7 (± 0.16)	—	5.7 (± 0.41)	5.5 (± 0.36)	5.3 (± 0.41)
Kidney	0	Male	3.9 (± 0.47)	3.6 (± 0.34)	3.5 (± 0.62)	3.1 (± 0.46)	3.0* (± 0.31)
		Female	1.6 (± 0.18)	1.8 (± 0.26)	1.8 (± 0.26)	1.9 (± 0.31)	1.8 (± 0.26)
	3	Male	4.1 (± 0.26)	4.0 (± 0.21)	3.1* (± 0.23)	3.0* (± 0.23)	2.6* (± 0.21)
	7	Male	5.2 (± 0.37)	3.8* (± 0.26)	4.1* (± 0.39)	3.4* (± 0.21)	3.3* (± 0.21)
	28	Male	4.6 (± 0.44)	4.2 (± 0.26)	4.1 (± 0.34)	4.0 (± 0.34)	4.4 (± 0.41)
Brain	0	Male	3.6 (± 0.52)	3.9 (± 0.37)	3.6 (± 0.31)	3.4 (± 0.44)	2.1* (± 0.34)
		Female	3.4 (± 0.34)	3.9 (± 0.41)	3.1 (± 0.31)	3.6 (± 0.6)	3.1 (± 0.49)
	3	Male	3.5 (± 0.26)	3.4 (± 0.16)	3.4 (± 0.16)	3.3 (± 0.18)	2.6* (± 0.26)
	7	Male	4.1 (± 0.49)	—	—	—	3.6 (± 0.48)
	28	Male	3.4 (± 0.16)	—	—	—	3.7 (± 0.39)

From Ebert et al. (1986b)

* $P < 0.05$ (according to Dunnett); ** $P < 0.05$ (according to Nemenyi/Dunnett); *** $P < 0.05$ (according to Sidak)

[a] Expressed as milligrams of γ-glutamylhydroxamate per gram tissue.

Table 21. Effect of glufosinate-ammonium on glutamine level in males

Organ	Recovery (days)	Glutamine level (mean ± standard deviation) (mg/g amino acid)				
		0 ppm	40 ppm	200 ppm	1000 ppm	5000 ppm
Liver	0 c, d	2.778 (± 0.497)	2.460 (± 0.561)	2.090* (± 0.355)	1.825* (± 0.225)	1.082* (± 0.320)
	3 a, d	2.116 (± 0.592)	2.404 (± 0.535)	2.276 (± 0.178)	2.092 (± 0.184)	2.052 (± 0.419)
Kidney	0 c, d	0.714 (± 0.357)	0.594 (± 0.413)	0.624 (± 0.392)	0.550 (± 0.285)	0.448* (± 0.156)
	3 b	0.328 (± 0.052)	0.322 (± 0.033)	0.232 (± 0.063)	0.252 (± 0.044)	0.350 (± 0.044)
Brain	0 e	2.794 (± 1.282)	2.464 (± 1.174)	1.720 (± 0.955)	1.364 (± 0.555)	1.264* (± 0.592)
	3 d	2.958 (± 0.629)	2.956 (± 0.63)	2.606 (± 0.742)	2.444 (± 0.728)	2.432 (± 0.636)

From Ebert et al. (1986b)

* $P < 0.05$; a: significant according to the parametric procedure of Dunnett; b: significant according to the distribution-free procedure of Nemenyi/Dunnett; c: significant according to the parametric procedure of Sidak; d: significant according to the parametric procedure of Dunnett (one-sided for decrease); e: significant according to the distribution-free procedure of Nemenyi/Dunnett (one-sided for decrease)

(b) Mechanistic studies

Mice

In a published study, the role of glutamine synthetase (GS) in early mouse embryogenesis was examined, because glutamine synthetase is expressed in a tissue-specific and developmentally controlled manner, functions to remove ammonia and glutamate and is the only enzyme that can synthesize glutamine, and because congenital deficiency of glutamine synthetase has not been reported. Because glutamine synthetase is expressed in embryonic stem cells, a null mutant mouse was generated by replacing one glutamine synthetase allele in-frame with a β-galactosidase-neomycin fusion gene. $GS^{+/LacZ}$ mice were phenotypically normal and fertile, but $GS^{LacZ/LacZ}$ mice died at embryonic day (ED) 3.5, demonstrating that glutamine synthetase is essential in early embryogenesis. Cells from ED 2.5 $GS^{LacZ/LacZ}$ embryos survive in vitro in glutamine-containing medium. Chimeric embryos constructed by injecting wild-type +/+ blastocysts with +/− or −/− cells were viable, although the glutamine synthetase–deficient cells showed a reduced fitness in chimera analysis. This indicates that the wild-type cells in the blastocyst were partly able to compensate for glutamine synthetase–deficient cells. In contrast, maternal glutamine synthetase activity could not compensate for glutamine synthetase–deficient cells. The survival of heavily (> 90%) chimeric mouse embryos up to at least ED 16.5 indicated that, after implantation, glutamine synthetase activity is not essential until at least the fetal period. The study authors hypothesized that glutamine synthetase–deficient embryos die when they move from the uterine tube to the harsher uterine environment, where the embryo has to catabolize amino acids to generate energy and, hence, needs to detoxify ammonia, two processes that require glutamine synthetase activity (He et al., 2007).

In vitro exposure of 8-day-old mouse embryos, micromass cultures in midbrain and limb bud cells to glufosinate-ammonium for 48 hours caused significant overall embryonic growth retardation and increased embryolethality to 37.5% at 10 µg/ml (5.0×10^{-5} mol/l). All embryos in the treated groups exhibited specific morphological defects, including hypoplasia of the prosencephalon (forebrain) (100%) and visceral arches (100%). In 10-day-old embryos cultured for 24 hours, glufosinate reduced the crown–rump length and the number of somite pairs and produced a high incidence of morphological defects (84.6%) at 10 µg/ml. In micromass culture, glufosinate-ammonium inhibited the differentiation of midbrain cells in day 12 embryos (Watanabe & Iwase, 1996).

In vitro exposure of mouse embryos to glufosinate-ammonium induced chromatin condensation and segregation, extracellular apoptotic bodies and cell fragments phagocytosed in macrophages in the neuroepithelium of the brain vesicle and neural tube (Watanabe, 1997).

Rats

In a study from the public literature, 7-day-old female Wistar-Immamichi rats (6–11 per group) received subcutaneous injections of glufosinate-ammonium at 0, 1, 2 or 5 mg/kg bw per day for 7 days. At 5 or 6 weeks of age, all the treatment groups showed a non-dose-dependent decreased frequency of kainic acid–induced wet-dog shakes. At 5 mg/kg bw per day, a reduction in body weight was found. The study suggests that glufosinate-ammonium treatment during the infantile period in the rat induces alterations in the kainin receptor in the brain (Fujii, Ohata & Horinaka, 1996).

In a study from the public literature, glufosinate-ammonium (30–3000 nmol/10 µl, purity unknown) was administered in vivo in the rat (Fischer 344) cerebellum through the microdialysis probe at a rate of 1 µl/minute for 10 minutes. Glufosinate-ammonium stimulated nitric oxide production, which was suppressed by an inhibitor of nitric oxide synthase and was antagonized by *N*-methyl D-aspartate (NMDA) receptor antagonists. These results suggest that glufosinate-ammonium stimulates nitric oxide production through NMDA receptors (Nakaki et al., 2000).

The effect of intravenous or intracerebroventricular injection of glufosinate-ammonium on catecholamine levels and glutamine synthetase activity in the brain was investigated in male Wistar rats.

In a preliminary study, groups of two animals each received intracerebroventricular injections of 10 or 20 µg of either glufosinate-ammonium (batch Lfd 13143; purity 96.9%) or MPP (batch Lfd 12956; purity > 99%) and were kept under observation for up to 24 hours.

After this preliminary study, groups of six animals received intracerebroventricular injections of 10 or 20 µg glufosinate-ammonium, 20 µg MPP or 10 µl physiological saline. The animals were killed 3 hours after treatment and the brains removed for determination of catecholamine (noradrenaline, dopamine and dihydroxyphenyl acetic acid) levels and glutamine synthetase activity. A parallel study was conducted with intravenous injections of glufosinate-ammonium at 0, 10 and 100 mg/kg bw in three groups of five rats each. The animals were killed 2 hours after injection, brains were dissected and catecholamine levels and glutamine synthetase activity were measured.

In the preliminary study, intracerebroventricular injection of 20 µg of glufosinate-ammonium resulted 3.5–4 hours later in sustained (> 24 hours) convulsions, which were to some extent successfully antagonized by diazepam (10 mg/kg bw intraperitoneally). From 10 minutes to 2 hours after intracerebroventricular injection of 10 µg glufosinate-ammonium, clonic spasms of forelimbs and opisthotonus were noted in one out of the two treated animals; these clinical signs disappeared rapidly after injection of diazepam. After intracerebroventricular injection of 20 µg MPP, relatively slight spasms of the forelimbs and opisthotonus were briefly observed 5 and 30 minutes after injection, respectively, in only one out of two animals. No clinical signs were observed 1 or 24 hours after injection or in the other animal at the same dose level.

In the main study, catecholamine determination on various cerebral regions—frontal cortex, striatum and hippocampus—revealed a 63% higher dihydroxyphenyl acetic acid concentration in the striatum and a 31% lower noradrenaline concentration on the frontal cortex 3 hours after intracerebroventricular injection of 20 µg of glufosinate-ammonium. Following 10 µg of glufosinate-ammonium and 20 µg of MPP, there were no changes in the catecholamine concentrations in any of the cerebral regions examined. There were also no changes in the catecholamine concentrations in any of these cerebral regions 2 hours after intravenous injection of glufosinate-ammonium at 10 or 100 mg/kg bw.

After intravenous injection, only one out of five animals from the highest dose tested (100 mg/kg bw) showed a decreased glutamine synthetase activity. When administered by intracerebroventricular injection, glufosinate-ammonium induced a dose-related inhibition of glutamine synthetase activity. Intracerebroventricular injection of MPP at the same high dose as glufosinate-ammonium (20 µg/animal) did not produce any inhibition of glutamine synthetase activity, and no clinical signs of intoxication were observed in any of the animals treated with MPP (Gerhards, Koecher & Ulm, 1986).

In a supplemental study, the sensitivity of the Fischer rat strain with regard to the inhibition of glutamine synthetase activity in the liver was investigated. Groups of 10 male and 10 female Fischer 344 rats were administered glufosinate-ammonium (purity 92.1%) via the diet over 90 days at a concentration of 0, 8, 64, 500 or 4000 ppm. Five rats of each sex per group were killed at the end of the 13-week study period, and five animals of each sex per group were killed after an additional 4-week post-dosing recovery period. At termination, serum and liver samples were obtained for measurement of liver glutamine synthetase activity and serum ammonia concentration.

Glutamine synthetase activity was statistically significantly inhibited at the end of the 13-week treatment period in the liver of both sexes from the 4000 ppm group and from the 500 ppm females. After the 4-week recovery phase, no inhibition was noted. Serum ammonia levels were not affected by treatment (Ohashi, Nakayoshi & Abe, 1982).

Glufosinate-ammonium (purity 96.9%) was tested in various in vitro receptor binding assays for γ-aminobutyric acid, noradrenaline (NA-α2 and NA-β), dopamine and serotonin (5-HT1 and 5-HT2). The affinity of glufosinate-ammonium for the benzodiazepine-binding site and the calcium ion channel was also investigated.

Glufosinate-ammonium (1 µmol/l) caused no displacement of the ^{3}H ligands from the receptor in any of the investigated receptor systems (Schacht, 1986).

The effect of glufosinate-ammonium (purity 99.5%) on the substrates of the Krebs cycle in isolated rat liver mitochondria and the oxidative phosphorylation of intact mitochondria was investigated.

No indication that glufosinate-ammonium affects oxidative metabolism in mitochondria was found. It was concluded that glufosinate-ammonium had no direct influence on the metabolism of intact rat liver mitochondria, or glufosinate-ammonium had not been taken up by these organelles (Metzger, 1986).

In a study in vitro, glufosinate-ammonium (10 mmol/l) did not inhibit the activity of ALT, AST, glutamate dehydrogenase or γ-glutamyl transpeptidase (Koecher, 1986).

In vitro, phosphinothricin, the free acid form of glufosinate-ammonium, was found to be a strong inhibitor of mammalian glutamate decarboxylase, which converts glutamate to γ-aminobutyric acid (Lacoste et al., 1985).

In in vitro tests, phosphinothricin, the free acid form of glufosinate-ammonium, as well as other analogues of glutamic acid were shown to be effective inhibitors of glutamine synthetase, acting competitively against L-glutamate. No effect was observed on the activity of glutamate dehydrogenase, whereas inhibition of decarboxylase and transaminases was insignificant (Lejczak, Starzemska & Mastalerz, 1981).

The effect of glufosinate-ammonium (concentration 50.2% w/w) at doses up to 500 µg/ml on the in vitro activity of glutamine synthetase was investigated in samples of liver, kidney and brain (neocortex, medulla oblongata and hypothalamic region) of Wistar rats. Statements of adherence to QA and GLP were included.

Glufosinate-ammonium induced a significant dose-related inhibition of glutamine synthetase from 10 µg/ml onwards. The maximal level of inhibition was 72%. Glufosinate-ammonium induced no inhibition of kidney glutamine synthetase activity at 1, 3 or 10 µg/ml. A dose-related inhibition was observed from 100 µg/ml onwards, with a maximum of 17%.

In the brain (neocortex, medulla and hypothalamus), glufosinate-ammonium induced a dose-related inhibition from 30 µg/ml onwards The maximal level of inhibition was in the range of 41–53% (Luetkemeier, 1999).

The kinetics of the decrease in serum glutamine levels after a single oral dose of glufosinate-ammonium (purity 95.2%) was investigated. Groups of four non-pregnant female Wistar rats received a single oral (gavage) dose of 200 mg/kg bw of glufosinate-ammonium or vehicle (0.5% methylcellulose). All animals were observed for mortality and clinical signs. Body weights were recorded before the study start, on the day of treatment (study day 1) and on study day 2. Blood

samples were collected from all animals in each group prior to treatment and then 1, 2, 4, 8 and 24 hours after treatment. Glutamine levels in the serum were determined by HPLC.

No mortalities and no clinical signs were observed. A progressive decrease in the mean serum glutamine levels was observed in the group treated with glufosinate-ammonium compared with the control group. The lower glutamine level was reached between 4 and 8 hours after treatment and remained stable until the 24-hour time point. When compared with the control group, a 24–31% decrease in glutamine level was observed between 4 and 24 hours after a single oral dose of glufosinate-ammonium (Kennel, 2003a).

The kinetics of the decrease in serum glutamine levels after a single oral dose of glufosinate-ammonium was investigated. Groups of 18 pregnant Wistar rats received a single oral (gavage) dose of 200 mg/kg bw of glufosinate-ammonium (batch 2/93; purity 95.2%) or vehicle (0.5% methylcellulose) on GD 6. All animals were observed for mortality and clinical signs. Body weights were recorded before the study start, on the day of treatment (study day 1) and on study day 2. Blood samples were collected from all animals 24 hours after treatment. Glutamine levels in the serum were determined by HPLC. Statements of adherence to QA and GLP were included.

No mortalities and no clinical signs occurred during the study. The mean serum glutamine level in the group treated with glufosinate-ammonium was significantly ($P < 0.01$) reduced by 21% compared with the control group (Kennel, 2003b).

(c) The significance of glutamine synthetase inhibition to humans

A number of studies concerning the significance of glutamine synthetase activity in humans were available. Based on the available information, JMPR in 1999 came to the following conclusion:

> Glutamine synthetase is a key enzyme in the metabolism of nitrogen and glutamate, catalysing the multi-step reaction of
>
> (−)-glutamate + ATP + NH_3 ↔ (−)-glutamine + ADP + P
>
> In plants, glutamine synthetase is the main enzyme involved in the control of ammonia concentrations, and its inhibition is the mechanism of action of glufosinate-ammonium in plants. In mammals, other pathways exist for the homeostatic control of ammonia, such as reverse reaction of amino acid dehydrogenases and the carbamoyl phosphate synthetase–urea cycle. Glutamate and glutamine can, however, play significant roles in other biochemical and physiological processes in mammals, such as neurotransmission (glutamate and gamma-aminobutyric acid (GABA)). The activity of glutamine synthetase varies between tissues and species (see below), but the amino acid sequence is reported to be well conserved (Lie-Venema et al., 1998; Purich, 1998; Ernst & Leist, 1999a).
>
> The liver has two distinct systems for dealing with ammonia. A high-capacity, low-affinity system exists in the periportal hepatocytes which is based on carbamoyl phosphate synthetase and the urea cycle. In central vein hepatocytes, a low-capacity, high-affinity system exists which is based on glutamine synthetase and ornithine aminotransferase. [Hack, Ebert & Ehling] (1994) showed that doses of glufosinate-ammonium did not increase ammonia concentrations in liver at a dose (5000 ppm) that inhibited glutamine synthetase activity by 50%. While a 60% reduction in liver glutamine was seen at day 1, the concentration had returned to normal by day 4, indicating the induction of alternative pathways. Inhibition of liver glutamine synthetase by up to 50% is therefore not considered to be adverse in isolation.
>
> The activity of this enzyme in kidney varies considerably between species (Lie-Venema et al., 1998; see below), with relatively high activity in rodents but negligible activity in dogs and humans. Inhibition of kidney glutamine synthetase in the absence of pathological findings is not considered to be relevant to human risk assessment.
>
> In the brain and central nervous system, ammonia homeostasis is controlled by a number of enzymes including glutamine synthetase and glutamate dehydrogenase. Under normal conditions (~ 100 µmol/L of

ammonium and 3 mmol/L of glutamate), the flux through glutamine synthetase in brain is 2–10% of its theoretical capacity and that of glutamate dehydrogenase is approximately 0.1% of its capacity (Lie-Venema et al., 1998). With such excess capacity, inhibition of brain glutamine synthetase is unlikely to result in significant increases in brain ammonia concentrations. This conclusion is supported by the finding of [Hack, Ebert & Ehling] (1994) that brain ammonia concentrations were not increased at doses of glufosinate-ammonium that produced a 40% reduction in brain glutamine synthetase activity in rats. However, the glutamine–glutamate shunt between GABA and glutamate in neurons and glutamine in astrocytes plays a role in both excitatory and inhibitory neurotransmission. The results of [Hack, Ebert & Ehling] (1994), although somewhat inconsistent, indicate that significant changes in a range of biogenic amines in regions of the dog brain are associated with changes of ≥ 8% in glutamine synthetase activity after administration of glufosinate-ammonium at 8 mg/kg bw for 28 days, a dose that produced "increased gait activity". It is thus proposed that any statistically significant, > 10% inhibition of glutamine synthetase activity in brain is a marker of potentially adverse effects on brain biochemistry and behaviour.

3. Observations in humans

Medical examinations of plant production personnel have not found effects related to the production of glufosinate-ammonium.

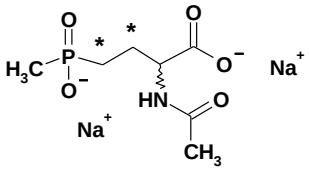
Several human poisoning cases due to (suicidal) ingestions of glufosinate-ammonium have been described in the literature. Observed symptoms were nausea, vomiting, diarrhoea, abdominal pain, tremor, hypotonia, bradycardia or tachycardia, muscle weakness, drowsiness, loss of consciousness and coma, convulsions and respiratory arrest. Rarely, hyperthermia has been described. The neurological symptoms may suddenly appear with a delay (latency period) of 8–48 hours without prior warning. Both cytotoxic and vasogenic oedema have been reported in brain regions. The fatality rate in reported poisonings is about 18%. It is not clear whether the toxicity is due to the active ingredient, to the surfactant contained in relatively high amounts in the formulation or to the combination of both (Koyama et al., 1994).

B. *N*-ACETYL-GLUFOSINATE (NAG)

N-Acetyl-L-glufosinate (NAG) is the major metabolite in genetically modified crops sprayed with racemic glufosinate-ammonium. Following oral administration of glufosinate-ammonium to rats, NAG is observed in the urine at extremely low trace levels. It was not detected in any organ extracted. Therefore, NAG is considered as a non-common metabolite. It cannot be assessed as a bioavailable metabolite in studies with parent glufosinate-ammonium and is consequently not covered in toxicity studies with glufosinate-ammonium in rats. Therefore, a toxicology dossier as would typically be required for non-common metabolites was submitted. It is noted that NAG is usually supplied as an aqueous solution. The dose levels referred to in the studies refer to the active ingredient unless stated otherwise.

The structure of ^{14}C-labelled NAG is shown in Figure 3.

Figure 3. Chemical structure of [^{14}C]N-acetyl-glufosinate (NAG). The positions of the radiolabel ^{14}C (C*) used in the toxicokinetics studies are shown.



4. Biochemical aspects

4.1 Absorption, distribution and excretion

Rats

Groups of male and female Wistar rats were administered a single oral or intravenous dose of [3,4-^{14}C]NAG (purity ~98%) at 3 mg/kg bw. The substance was dissolved in a pyrogen-free saline. The positions of the ^{14}C labels are shown in Figure 3. An overview of the study groups is presented in Table 22. In four groups of five males or five females, excretion was studied over a period of 4 days. At the end of the study, animals were sacrificed and dissected to sample different organs and tissues for determination of the distribution of the radioactive residues. Whole-body autoradiography was performed in another two males 4 days after oral and intravenous administration, respectively. Metabolism was investigated by radio-HPLC of the contents of stomach and intestine in two additional male rats dosed orally and sacrificed, respectively, 4 hours and 24 hours after dosing.

Table 22. Overview of experimental groups

Test group	Number and sex	Administration route	Targeted dose (actual applied doses)	Sampling regimen
1	5M/5F	Oral gavage	3 mg/kg bw (single), [^{14}C]NAG (2.5–3.0 mg/kg bw)	Excretion and residues Urine, cage washings and faeces collected at 4 and 8 h and 1, 2, 3 and 4 days after dosing. Sacrifice and tissue and blood collection on day 4.
	2M	Oral gavage	3 mg/kg bw (single), [^{14}C]NAG (3.0 mg/kg bw)	Metabolism One rat was killed 4 h after dosing and one 24 h after dosing. Gastrointestinal contents were removed and investigated for metabolism.
	1M	Oral gavage	3 mg/kg bw (single), [^{14}C]NAG (2.8 mg/kg bw)	Whole-body autoradiography 96 h after dosing
2	5M/5F	Intravenous	3 mg/kg bw (single), [^{14}C]NAG (2.8–3.3 mg/kg bw)	Excretion and residues Urine, cage washings and faeces collected at 4 and 8 h and 1, 2, 3 and 4 days after dosing. Sacrifice and tissue and blood collection on day 4.
	1M	Intravenous	3 mg/kg bw (single), [^{14}C]NAG (3.1 mg/kg bw)	Whole-body autoradiography 96 h after dosing

From Kellner, Stumpf & Braun (1993)
F, female; M, male

Organs/tissues (blood, bone, brain, carcass, fat, gonads, heart, kidney, liver, lung, plasma, skeletal muscle, spleen) of each rat were collected. Radioactivity levels were determined by LSC. Metabolite characterization was performed in stomach and intestines. NAG dosing solutions were homogeneous, and radiolabelled solutions remained stable during the study. Statements of adherence to QA and GLP were included.

No abnormalities were observed in the rats upon oral or intravenous exposure. The kinetics results are summarized in Table 23. Absorption by orally treated rats was slight, with mean values of 5.5% for males and 6.3% for females. Over a period of 96 hours, orally administered radioactivity was excreted mainly via faeces, with values of 98% ± 5% in males and 109% ± 11% in females. The mean renal portion was about 6% in both males and females. Following intravenous administration,

radioactivity was mainly excreted via urine, with 97% ± 5% in males and 95% ± 7% in females. The mean faecal portion was between 1.8% and 4.1%.

Table 23. Half-lives for excretion in urine and faeces after oral and intravenous administration of [^{14}C]NAG

	Half-life (mean ± standard deviation) (h)			
	Oral administration, single dose (3 mg/kg bw)		Intravenous administration, single dose (3 mg/kg bw)	
	Males	Females	Males	Females
Phase I, urine	3.6 ± 0.7	3.8 ± 0.3[a]	1.7 ± 0.6	1.4 ± 0.4
Phase II, urine	18.0 ± 3.5	25.6 ± 9.3[a]	18.2 ± 2.0	42.3 ± 39.2[b] 24.8 ± 3.9[b]
Phase I, faeces	5.1 ± 0.3	6.2 ± 0.8[a]	12.1 ± 2.4	12.6 ± 1.5

From Kellner, Stumpf & Braun (1993)

[a] One female not included, as it showed an inverse excretory pattern (112.1 hours for phase II).

[b] Values with and without one female showing very high phase II half-life of 112.1 hours (others range from 19.8 to 28.5 hours).

In the orally dosed rats (test group 1), total recovery in urine, cage washings and faeces ranged from 100% to 125% at 96 hours, of which 94–119% (one rat [no. 13] only 20%) was found in the faeces and 5.2–6.7% in the urine (one rat [no. 13] 85%). In the intravenously dosed rats (test group 2), total recovery in urine, cage washings and faeces ranged from 94% to 105% at 96 hours, of which 88–104% was found in the urine and 1.0–7.3% in the faeces. Mean values per sex are presented in Table 24.

The mean total radioactivity in tissues and blood of rats dosed orally was 0.097% (excluding rat no. 13, for which it was 0.14% of the total administered radioactivity) on day 4. The mean total radioactivity in tissues and blood of rats dosed intravenously was 0.22% on day 4. Measurable concentrations of administered radioactivity were found only in kidneys, testes, liver, spleen, bones and carcasses of the males and in kidneys, liver, spleen and carcasses, but not gonads, of the females. In the other organs, no detectable concentrations were observed. One orally dosed female rat differed from all the other animals. This was probably caused by faulty intubation, as suggested by the relatively high concentration of radioactivity in the lungs. However, no abnormalities were observed during the study or at necropsy.

Whole-body autoradiography showed radioactivity in the kidneys (concentrated in the medulla), spleen (accumulated in the red pulp), salivary glands and thymus (homogeneous distribution), testes (band-like) and epididymides (circular). It was not possible to assign the radioactivity to any particular tissue structure. This also applies to the occasionally observed, but extensive, accumulations in the liver.

Except for gonads and kidneys, where concentrations were slightly higher in males than in females, no definite sex-specific differences were found (Kellner, Stumpf & Braun, 1993).

The blood-level kinetics of NAG was investigated in male and female Wistar rats following an oral or intravenous administration of [^{14}C]NAG at a targeted common dose rate of 3 mg/kg bw. Three rats of each sex per exposure group received a single dose via either gavage or injection into a tail vein. Blood samples were taken 0.25, 0.5, 1, 2, 4, 6 and 8 hours and 1, 2, 3 and 4 days after dosing. Statements of adherence to QA and GLP were included.

Table 24. Mean percentage of administered [^{14}C]NAG after a single dose (oral and intravenous)

Time after dosing (h)	Mean % of administered radioactivity					
	Oral			Intravenous		
	Faeces	Urine	Cage wash[a]	Faeces	Urine	Cage wash
Males						
0–4	—	1.956	—	—	85.6	—
4–8	—	0.606	—	—	8.89	—
8–24[a]/0–24[b]	95.21	2.476	0.0428	1.416	1.71	0.092
24–48	2.242	0.140	0.0035	0.269	0.37	0.023
48–72	0.047	0.031	—	0.057	0.13	—
72–96[a]/48–96 [b]	0.0072	0.012	—	0.025	0.06	0.008
Total	97.51	5.222	0.0463	1.768	96.76	0.123
Total excreted	102.78			98.66		
Females[c]						
0–4	—	1.933	—	—	91.24	—
4–8	—	0.831	—	—	1.95	—
8–24[a]/0–24[b]	102.6	2.825	0.0746	3.44	0.79	0.122
24–48	4.37	0.219	0.0092	0.49	0.37	0.086
48–72	2.01	0.053	—	0.14	0.28	—
72–96[a]/48–96[b]	0.041	0.026	—	0.06	0.15	0.083
Total	109.1	5.887	0.0838	4.14	94.78	0.27
Total excreted	115.02			99.23		

From Kellner, Stumpf & Braun (1993).
[a] Urine and cage wash.
[b] Faeces.
[c] Means do not include the findings of rat no. 13.

Following oral administration (actual rates vary from 2.5 to 3.6 mg/kg bw), the maximum blood levels were reached after approximately 1 hour, amounting to mean concentrations of 0.052 and 0.051 mg Eq/kg in male and female rats, respectively. The last measurable concentrations were found 8 hours after dosing. The elimination kinetics was biphasic, with half-lives of 0.8 and 0.9 hour (phase I) and 6.3 and 7.4 hours (phase II) for males and females, respectively. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) values amounted to 0.214 ± 0.031 mg Eq·h/kg for male rats and to 0.192 ± 0.064 mg Eq·h/kg for female rats. Following intravenous administration, the blood level at the first sampling interval, 5 minutes after injection, amounted to 6.19 and 7.44 mg Eq/kg in male and female rats, respectively, at the mean. The blood levels declined rapidly. At 8 hours post-dosing, levels of 0.008 and 0.010 mg Eq/kg were measured in male and female rats, respectively. The elimination kinetics was also biphasic, with half-lives of 0.35 and 0.30 hour (phase I) and 12.9 and 15.4 hours (phase II) for males and females, respectively. The $AUC_{0-\infty}$ values amounted to 3.66 ± 0.158 mg Eq·h/kg for male rats and 3.86 ± 0.207 mg Eq·h/kg for female rats. The kinetics results are summarized in Table 25.

The bioavailable portion after oral administration (oral absorption) took place rapidly after oral administration, but was only slight, calculated from the $AUC_{0-\infty}$ values after oral and intravenous administration. This calculation yielded a bioavailable portion of orally administered NAG of 5.9% of the dose for male rats and 5.0% of the dose for female rats (Kellner & Braun, 1993).

Table 25. Toxicokinetic data in blood after a single oral or intravenous administration of [^{14}C]NAG

Parameter	Oral administration, single dose (3 mg/kg bw)		Intravenous administration, single dose (3 mg/kg bw)	
	Males	Females	Males	Females
C_{max} (mg Eq/kg)	0.052	0.051	6.19	7.44
T_{max} (h)	1	1.17	0.083	0.083
Phase I $t_{½}$ (h)	0.82	0.86	0.35	0.3
Phase II $t_{½}$ (h)	6.3	7.4	12.9	15.4
$AUC_{0-\infty}$ (mg Eq·h/kg)	0.21	0.19	3.66	3.86
Bioavailability (%)[a]	5.9	5.0	—	—

From Kellner & Braun (1993)
$AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero to infinity; C_{max}, maximum plasma concentration; Eq, equivalents; $t_{½}$, half-life; T_{max}, time to reach C_{max}
[a] Bioavailability (%) = 100 × (AUC (oral) / AUC (intravenous)).

In a parallel study, groups of male and female Wistar rats were treated with a single oral gavage dose of [3,4-^{14}C]NAG (purity > 90%) at 3 mg/kg bw. The study was aimed at determining the rate and extent of excretion of radioactivity, the metabolite profile in excreta and the identity of the major excreted metabolites. The results of the metabolite profile and the identity of the major excreted metabolites are presented in the next section. The substance was stocked as an aqueous solution, which was diluted with sodium chloride solution before administration. The positions of the ^{14}C labels are shown in Figure 3 above. Five male and five female animals were treated. Excreta were collected up to 96 hours after dosing. Native urine was investigated by radio-HPLC using an anion exchange system or by radio-TLC using silica plates. Radioactivity in cage wash and carcass was not measured, because a parallel toxicokinetic study (Kellner & Braun, 1993) had shown that radioactivity in these fractions was less than 0.5% of the dose. Statements of adherence to QA and GLP were included.

Total renal excretion accounted for 5.5% and 9.3% of the administered dose for males and females, respectively. The majority of the radioactivity was eliminated in the 0- to 24-hour interval. Total faecal excretion accounted for 100% and 95.8% of the administered dose in males and females, respectively, with the majority being excreted in the 0- to 24-hour interval. Total recoveries of the radioactivity were 105.4% and 105.1% for males and females, respectively (Stumpf, 1993b).

Pharmacokinetics in rats following a single oral administration of NAG at a high dose level was investigated. ^{14}C-labelled NAG was dissolved in saline and orally administered to different groups of male and female rats by gavage at a target dose rate of 1000 mg/kg bw (actual dose levels ranged from 1017 to 1196 mg/kg bw). Different animals of both sexes were sacrificed 2 hours (one animal of each sex), 6 hours (one animal of each sex), 24 hours (five animals of each sex) and 96 hours (five animals of each sex) after dosing. Urine, faeces and cage wash were collected on a daily basis or until sacrifice for a maximum period of 4 days. After humane killing, the animals were dissected, and selected organs and tissues were sampled. All samples were radioassayed by LSC. Statements of adherence to QA and GLP were included.

The material balance was effectively complete and amounted to 95.3% of the dose for both sexes 96 hours after dosing (Table 26). The excretion was rapid, as approximately 71% and 66% of the dose were excreted during the 1st day after dosing by male and female rats, respectively. The faeces proved to be the predominant excretion route, accounting for approximately 87% and 77% of the dose 96 hours after administration to male and female rats, respectively. Total renal excretion (urine and cage wash combined) accounted for 8.5% and 18.6% of the dose in males and females, respectively. The high figure for female rats was assessed by the study director to be a result of contamination by the faeces. Considering the excretion pattern of all animal groups in this and other

studies, a lower value for oral absorption, of 8–10% of the dose, was assumed to be realistic. The overall data also indicate that the oral absorption of NAG does not depend on the dose rate.

Table 26. Material balance and excretion of radioactive residues 0–96 hours after an oral administration of [^{14}C]NAG at 1000 mg/kg bw to male and female rats

Matrix	% of administered dose[a]							
	Males				Females			
	0–24 h		0–96 h		0–24 h		0–96 h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Urine	6.20	2.26	6.79	1.71	7.01	2.35	12.71	6.24
Faeces	62.24	5.89	86.76	9.11	56.95	12.5	76.68	14.4
Cage wash	2.18	1.16	1.71	0.65	2.24	1.00	5.89	4.30
Total excreted	70.62	3.65	95.26	7.78	66.09	13.26	95.28	4.57
Organs/tissues	0.04	0.03	< LOD	< LOD	0.01	0.003	< LOD	< LOD
Total recovery	70.66	3.67	95.26	7.78	66.27	13.34	95.28	4.57

From Maas & Braun (1995)
LOD, limit of detection; SD, standard deviation
[a] Mean and standard deviation of five rats of each sex per collection period.

The total radioactive residue detected in organs and tissues 2 hours after administration was below 0.5% of the dose and declined rapidly. The highest residue levels were consistently observed in the kidneys and liver at all sampling times. Four days after administration, radioactivity was detectable only in the kidneys of three males, whereas in females, no radioactivity was detectable in any of the organs or tissues. These results did not indicate any potential for bioaccumulation of NAG. No marked sex-specific differences were observed. The average half-lives estimated for faecal elimination were 11 hours in males and 10 hours in females. The half-life for renal excretion was 17 hours in males and 19 hours in females (Maas & Braun, 1995a).

The metabolism of a single high-dose oral administration of [^{14}C]NAG in rats was investigated. ^{14}C-labelled NAG was dissolved in saline and orally administered to different groups of male and female rats by gavage at a target dose rate of 1000 mg/kg bw (actual dose levels ranged from 999 to 1594 mg/kg bw). Different animals of both sexes were sacrificed 2 hours (one animal of each sex), 6 hours (one animal of each sex), 24 hours (five animals of each sex) and 96 hours (five animals of each sex) after dosing. Urine, faeces and cage wash were collected on a daily basis or until sacrifice for a maximum period of 4 days. After humane killing, the animals were dissected, and selected organs and tissues were sampled. Metabolite characterization of all samples is described in section 4.2 in the evaluation of the study of Lauck-Birkel (1995b). Statements of adherence to QA and GLP were included.

The total recovery (urine, faeces and cage wash) was 98.9% and 97.7% for male and female rats, respectively. The renal excretion (including cage wash) accounted for 10% of the administered dose for both sexes. The total faecal excretion accounted for 89% and 88% of the total dose for males and females, respectively. Most of the radioactivity was eliminated within 0–48 hours (90–92% of the administered dose). Residue levels in the organs and tissues were insignificant (< 0.01% of the dose already at 24 hours after administration) (Maas & Braun, 1995b).

The absorption, distribution and elimination of a single intermediate dose (30 mg/kg bw) of [3,4-^{14}C]NAG were investigated by Maas & Braun (1999c). Three groups of five male Wistar rats received an actual NAG dose of 31.6–35.1 mg/kg bw dissolved in water by stomach tube. Animals were killed 1, 6 and 24 hours after administration. Blood and plasma, kidneys, liver and brain were

sampled. These organs were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the third group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic profiling results are summarized in section 4.2 below on biotranformation (Lauck-Birkel & Strunk, 1999d). Statements of adherence to QA and GLP were included.

Twenty-four hours after the oral administration, more than 90% of the dose was excreted: approximately 2% in the urine and 88% of the dose with the faeces. One hour after oral administration, the highest residue level was observed in the kidneys, amounting to approximately 2.01 mg Eq/kg, followed by the liver, with 0.36 mg Eq/kg, as well as blood and plasma, with 0.20 and 0.33 mg Eq/kg, respectively. Similar residue levels in blood and plasma indicated no adsorption of NAG residues to the blood cells. The lowest residues were detected in the brain, amounting to 0.02 mg Eq/kg, suggesting poor penetration of the NAG residues through the blood–brain barrier. Six hours after administration, the maximum residue level was again observed in the kidneys, accounting for 1.09 mg Eq/kg. Compared with the first sampling time, the residues slightly increased in liver and brain, amounting to 0.46 and 0.03 mg Eq/kg, whereas the residues in blood and plasma dropped to 0.10 and 0.14 mg Eq/kg, respectively.

Twenty-four hours after administration, all residue levels were less than 1 mg Eq/kg in the selected organs. The highest residue level was observed in kidneys, accounting for 0.66 mg Eq/kg, followed by the liver, with 0.21 mg Eq/kg. Other residue levels were less than 0.04 mg Eq/kg (Maas & Braun, 1999c).

The absorption, distribution and elimination of a single intravenous low dose (3 mg/kg bw) of NAG in rats were investigated by Maas & Braun (1999d). Two groups of five male Wistar rats received an actual dose of [3,4-^{14}C]NAG at 3.1–3.4 mg/kg bw dissolved in saline injected into the tail vein. Animals were sacrificed 2 and 24 hours after administration. Sacrificial blood and plasma, kidneys, liver and brain were sampled. These organs were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the second group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic profiling results of tissues, organs and excreta are summarized in section 4.2 on biotranformation (Lauck-Birkel & Strunk, 1999c). Statements of adherence to QA and GLP were included.

Twenty-four hours after intravenous administration, more than 87% of the dose was excreted. Approximately 85% of the dose was excreted with the urine and 2% with the faeces, suggesting that the biliary excretion is low. Therefore, the renal excretion after oral administration of NAG approximates the oral absorption.

Two and twenty-four hours after intravenous administration, the highest residue level was observed in the excretory organs, the kidneys, followed by the liver. Even lower levels were measured in blood and plasma (Maas & Braun, 1999d).

4.2 Biotransformation

Rats

In the study of Stumpf (1993b; see study description under section 4.1), metabolites were analysed in urine and faeces of Wistar rats dosed orally with [^{14}C]NAG (purity > 90%) at a targeted rate of 3 mg/kg bw. Metabolites were isolated by HPLC and identified by co-chromatography.

After oral administration of NAG at actual dose rates of 2.1–3.4 mg/kg bw, the main metabolite was the unchanged NAG (70% of applied dose), predominantly in the (0- to 24-hour) faeces. In urine, NAG was the main component, accounting for 3.5% of the dose in males and 6.6% in females. Two impurity peaks (MPP and MPB) of the test substance were observed in urine at very low dose levels (0.6–0.7% of the dose). It is unclear whether these levels should be attributed to metabolism or to impurities present in the test substance. Free glufosinate in urine was detected at or

slightly above the limit of detection of 0.05% of the dose. Radio-HPLC and radio-TLC of the purified faeces extract showed that NAG formed the main residue, with 68.2% of the dose in males and 68.4% of the dose in females. Free glufosinate was detected in faeces at 10.8% and 9.3% of the dose, respectively, for males and females. In addition, two minor metabolites, MHB and MPP, accounted for, respectively, 0.7–1% and 0.2–0.6% of the dose (Stumpf, 1993b).

The metabolism of a single high-dose oral administration (1000 mg/kg bw) of [^{14}C]NAG in urine, faeces and cage wash samples of rats was investigated. The study design is described in section 4.1 (Maas & Braun, 1995b). All samples were radioassayed by LSC. Urine and aqueous extracts of faeces were investigated by radio-HPLC and/or radio-TLC. Statements of adherence to QA and GLP were included.

The metabolic profile in the excreta was the same in both sexes. Unchanged NAG was the predominant residue component in the 0- to 96-hour excreta, accounting for 6.7% and 7.4% of the dose in the urine and 84.9% and 88.5% of the dose in the faeces of males and females, respectively. Minor metabolites (< 0.5% of the dose) detected in urine and faeces were MHB, MPP, MPA and MPB. The low levels suggest that NAG was essentially not metabolized in the rat. No deacetylated NAG (glufosinate) was detected in urine. Deacetylation of NAG was observed in the intestine, but only up to 1% of the dose (Lauck-Birkel, 1995b).

The metabolism of a single intermediate oral dose (30 mg/kg bw) of [3,4-^{14}C]NAG was investigated in the tissues, organs and excreta collected in the study described in the previous section (Maas & Braun, 1999c). These organs were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the third group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic profile in excreta, blood and plasma, liver and kidney is described here. Statements of adherence to QA and GLP were included.

Unchanged NAG was the predominant residue component in urine (1.7% of the dose) and faeces (82% of the dose). Minor metabolites were detected in urine: MPP (0.15%), MPA (0.03%), MPB (0.18%) and L-glufosinate (0.02% of the dose). In the faeces, the deacetylated substance, L-glufosinate, amounted to 5.2% of the dose. MPB was again detected in the faeces, amounting to 1.4% of the dose, possibly resulting from the MPB impurity of the test substance. In the kidneys, the unchanged test substance NAG, representing 0.031% of the dose, was the main compound 1 hour after dosing. In addition, traces of MPP, L-glufosinate (free acid) and MPB were also detected. Six hours after dosing, the metabolite profile was comparable, whereas 24 hours after dosing, L-glufosinate (free acid) was the major residue component, amounting to 0.018% of the dose. Some traces of NAG and MPP were still observed. In the liver, NAG and MPP proved to be the two main residue components 1 hour after administration, accounting for 0.018% of the dose each. Traces of MPB and L-glufosinate (0.001% of the dose) were also detected. Six hours after administration, MPP was the main metabolite in the liver, amounting to 0.038% of the dose. Unchanged NAG accounted for 0.011% of the dose, and traces of MPB were again observed. Twenty-four hours after dosing, MHB was the main metabolite in the liver, representing 0.015% of the dose, although it was not detected at earlier sampling periods. Glufosinate was no longer detected 6 and 24 hours after administration (Lauck-Birkel & Strunk, 1999d).

The metabolism of a single low intravenous dose (3 mg/kg bw) of [3,4-^{14}C]NAG was investigated in the tissues, organs and excreta collected in the study of Maas & Braun (1999d). These samples were radioassayed, extracted and profiled for residue composition by HPLC with co-elution of authentic reference substances. Excreta were collected for 24 hours only from the second group, radioassayed by LSC and analysed for the composition of residues by radio-HPLC. The metabolic

profile in excreta, blood and plasma, liver and kidney is described here. Statements of adherence to QA and GLP were included.

The unchanged test substance NAG was the principal residue component in urine (approximately 85% of the dose) and faeces (approximately 2% of the dose). A minor metabolite detected in urine was MPB, amounting to approximately 1% of the dose. However, MPB was reported to be an impurity of the test substance (1.2%). Apart from the test substance NAG, two minor metabolites were additionally observed in the faeces: i.e. L-glufosinate (representing the free acid) and MPB, each amounting to less than 0.1% of the dose.

In kidneys and liver, the unchanged test substance NAG proved to be the main residue component, amounting to 0.79% and 0.01% of the dose, respectively, 2 hours after intravenous administration. Apart from NAG, L-glufosinate (representing the free acid) was detected as the main metabolite in the kidneys (but not in the liver), accounting for 0.05% and 0.06% of the dose 2 and 24 hours after dosing, respectively. Additionally, MPP was detected in the liver at a low level of 0.04% of the dose. With the exception of glufosinate, all residue levels had decreased significantly by the 24-hour sampling (Lauck-Birkel & Strunk, 1999c).

The results of the above studies suggest the same metabolic pathway of NAG in the rat for both oral and intravenous routes of exposure (Figure 4). NAG is partly deacetylated to form glufosinate, which is further metabolized via oxidative deamination and decarboxylation, resulting in MPP and MPA (Lauck-Birkel & Strunk, 1999c,d).

5. Toxicological studies

5.1 Acute toxicity

(a) Lethal doses

Two acute oral toxicity studies with NAG were performed in rats and mice, respectively. The results are summarized in Table 27. No treatment-related mortality occurred in either study, and no macroscopic findings were reported. Treatment-related clinical signs of toxicity observed in surviving rats were reduced spontaneous activity, high-legged gait, contracted flanks, squatting position, piloerection, irregular breathing and increased respiratory rate on day 1 only. After the day of treatment, animals were free of clinical signs. In mice, largely the same clinical signs were observed (reduced spontaneous activity, contracted flanks, squatting position, piloerection and increased respiratory rate), but some of the signs were still observed 14 days after treatment.

(b) Dermal sensitization

A dermal sensitization study (maximization test) was performed according to OECD Test Guideline No. 406. Female Pirbright-White guinea-pigs were used: 9 in the dose range–finding tests, 10 in the control group and 20 in the treatment group. An additional escort group of five animals was used to test the effect of Freund's adjuvant on lowering the primary irritation determined in the preliminary tests. Five per cent of the test substance (corresponding to 2.9% of water-free substance) was used for the intradermal induction phase, followed by an epidermal induction on day 9 and epidermal challenge on day 22 with the undiluted test substance (corresponding to 57.9% water-free test substance). The treated animals showed no clinical signs during the study. Intradermal injections with Freund's adjuvant caused only very slight erythema and oedema. The treated skin was dry-chapped, developed fine and coarse scales, was light encrusted, was indurated and had white lumps and necrosis. Following the challenge application, 24 and 48 hours after removal of the occlusive bandage, the animals showed no effects (Schollmeier & Leist, 1989c).

Figure 4. The proposed metabolic pathway of NAG in rats

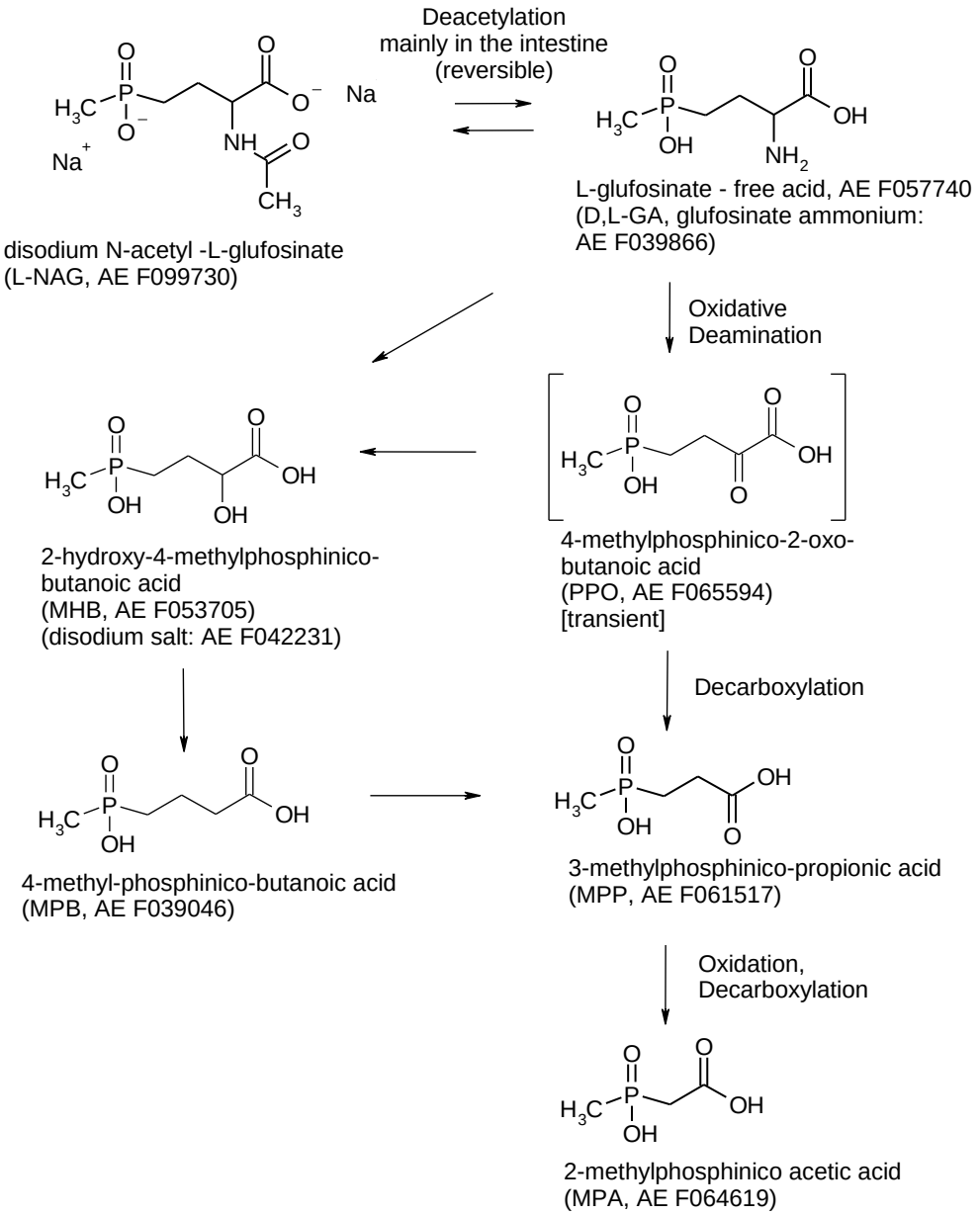


Table 27. Results of studies of acute toxicity with NAG

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	NMRKf (SPF71)	M+F	Oral	Deionized water	95.9	> 2895	Schollmeier & Leist (1989a)[a]
Rat	WISKf(SPF71)	M+F	Oral	Deionized water	82.6	> 2895	Schollmeier & Leist (1989b)[a]

F, female; LD_{50}, median lethal dose; M, male

[a] Performed according to GLP and OECD Test Guideline No. 401 using NAG (purity 82.6%) supplied as an aqueous solution with a water content of 42.1%. Presented NAG doses are corrected for water content in the aqueous solution.

5.2 Short-term studies of toxicity

Mice

NMRI mice (five of each sex per group) were fed diets containing NAG (purity 79.4%, delivered in aqueous solution with water content of 42.1%, active ingredient content 57.9%) for 4 weeks at a nominal concentration of 0, 116, 579, 2895 or 5790 ppm (equal to average concentrations of 0, 21.7, 124, 600 and 1126 mg/kg bw per day for males and 0, 22.9, 115, 591 and 1148 mg/kg bw per day for females, respectively). The study was performed in accordance with OECD Test Guideline No. 407. Mice were examined daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Animals were sacrificed at the end of the 4-week treatment period. Haematological examination, clinical chemistry and urine analysis were carried out at the termination of the study. In addition, the activity of glutamine synthetase was examined in liver and brain. At necropsy, animals were examined macroscopically and histologically. Statements of adherence to QA and GLP were included.

During the study, behaviour and general health conditions were unaffected. No signs of neurological disturbances, opacity of the refracting media of the eyes, lesions of the oral mucosa or disturbances of dental growth were observed in any of the treated groups. No clinical signs of systemic toxicity (specifically indicative of central nervous system intoxication) were observed, and no animals died during the study. No changes in body weight gain or feed and water consumption were reported.

There were a few statistically significant changes in the haematology and clinical chemistry parameters, without any toxicological significance, apart from the slight decrease in calcium levels in females from 2895 ppm (equal to 591 mg/kg bw per day) and higher, for which substance dependency cannot be excluded. Determination of glutamine synthetase activity revealed a significant inhibition in the liver (21–45%) and brain (37–43%) at 2895 and 5790 ppm (Table 28). A 14% reduction in brain synthetase activity in females at 579 ppm did not reach statistical significance.

Table 28. Effect of treatment with NAG on glutamine synthetase activity in mice

Organ	Sex	Glutamine synthetase activity (± SD) (nkat/mg protein)				
		0 ppm	116 ppm	579 ppm	2895 ppm	5790 ppm
Liver	Male	0.43 (± 0.06)	0.38 (± 0.03)	0.38 (± 0.03)	0.34* (± 0.04)	0.24* (± 0.04)
	Female	0.38 (± 0.04)	0.50* (± 0.06)	0.40 (± 0.03)	0.42 (± 0.05)	0.22* (± 0.02)
Brain	Male	1.24 (± 0.11)	1.44 (± 0.10)	1.52 (± 0.09)	1.12 (± 0.16)	0.71* (± 0.12)
	Female	1.47 (± 0.09)	1.41 (± 0.20)	1.26 (± 0.17)	0.92* (± 0.14)	0.89* (± 0.15)

From Ebert (1991a)
SD, standard deviation
* $P < 0.05$ (according to the parametric method of Dunnett)

The effect on glutamine synthetase activity was considered at the time the report was issued to be due to the contamination of the test article with glufosinate (4.5%), a proven inhibitor of glutamine synthetase. However, subsequent rat metabolism work shows that glutamine synthetase inhibition would also be expected as a result of glufosinate formed by in vivo deacetylation of NAG to glufosinate. The combination of test substance impurity and deacetylation provides the full explanation for the level of glutamine synthetase inhibition observed. Urine analysis showed no treatment-related changes in any of the groups. Analysis of organ weights indicated no substance-related changes in any group. There were no treatment-related macroscopic or microscopic changes.

The NOAEL was 579 ppm (equal to 115 mg/kg bw per day), based on a significant reduction (37%) in brain glutamine synthetase activity at 2895 ppm (equal to 591 mg/kg bw per day) (Ebert, 1991a).

A subchronic (13-week) oral feeding study was performed in mice, according to OECD Test Guideline No. 408. SPF-bred Hanover-derived NMRI mice (20 animals of each sex per group) were treated via diet with NAG (purity of technical ingredient 74.7%) in the diet. Dietary concentration levels were 0, 500, 2000 and 8000 ppm. Actual dose levels were 0, 83, 233 and 1296 mg/kg bw per day for males and 0, 110, 436 and 1743 mg/kg bw per day for females, respectively. Mice were examined at least once daily. Detailed clinical observations (including palpation for tissue mass) were done once a week. Body weights and feed consumption were recorded weekly. Blood and urine sampling was performed at week 13. Animals were sacrificed at the end of the 13-week treatment period. At necropsy, animals were examined macroscopically and histologically. In addition, the activity of glutamine synthetase was examined in liver, kidney and brain samples. Statements of adherence to QA and GLP were included.

One male died at 8000 ppm after blood sampling in week 13, but this was unrelated to treatment. No treatment-related clinical signs were observed. Body weight development and the absolute and relative feed intake were not affected. Blood analysis showed a statistically significant increase of lactate dehydrogenase in two high-dose males only. This effect was not observed in females, the values were within the normal historical control range and this effect was not seen in the 28-day mouse study and therefore not considered to be of biological significance. Glutamine synthetase activity was significantly inhibited in liver, kidney and brain (Table 29).

Table 29. Effect of NAG on glutamine synthetase activity in liver, kidney and brain of mice

Organ	Sex	Glutamine synthetase activity[a]			
		0 ppm	500 ppm	2000 ppm	8000 ppm
Liver	Male	3.98	3.74	3.34**	2.94**
		—	(94)	(84)	(74)
	Female	4.97	4.91	4.60*	3.98**
		—	(99)	(93)	(80)
Kidney	Male	1.42	1.01**	0.83**	0.71**
		—	(71)	(58)	(50)
	Female	1.73	1.29**	1.20**	1.10**
		—	(75)	(69)	(64)
Brain	Male	3.57	3.32**	3.17**	2.59**
		—	(93)	(89)	(73)
	Female	3.38	3.32	2.94**	2.18**
		—	(98)	(87)	(64)

From Tennekes, Schmid & Probst (1992)

* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

[a] Units of glutamine synthetase activity are micromoles of γ-glutamyl-hydroxamate formed per millilitre of reaction mixture per 20 minutes at 37 °C. Percentage of control value given in parentheses.

In the liver, slight to moderate inhibition of glutamine synthetase activity was seen at 2000 and 8000 ppm. In the kidney, slight to moderate inhibition was seen at all doses. In brain, glutamine synthetase activity was dose-dependently inhibited at 2000 and 10 000 ppm.

No treatment-related changes were seen in organ weights, necropsy findings or microscopic findings.

The NOAEL was 500 ppm (equal to 83 mg/kg bw per day), based on the inhibition of brain glutamine synthetase activity (11–13%) at 2000 ppm (equal to 233 mg/kg bw per day) (Tennekes, Schmid & Probst, 1992).

Rats

Wistar rats (five of each sex per group) were fed diets containing NAG (purity 79.4%, delivered in aqueous solution with water content of 42.1%, active ingredient content 57.9%) for 4 weeks at a nominal concentration of 0, 116, 579, 2895 or 5790 ppm (equal to 0, 12.1, 59.9, 309 and 593 mg/kg bw per day for males and 0, 11.2, 55.1, 283 and 561 mg/kg bw per day for females, respectively). The study was performed in accordance with OECD Test Guideline No. 407. Rats were examined daily. Body weights and feed consumption were recorded twice weekly, and water consumption was recorded once weekly. Animals were sacrificed at the end of the 4-week treatment period. Haematological examination, clinical chemistry and urine analysis were carried out at the termination of the study. In addition, the activity of glutamine synthetase was examined in liver and brain. At necropsy, animals were examined macroscopically and histologically. Statements of adherence to QA and GLP were included.

During the study, behaviour and general health conditions were unaffected. No signs of neurological disturbances, opacity of the refracting media of the eyes, lesions of the oral mucosa or disturbances of dental growth were observed in any of the treated groups. No clinical signs of systemic toxicity (specifically indicative of central nervous system intoxication) were observed, and no animals died during the study. No changes in body weight gain or feed and water consumption were reported.

There were a number of statistically significant changes in the haematology and clinical chemistry parameters; however, most of them were considered to reflect normal biological variation and were not caused by the test substance. However, a treatment-related effect cannot be excluded for lactate dehydrogenase, for which there was a statistically significant decrease at the high dose level in females. However, as this possible effect was a decrease in the enzyme, it is not of toxicological significance. Determination of glutamine synthetase activity revealed a significant inhibition (about 50%) in the livers of the animals of both sexes at the high dose level. This effect was observed at a dose level of 579 ppm and above in males and at 2895 ppm and above in females. Statistically significant decreases in glutamine synthetase (up to 39%) were found in the brain at the three highest dose levels in females and at 116 and 2895 ppm in males. However, the decreases showed no dose–response relationship. The effect on glutamine synthetase activity was considered at the time the report was issued to be due to the contamination of the test article with glufosinate (4.5%), a proven inhibitor of glutamine synthetase. However, subsequent rat metabolism work shows that glutamine synthetase inhibition would also be expected as a result of glufosinate formed by in vivo deacetylation of NAG to glufosinate. The combination of test substance impurity and deacetylation could explain the level of glutamine synthetase inhibition observed. In a 13-week dietary study by Schmid et al. (1998) with NAG containing glufosinate as an impurity at 0.1%, no effect on brain glutamine synthetase activity was found. Thus, the Meeting concluded that it is likely that the observed effects on brain synthetase in the present study can be attributed to the presence of glufosinate-ammonium as an impurity of NAG and the deacetylation of NAG to glufosinate. Urine analysis showed no treatment-related changes in any of the groups. Analysis of organ weights indicated a slight decrease in absolute and relative heart weights among the males from all dose groups, reaching statistical significance for absolute heart weight in the highest-dose group. As the effect was observed in all treatment groups and was not dose dependent and as no histopathological changes in the heart were observed, the Meeting considered this a fortuitous finding. No other changes that might be related to the test substance were detected. There were no treatment-related macroscopic or microscopic changes.

The NOAEL was 5790 ppm (equal to 561 mg/kg bw per day), the highest dose tested (Ebert, 1991b).

A subchronic (13-week) oral feeding study was performed in rats, according to OECD Test Guideline No. 408. SPF-bred Hanover-derived Wistar rats (10 animals of each sex per group and 10 animals of each sex in the control, mid-dose and high-dose groups for recovery) were treated via diet containing NAG (purity of technical ingredient 79.4%, supplied as a 44% w/w solution in water).

Dietary concentration levels were 0, 400, 2000 and 10 000 ppm, corresponding to 0, 900, 4500 and 22 500 mg test substance per kilogram diet. Actual dose levels were 0, 29.1, 147 and 738 mg/kg bw per day for males and 0, 31.7, 162 and 800 mg/kg bw per day for females, respectively. Rats were examined at least once daily. Detailed clinical observations (including palpation for tissue mass) were done once a week. Body weights and feed consumption were recorded weekly. Ophthalmoscopic examinations were performed on all animals pretest and at 11 and 16 weeks. Blood and urine sampling was performed at 13 and 17 weeks. Animals were sacrificed at the end of the 13-week treatment period or after recovery (17 weeks). At necropsy, animals were examined macroscopically and histologically. In addition, the activity of glutamine synthetase was examined in liver, kidney and brain samples. Statements of adherence to QA and GLP were included.

There were no unscheduled deaths during the study, and no treatment-related clinical signs were observed. Body weight development and absolute and relative feed intake were not affected. Very slight, but statistically significant, decreases in erythrocyte count (4%) and haemoglobin concentration (3%), a marginal increase in corpuscular volume index (3%) for males and a very slight increase in prothrombin time (2%) in males at 10 000 ppm were observed. No biological significance was attributed to these findings, as they were all marginally different from the control group values and within the range of the historical control data. Furthermore, at the end of the recovery period, these findings were no longer statistically different from the control values. For clinical chemistry, glutamine synthetase inhibition was observed in liver, kidney and brain tissues (Table 30).

In liver of high-dose males, glutamine synthetase activity was inhibited to 47% of control values. In males and females of the high-dose group, brain glutamine synthetase activity was decreased to 88–89% of control activity. After the recovery period, slight inhibition (6–8%) was still observed in the liver of females and in the brain (6–8%) of both sexes at 10 000 ppm. Statistically significantly higher kidney weights were observed in males at 400 and 10 000 ppm at 13 weeks. As no statistically significant increase was observed at the middle dose and there were no histopathological effects on the kidneys, they were considered not to be of toxicological significance. No effects were observed after the recovery period. There were no treatment-related necropsy findings or microscopic findings.

The NOAEL was 2000 ppm (equal to 147 mg/kg bw per day), based on statistically significant inhibition (11–12%) of brain glutamine synthetase activity at 10 000 ppm (equal to 738 mg/kg bw per day) (Tennekes, Probst & Luetkemeier, 1992).

Dogs

A subchronic (13-week) oral feeding study was performed in dogs, according to OECD Test Guideline No. 409. Purebred Beagles (four [group 2] or six [groups 1, 3 and 4] animals of each sex per group) were treated with NAG (purity of technical ingredient 74.7%) in the diet. Dietary concentration levels were 0, 500, 2000 and 8000 ppm, corresponding to an average test article intake of 0, 20, 76 and 294 mg/kg bw per day, respectively. Dogs were examined twice daily for viability and clinical signs. Body weights were recorded weekly, and feed consumption was recorded daily. Ophthalmoscopic examinations were performed 4 times (pretest and at 4 weeks, 13 weeks and 4 weeks after recovery), as was blood and urine sampling. Animals were sacrificed at the end of the 13-week treatment period or after the 4-week recovery period. At necropsy, animals were examined macroscopically and histologically. In addition, the activity of glutamine synthetase was examined in liver, kidney and brain samples. Statements of adherence to QA and GLP were included.

All animals survived to the end of the scheduled treatment or recovery periods, and no clinical signs were observed. Feed consumption and body weight were unaffected, and there were no treatment-related ophthalmoscopic changes. There were no treatment-related haematological findings. The plasma clinical biochemistry parameters were unaffected by treatment. A moderate to marked, dose-dependent inhibition of glutamine synthetase activity was observed in the liver (43–79%) and brain (16–65%) taken after 13 weeks of treatment from dogs at 2000 or 8000 ppm (Table 31).

Table 30. Efect of NAG on glutamine synthetase activity in liver, kidney and brain of rats

Organ	Sex	Sampling week	Glutamine synthetase activity[a]			
			0 ppm	400 ppm	2000 ppm	10 000 ppm
Liver	Male	13	3.76[a]	2.76**	2.18**	1.77**
			—	(73)	(58)	(47)
		17	3.17	—	3.49	3.42
			—	—	(110)	(108)
	Female	13	3.71	3.12*	2.57**	2.35**
			—	(84)	(69)	(63)
		17	3.73	—	3.53	3.32 *
			—	—	(95)	(89)
Kidney	Male	13	2.10	1.68**	1.49**	1.64**
			—	(80)	(71)	(78)
		17	2.13	—	2.09	1.88
			—	—	(98)	(88)
	Female	13	1.17	1.12	1.20	1.52**
			—	(96)	(103)	(130)
		17	1.27	—	1.28	1.32
			—	—	(101)	(104)
Brain	Male	13	3.19	3.26	3.02*	2.82**
			—	(102)	(95)	(88)
		17	3.14	—	2.98	2.89**
			—	—	(95)	(92)
	Female	13	3.12	3.06	3.02	2.77*
			—	(98)	(97)	(89)
		17	3.09	—	3.11	2.92*
			—	—	(101)	(94)

From Tennekes, Probst & Luetkemeier (1992)

* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

[a] Units of glutamine synthetase activity are micromoles of γ-glutamyl-hydroxamate formed per millilitre of reaction mixture per 20 minutes at 37 °C. Percentage of control value given in parentheses.

A slight, statistically significant decrease (31%) in liver glutamine synthetase activity was also seen in males at 500 ppm. Glutamine synthetase activity in the kidney was unaffected by treatment at all dose levels. Following 4 weeks of recovery, no statistically significant difference between control and test groups was observed. Urine analysis showed lower specific gravity and osmolality in females at 8000 ppm after 4 and 13 weeks of treatment and at the end of the recovery period. In the absence of histopathological changes in the kidney, these findings were not considered to be of toxicological relevance. No treatment-related changes were seen in organ weights, necropsy findings or microscopic findings.

The NOAEL was 500 ppm (equal to 20 mg/kg bw per day), based on reduction in brain glutamine synthetase activity (≥ 16%) at 2000 ppm (equal to 76 mg/kg bw per day) (Corney, Braunhofer & Luetkemeier, 1992).

Table 31. Effect of NAG on glutamine synthetase activity in liver, kidney and brain areas of dogs

Organ	Sex	Sampling week	Glutamine synthetase activity[a]			
			0 ppm	500 ppm	2000 ppm	8000 ppm
Liver	Male	13	2.72	1.88**	1.15**	0.58**
		17	2.61	—	2.39	2.03
	Female	13	1.85	1.63	1.05*	0.68**
		17	2.06	—	1.72	1.26
Kidney	Male	13	0.02	0.04	0.06	0.05
		17	0.07	—	0.05	0.06
	Female	13	0.04	0.10	0.06	0.09
		17	0.05	—	0.08	0.07
Brain (cortex)	Male	13	3.17	3.35	2.82	2.30*
		17	2.97	—	2.40	2.49
	Female	13	2.90	3.15	2.70	2.48
		17	3.11	—	2.74	2.35
Brain (midbrain)	Male	13	2.33	2.62	2.58	1.37*
		17	2.10	—	2.29	2.12
	Female	13	2.71	2.23	2.39	2.04
		17	2.09	—	2.07	1.83
Brain (cerebellum)	Male	13	1.58	1.42	1.26*	0.85**
		17	1.42	—	1.23	1.20
	Female	13	1.59	1.47	1.34**	0.87**
		17	1.49	—	1.30	1.14
Brain (brainstem)	Male	13	1.36	1.30	0.90**	0.47**
		17	1.32	—	0.95	0.93
	Female	13	1.20	1.19	0.91**	0.73**
		17	1.35	—	1.17	0.83

From Corney, Braunhofer & Luetkemeier (1992)

* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

[a] Units of glutamine synthetase activity are micromoles of γ-glutamyl-hydroxamate formed per millilitre of reaction mixture per 20 minutes at 37 °C.

A subchronic (52-week) oral dietary toxicity study was performed in dogs, according to OECD Test Guideline No. 452. Purebred Beagles (six animals of each sex per group) were treated with NAG (purity 92.4%, supplied as 35.7% solution in water) in the diet. Dietary concentrations were 0, 100, 1000 and 8000 ppm, corresponding to an average test article intake of 0, 4.0, 44 and 325 mg active ingredient per kilogram body weight per day for males and 0, 4.3, 43 and 346 mg active ingredient per kilogram body weight per day for females, respectively. Dogs were examined twice daily for viability and clinical signs and were physically examined once a week. Body weights were recorded weekly, and feed consumption was recorded daily. Ophthalmoscopic examinations were performed 3 times: at weeks 12, 25 and 51 of treatment. Prior to the start of treatment and in weeks 13, 26 and 52, blood was collected for haematology and clinical chemistry evaluation. Urine was collected in the same weeks for urine analysis. Two animals of each sex from each dose level were sacrificed after 26 weeks of treatment (at 2000 ppm only one female, because one female had already been killed in week 3). All remaining animals were killed after 52 weeks of treatment. Gross and histopathological examinations were performed on all animals. Statements of adherence to QA and GLP were included.

One female (mid-dose group) was killed in extremis during week 3 of treatment. Microscopic examination revealed a non-suppurative encephalitis of unknown origin, consistent with the clinically observed convulsions. Etiology could not be established, but a post-vaccine reaction cannot be excluded. In males receiving 1000 or 8000 ppm, soft faeces were observed more frequently. Feed consumption and body weight were unaffected, and there were no treatment-related ophthalmoscopic changes. There were no treatment-related haematological findings. Small, but statistically significant, increases (differential lymphocyte count) or decreases (haematocrit and monocyte count) were occasionally observed, but were not found to be dose related and therefore were considered to be incidental. There were no changes in the plasma clinical biochemistry parameters. After weeks 13, 26 and 52, decreased lactate dehydrogenase values were observed in high-dose animals compared with controls. Lower lactate dehydrogenase values (50%) were also seen in mid-dose females (pretreatment and at weeks 26 and 52). As these decreases were not associated with any other changes and because decreased mean lactate dehydrogenase values were initially seen pretreatment in mid- and high-dose females, these differences were considered to be of no toxicological significance. Small and transient changes were seen in creatinine, creatine phosphokinase, glucose, blood urea nitrogen and chloride, but these were inconsistent between sampling occasions. Likewise, the transient increases in ALP and ALT levels observed in week 26 in a low-dose male and a high-dose female were considered incidental. Urine analysis showed no treatment-related changes. Decreased absolute and relative spleen weights were observed in males at 1000 and 8000 ppm after 12 months. The changes were not clearly dose related, and no gross pathological or histopathological findings were reported, and they were therefore considered not to be of toxicological significance. Glutamine synthetase activity was not determined in this study.

The NOAEL was 8000 ppm (equal to 325 mg/kg bw per day), the highest dose tested (Bernier, 1996).

5.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year dietary carcinogenicity study, technical NAG (purity 92.43%, supplied as a 35.7% solution in water) was administered to groups of 70 male and 70 female CRL:CD mice at 0, 100, 1000 or 8000 ppm (equal to 0, 15, 148 and 1188 mg/kg bw per day for males and 0, 19, 187 and 1460 mg/kg bw per day for females, respectively). An additional 20 mice of each sex per group were designated for interim sacrifice after 52 weeks of treatment. The mice were checked daily for mortality and clinical signs. A detailed clinical examination and palpation for nodules and masses were performed weekly. Feed consumption and body weight were recorded weekly during months 1–3 and biweekly thereafter. Blood was collected after 52 weeks (interim group) and 78 and 104 weeks of treatment in the carcinogenicity group. Glutathione levels in whole blood and liver tissue were measured in the remaining fasted animals at termination of the study. At termination at 104 weeks, all surviving animals were killed and organs were weighed. Gross pathological examination was performed for all animals. Histopathological examinations were performed on all control and high-dose animals, on low- and intermediate-dose animals that were found dead or were killed before the termination of the study, on the lungs, kidneys, liver and adrenal glands in all low- and intermediate-dose animals and on any gross lesions observed macroscopically for animals in all dose groups. Statements of adherence to QA and GLP were included.

The survival rates of control and treated animals were similar. No treatment-related clinical signs were observed. No treatment-related effects were seen on feed consumption. There were no treatment-related effects on haematology or on the clinical biochemistry values of the interim sacrifice or carcinogenicity animals when compared with the control group. At termination of the study, statistically significant increases in total protein and globulin levels were seen in males treated at 10 000 ppm. These increases were not considered to be of toxicological significance, as there was no evidence of an effect at this dose level, nor was there a correlation with any histopathological finding. There was no effect on organ weights. There were no macroscopic or microscopic neoplastic or non-neoplastic findings in either the interim sacrifice or carcinogenicity group animals that could be associated with the administration of the test substance. The Meeting noted that salivary gland

amyloidosis tended to be dose-dependently increased in females. However, such an increase was not observed in males. It was further noted that amyloidosis occurred frequently in other organs in males and females of all dose groups, generally without dose dependency. The Meeting therefore considered the increased incidence of amyloidosis in salivary glands in females to be a fortuitous finding.

The NOAEL was 8000 ppm (equal to 1188 mg/kg bw per day), the highest dose tested (Farrell, 1997; Ernst & Stumpf, 1999a).

Rats

A 52/104-week combined toxicity and carcinogenicity study was performed on Sprague-Dawley Crl:CDR(SD)BR rats (100 of each sex per dose). Ten rats of each sex per group were killed after 52 weeks of treatment, and 70 rats of each sex per group after 104 weeks; 20 rats of each sex per group were used as satellite groups. The groups of rats were fed diet with NAG (purity not given, supplied as a 35.7% solution) at a technical dose level (dry portion of the material) of 0, 200, 2000 or 20 000 ppm (equal to 0, 9, 91 and 998 mg/kg bw per day for males and 0, 11, 108 and 1212 mg/kg bw per day for females of the carcinogenicity groups [104 weeks]). No separate calculations were reported for the toxicity groups (52 weeks). The rats were examined daily for mortality and clinical signs. Additionally, a detailed clinical examination was performed weekly. Body weight and feed consumption were recorded weekly for the first 3 months and every 2 months thereafter. Ophthalmoscopic evaluations were performed on all animals in the pretreatment period and additionally in week 50 (toxicity subgroup) and week 101 (satellite subgroup). Haematology, clinical chemistry and urine analysis were performed at weeks 25 and 51 (10 animals of each sex per dose from the toxicity subgroup and 10 animals of each sex per dose from the satellite subgroup) and during weeks 78 and 102 on the surviving animals in the satellite subgroups. No additional glutamine synthetase measurements were performed. Weights of adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes and thyroid were determined at scheduled necropsy from each animal euthanized after 52 weeks. All remaining animals were sacrificed after 104 weeks of treatment. Gross pathological examination was performed for all animals, and histopathological examination was performed on the control and high-dose animals of the carcinogenicity, toxicity and satellite subgroups, regardless of mode of death. In addition, histopathology was performed on all animals that died or were killed in extremis during the conduct of the study and on any gross lesions observed macroscopically for animals in the low- and mid-dose groups. Macroscopic and microscopic examinations of a wide range of organs and tissues were performed on all animals. Statements of adherence to QA and GLP were included.

There were no significant differences in mortality between treatment and control groups of the chronic toxicity animals for the major duration of the study. A high incidence of soft faeces was noted at 20 000 ppm in both sexes after approximately 8 weeks. Lower body weight gains were observed in both sexes at 20 000 ppm (about 10%), although feed consumption was consistently higher throughout the first 88 (males) or 68 weeks (females) than that of controls. There was no evidence that treatment resulted in any ophthalmological changes. At week 102, statistically significantly increased haematocrit values and mean cellular volumes as well as decreased mean haemoglobin concentrations were seen in the mid- and high-dose females (Table 32). However, these changes were of small magnitude, and the values were within the normal physiological range for rats of this age and strain.

Other statistically significant intergroup differences observed in clinical chemistry parameters were considered not to be treatment related, because the changes were within the normal physiological range for rats of this age and strain, did not show a dose–response relationship or were inconsistent between sampling occasions. Evaluation of the urine analysis results indicated no treatment-related effects. Evaluation of the organ weight data obtained from animals sacrificed after 52 weeks of treatment revealed a slight increase in kidney weights (absolute, relative to body weight and to brain weight, 14–21%) in females at 20 000 ppm. An increased incidence, statistically significant, of renal chronic progressive nephropathy was seen in female rats killed after 52 weeks of treatment at 20 000 ppm in the carcinogenicity subgroup (41/70) compared with controls (16/70).

However, chronic progressive nephropathy is a kidney alteration that occurs spontaneously in ageing rats and is not considered relevant for humans. A dose-related statistically significant difference was seen in the incidence of urolithiasis in females at 20 000 ppm in the carcinogenicity and satellite subgroups. In the high-dose animals (main plus satellite groups), there was an increased incidence of polyarteritis nodosa in testes (18/90 [20%] in controls; 33/90 [37%] at 20 000 ppm, historical control range 16–31%) and blood vessels (males: 4/7 in controls; 11/18 at 20 000 ppm; females: 4/4 in controls; 9/11 at 20 000 ppm; no historical control data). Although within the historical control ranges (8–46% for males; 15–36% for females), an increased incidence of extramedullary haematopoiesis (males: 12/90 [13%] in controls; 25/90 [28%] at 20 000 ppm; females: 10/90 [11%] in controls; 28/90 [31%] at 20 000 ppm) was observed.

Table 32. Effects seen in rats exposed to NAG in the diet

Parameter	Week	Mean value							
		Males				Females			
		0 ppm	200 ppm	2000 ppm	20 000 ppm	0 ppm	200 ppm	2000 ppm	20 000 ppm
Haematocrit (%)	25	42.4	41.9	41.9	40.6**	41.3	41.4	41.5	39.9*
	51	40.6	40.5	40.0	39.4	40.2	39.3	39.7	38.7
	78	39.9	39.7	40.1	39.7	39.1	38.9	36.4	36.6
	102	33.6	35.3	33.5	36.9	36.2	42.5	55.7***	49.4*
Mean cell volume (μm^3)	25	50.1	50.2	49.8	49.7	53.9	53.7	53.3	53.5
	51	51.3	51.3	51.0	51.0	54.9	54.6	54.8	55.0
	78	52.7	52.7	52.7	52.4	55.7	54.6	56.0	56.0
	102	55.3	51.3	54.3	53.8	57.4	64.3	79.3***	82.1***
Mean cell haemoglobin concentration (pg)	25	36.2	36.7	36.8*	37.0**	36.1	36.4	36.7*	37.2**
	51	36.6	36.5	36.8	36.9	36.6	36.7	37.0*	37.1**
	78	36.3	36.3	36.4	36.3	36.2	36.7*	36.9**	36.4
	102	36.4	36.6	36.5	36.8	36.8	33.7	25.5***	25.1***

From Bernier (1997); Ernst & Stumpf (1999b); Chevalier (2001)
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Analysis of the tumour data did not reveal any biologically significant differences in the type or distribution of tumours observed in controls or treated animals.

The NOAEL was 2000 ppm (equal to 91 mg/kg bw per day), based on decreased body weight gain, increased incidence of soft faeces and increased incidences of poliarteritis nodosa in blood vessels and testes and urolithiasis at 20 000 ppm (equal to 998 mg/kg bw per day) (Bernier, 1997; Ernst and Stumpf, 1999b; Chevalier, 2001).

5.4 Genotoxicity

NAG was tested for genotoxicity in a range of guideline-compliant assays. No evidence for genotoxicity was observed in any test.

The results of the genotoxicity tests are summarized in Table 33.

Table 33. Overview of genotoxicity tests with NAG

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 *uvrA*	2.33–2910 µg/plate (±S9)	82.6	Negative	Müller (1989a)[a]
Chromosomal aberrations	V79 Chinese hamster ovary cells	29–1546.5 µg/ml (±S9, cytotoxicity), 154.6, 773.5 and 1546.5 µg/ml (±S9, chromosomal aberration study)	79.4	Negative	Müller (1989b)[b]
Chromosomal aberrations	Human lymphocytes	600, 3000 and 5000 µg/ml (−S9, 24 h), 5000 µg/ml (−S9, 28 h), 600, 3000, 4750 µg/ml (+S9, 24 h), 4750 µg/ml (+S9, 48 h)	74.7	Negative	Heidemann & Voelkner (1992)[c]
Gene mutation	V79 Chinese hamster lung cells, HGPRT test	1000, 1500, 2000, 2671 µg/ml (±S9), equivalent to 582, 873, 1164 and 1555 µg/ml on a water-free basis	82.6	Negative	Müller (1989c)[d]
Gene mutation	V79 Chinese hamster lung cells, HGPRT test	1000, 1500, 2000, 2671 µg/ml (±S9), equivalent to 444, 666, 888 and 1186 µg/ml on a water-free basis	74.7	Negative	Müller (1991a)[e]
Unscheduled DNA synthesis	Human cell line A549	0.582–582 µg/ml (±S9)	79.4/82.6	Negative	Müller (1989d)[f]
Unscheduled DNA synthesis	Human cell line A549	3–3000 µg/ml (±S9), equivalent to 1.332–1332 µg/ml on a water-free basis	74.7	Negative	Müller (1991b)[g]
In vivo					
Micronucleus	NMRI mouse (5/sex/dose/time point) (3/sex/dose in preliminary dose range–finding study)	0, 500, 2500 and 5000 mg/kg bw (single gavage doses of technical substance) at each time point (24, 48 and 72 h after administration), equivalent to 0, 222, 1100 and 2220 mg/kg bw on a water-free basis	74.7	Negative	Müller (1991c)[h]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Performed in accordance with OECD Test Guideline No. 471. Dose levels selected based on absence of toxicity in a toxicity test with dose levels up to 5820 µg/plate. Positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene and *N*-methyl-*N*-nitroguanidine in the absence of S9 and benzo[*a*]pyrene and 2-aminoanthracene in the presence of S9.

Table 33 (continued)

[b] Performed in accordance with OECD Test Guideline No. 473. Positive controls used were ethylmethanesulfonate (−S9) and cyclophosphamide (Endoxan) (+S9). Two hundred cells per plate were analysed. NAG induced a significant increase in the aberration rate, but only in cells inclusive with gaps 7 hours after treatment with 1546.5 µg/ml with metabolic activation (S9 mix). Also at the preparation time of 18 hours without S9 mix, all three concentrations produced a very slight increase in the number of aberrations in cells inclusive of gaps. Additionally, the number of cells with aberrations inclusive of gaps was very slightly increased at the 28-hour preparation time at the highest dose level with metabolic activation. The observed aberrations at the 18-hour and 28-hour preparation times were considered of no relevance because of the very slight increase in the numbers and the fact that solvent control values were lower than usual.

[c] Performed in accordance with OECD Test Guideline No. 473. Positive controls used were ethylmethanesulfonate (−S9) and cyclophosphamide (+S9). For each culture, 100 metaphases were scored for structural chromosomal aberrations (positive control 25 metaphases). Treatment of the cells even with the highest concentration (5.00 mg/ml) did not reduce the mitotic indices at both fixation intervals in either the presence or absence of S9 mix. In both experiments, there was no biologically relevant increase in cells with aberrations after treatment with the test article at any fixation intervals.

[d] Performed in accordance with OECD Test Guideline No. 476. NAG was supplied as an aqueous solution (water content 41.8%). Positive controls used were ethylmethanesulfonate (−S9) and 9,10-dimethyl-1,2-benzanthracene (+S9). The test substance produced no significant cytotoxic effect from 50 µg/ml up to the highest concentration of 2671 µg/ml. The test compound did not induce a significant increase in the number of mutant colonies or in the mutation frequency at any dose level of the test substance either with or without S9 mix. No cytotoxic effect was observed in the main experiment. Marked increases in mutation frequency were obtained with the positive control substances.

[e] Performed in accordance with OECD Test Guideline No. 476. NAG was supplied as an aqueous solution (water content 55.6%). Positive controls used were ethylmethanesulfonate (−S9) and 9,10-dimethyl-1,2-benzanthracene (+S9). The test substance produced no significant cytotoxic effect from 50 µg/ml up to the highest concentration of 2671 µg/ml (10 mmol/l). No increase in mutant colony numbers was obtained at any dose level in the two independent experiments. The positive control substances showed an increase in induced mutant colonies.

[f] Performed in accordance with OECD Test Guideline No. 482. NAG was supplied as an aqueous solution (water content 42.1%/41.8%). Positive controls used were 4-nitroquinoline-*N*-oxide (−S9) and benzo[*a*]pyrene (+S9). No cytotoxicity and no increase in unscheduled DNA synthesis were observed at any of the test substance concentrations in either the presence or absence of S9 mix. A significant induction of unscheduled DNA synthesis was obtained with the positive control substances.

[g] Performed in accordance with OECD Test Guideline No. 482. NAG was supplied as an aqueous solution (water content 55.6%). Positive controls used were 4-nitroquinoline-*N*-oxide (−S9) and benzo[*a*]pyrene (+S9). No cytotoxicity and no increase in unscheduled DNA synthesis were observed at any of the test substance concentrations in either the presence or absence of S9 mix. A significant induction of unscheduled DNA synthesis was obtained with the positive control substances.

[h] Study design resembles OECD Test Guideline No. 474. NAG was supplied as an aqueous solution (water content 55.6%). The number of polychromatic and normochromatic erythrocytes containing micronuclei was not increased. The ratio of polychromatic to normochromatic erythrocytes in both male and female animals remained unaffected by the treatment with NAG and was not statistically different from the control values. The positive control used was cyclophosphamide (Endoxan). It induced a marked statistically significant increase in the number of polychromatic cells with micronuclei in both sexes, indicating the sensitivity of the system. The ratio of polychromatic erythrocytes to normocytes was not changed to a significant extent.

5.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a range-finding one-generation reproductive toxicity study, NAG was administered in the diet during a 3-week premating period and continuing throughout the mating, gestation and lactation periods to groups of 10 Sprague-Dawley rats of each sex per group. The purity was 92.4%. The raw test material had a water content of 64.3%. This was taken into account when calculating the dose level of technical material to be administered. Dietary concentrations were 0, 200, 2000 and 10 000 ppm (equal to 0, 13, 129 and 670 mg/kg bw per day for males and 0, 16, 156 and 799 mg/kg bw per

day for females, respectively). The rats were checked daily for mortality and clinical signs. Body weight and feed consumption were measured weekly. In dams that littered, body weight and feed consumption were measured on LDs 1, 4, 7, 14 and 21. Litter size, sex of pups, number of live and dead pups and malformations were recorded. Maternal rats were killed and necropsied at the end of lactation. The number of implantation sites was recorded. Ovaries, uterus, testes, epididymides and seminal vesicles of all parental animals were weighed. At the end of the lactation period, the pups were examined externally and killed. Statements of adherence to QA and GLP were included.

No mortality was observed. One mid-dose male was killed in a moribund state. His condition was considered not to be treatment related, as no high-dose animals showed similar clinical effects or gross changes to internal organs. Occasionally observed clinical signs occurred in all groups, including controls, and were considered not related to treatment. No significant reductions in body weight gain were observed. During the pretreatment and premating periods, feed consumption was increased in high-dose females. This finding was considered to be incidental. The organ weights, mating and fertility indexes, conception rate, duration of pregnancy, gestation index, number of implantations, post-implantation loss, sex ratio and number of live and dead pups were not affected by treatment. Parental animals showed no treatment-related gross changes. Pup viability, survival and lactation indices, clinical signs, body weights and macroscopic appearance in the treated groups were not different from controls (Beyrouty, 1996a).

In a two-generation dietary reproductive toxicity study, performed in accordance with OECD Test Guideline No. 416, Sprague-Dawley rats (30 of each sex per group for the F_0 generation, 26 of each sex per group for the F_1 generation) were fed NAG (purity 92.4%) at a dietary concentration of 0, 200, 2000 or 10 000 ppm. The corresponding NAG intakes during the different phases of the study are presented in Table 34.

Table 34. NAG intake in parental rats during different phases of the multigeneration reproductive toxicity study

Test group	Generation	Dietary concentration (ppm)	Test material intake (mg/kg bw per day)		
			Premating		Post-mating
			Males	Females	Females (gestation)
1	F_0	0	0	0	0
2		200	13	18	13
3		2000	137	173	126
4		10 000	702	891	622
1	F_1	0	0	0	0
2		200	16	19	12
3		2000	162	197	124
4		10 000	821	1008	652

From Beyrouty (1996b)

All adult animals were examined daily for clinical signs. Body weights were measured weekly for the males throughout the study and for the females during the premating period. For mated females, body weights during gestation and lactation were also recorded. Feed intake was measured during the premating period for both sexes, post-mating for males and during gestation for the mated females. Following up to 14 days of mating, the males and females were separated and the dams then allowed to litter. Pup clinical signs and body weights were recorded throughout lactation. On day 21 postpartum, the pups were weaned and sacrificed (except those forming the F_1 adult generation). The adults were killed soon after weaning of the pups, and each adult rat, together with one weanling of

each sex per litter, where possible, received a complete necropsy. For adult animals, organ weight measurements and epididymal and testicular sperm assessments were also conducted, and for adult rats in the control and high-dose groups, a histopathological examination was performed on a range of tissues. Statements of adherence to QA and GLP were included.

In the F_0 and F_1 adult generations, no treatment-related mortality occurred. Most high-dose males and some high-dose females showed brown fur staining (males only) and soft faeces. Males in the 2000 ppm group had a significantly ($P < 0.05$) reduced body weight at the end of week 1 when compared with the control group. Females in the 2000 ppm group had significantly ($P < 0.05$) lower body weights at the end of weeks 0 and 1. The body weights were also significantly ($P < 0.05$ or $P < 0.01$) reduced for the 200 ppm group females for weeks 1, 2 and 4. No significant differences were noted during gestation. Significant ($P < 0.05$) reductions in body weights were noted for the 200 ppm group females during lactation on days 0 and 21. These differences were considered not to be treatment related, as no significant differences were observed between the control and high-dose groups. In high-dose animals, feed consumption was occasionally slightly, but statistically significantly, increased during the premating period. Mating and fertility indices, gestational index, length of gestation, number of implantations, post-implantation loss, pup sex ratio, numbers of live or dead pups at birth and estrous cycle were not affected by treatment. Examination of sperm parameters, organ weights and gross and histological examination revealed no effect of treatment. A dose-related increase in the weight of the left seminal vesicles was seen in F_1 adults (up to 14%), reaching statistical significance in males at the high dose. As there were no associated abnormal histological findings and no functional deficit, the effect was considered not to be adverse. An increased incidence of extramedullary haematopoiesis in the livers of high-dose F_1 males only (8/30 versus 2/30 in controls) was also considered not to be adverse, as it is a common finding in rats and it was not seen in females or in F_0 males.

Litter sizes, pup viability, survival, lactation indices, body weights and body weight gains were not significantly different between the control and treated groups. No treatment-related clinical signs were seen in pups. Gross external and internal examination of the pups revealed no effect of treatment.

The NOAEL for parental, offspring and reproductive toxicity was 10 000 ppm (equal to 622 mg/kg bw per day), the highest dose tested (Beyrouty, 1996b).

(b) Developmental toxicity

Rats

In a developmental toxicity study, performed according to OECD Test Guideline No. 414, groups of 20 mated female Wistar rats were treated orally, by gavage, with NAG (purity unknown, supplied as 77.5% solution) in distilled water at a limit dose level of 1000 mg/kg bw per day or with vehicle only from day 7 through day 16 of gestation (day 0 = day of observation of copulatory plug). All rats were observed for clinical signs pretest and daily during the study. Body weight was recorded at days 0, 7, 14, 17 and 21 of pregnancy. The dams were killed and delivered by caesarean section on day 21 of pregnancy and examined macroscopically for abnormalities. The numbers of live and dead fetuses and the conceptuses undergoing resorption were determined. Body weight, crown–rump lengths, sex ratios of the fetuses and placental weights were recorded, and external, visceral and skeletal examinations of the fetuses were performed. In a range-finding study conducted beforehand, NAG had been administered orally to two groups of three gravid Wistar rats at dose levels of 111.1 and 1000 mg/kg bw per day from day 7 to day 16 of pregnancy. On day 21 of gestation, the dams were killed. Both doses were tolerated without untoward effects; therefore, a limit test at 1000 mg/kg bw per day was conducted. Statements of adherence to QA and GLP were included.

All rats survived until the termination of the study. There were no signs of disturbance of behaviour or general health, and NAG had no effect on body weight gain. Feed consumption of the dams treated with NAG was slightly lower during the treatment period than that of the controls. However, as feed consumption remained within the range of historical control values and body weight gain was not adversely affected, no toxicological significance was attached to this finding. No adverse

effects were observed at examination after caesarean section. At necropsy and body cross-sectional examination of fetuses, it was found that one eyelid of a fetus in the substance group was half open. Blood was present in the pericardium or abdominal cavity of some fetuses in the substance group and isolated fetuses in the control group. Some of the fetuses in both the substance and control groups exhibited unilateral or bilateral distension of the renal pelvis, accompanied frequently by distension of the corresponding ureter(s). Distension of the ureter was found in one fetus from the treatment group. The skeletons of the treatment group fetuses were at much the same stage of development as those of the control fetuses. The degree of ossification corresponded to day 21 of gravidity. One NAG-exposed fetus had only six vertebral arches on the right side in the cervical region of the vertebral column, and one control fetus showed thickened ribs on both sides. Otherwise, no adverse effects were seen. The terminal necropsy revealed no macroscopically visible abnormalities in the internal organs of any of the dams. The heart, liver, kidney and spleen weights of the NAG-treated dams were comparable to those of control animals.

NAG was not teratogenic to the rat when dosed at 1000 mg/kg bw per day. The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the only dose tested (Horstmann & Baeder, 1992; Baeder, Albrecht & Mayer, 1993).

Rabbits

An oral developmental toxicity study was performed according to OECD Test Guideline No. 414 with Himalayan rabbits (15 per group) exposed to NAG (purity 92.4%, supplied as 77.5% w/w aqueous solution) at 0, 64, 160 or 400 mg/kg bw per day by oral gavage once daily. Rabbits were mated with sexually mature males at a ratio of 1:1 and again 6 hours later. Dams were administered once daily with NAG from GD 6 to GD 18 and sacrificed on day 29 of pregnancy. All rabbits were observed daily for clinical signs. Body weights and feed consumption were recorded on days 0, 6, 13, 19 and 29 of gestation. At necropsy, dams were examined for macroscopically visible changes. The uterus was examined to determine the reproductive status (number of live and dead fetuses and the number of conceptuses undergoing resorption). Live fetuses were removed and checked for viability during 24 hours. Body weights, crown–rump lengths, sex ratios of the fetuses and placental weights were determined, and external, visceral and skeletal examinations of the fetuses were performed. Statements of adherence to QA and GLP were included.

None of the females died. Body weight development of the dams remained unaffected.

Reduced water consumption and pultaceous faeces were observed sporadically in two animals and one animal of the 400 mg/kg bw per day group, respectively. Body weight gain was not impaired by administration of the test substance. Feed consumption was slightly to moderately decreased in the animals of the intermediate-dose group (160 mg/kg bw per day) and high-dose group (400 mg/kg bw per day) during the treatment period (Table 35).

Table 35. Mean feed consumption in a teratogenicity study in rabbits exposed to NAG

Days of gestation	Mean feed consumption (± standard deviation) (g/100 g bw)			
	0 mg/kg bw per day	64 mg/kg bw per day	160 mg/kg bw per day	400 mg/kg bw per day
0–6	4.21 (± 0.45)	4.23 (± 0.60)	4.16 (± 0.50)	4.26 (± 0.27)
6–13	3.69 (± 0.81)	3.17 (± 0.82)	2.79* (± 0.63)	2.20* (± 0.59)
13–19	3.81 (± 0.89)	3.41 (± 0.83)	2.97* (± 0.65)	2.67* (± 1.26)
19–29	4.03 (± 0.56)	3.90 (± 0.67)	3.96 (± 0.48)	4.34 (± 0.90)

From Baeder & Hofmann (1994)
* $P < 0.05$ (according to a non-parametric linear model)

No compound-related changes were observed at necropsy. All animals became pregnant. One female in the low-dose group had an abortion, and one in the intermediate-dose group had an early intrauterine death. Dead and stunted fetuses were observed in one animal of the low-dose group and in two animals of the intermediate- and high-dose groups, respectively. As these incidences were within the normal range of the rabbit strain used and there was no clear dose–response relationship, it was considered that these observations were not due to administration of the compound. Morphological examination of the fetuses revealed a statistically significant increase in the incidence of supernumerary thoracic ribs at 160 and 400 mg/kg bw per day. The incidence was within the normal range in the intermediate-dose group and slightly above the upper limit of the normal range in the high-dose group (Table 36).

Table 36. Intergroup comparison of extra thoracic rib incidence (by fetus/litter) in a teratogenicity study in rabbits exposed to NAG

	Incidence of extra rib (number/number examined (%))			
	0 mg/kg bw per day	64 mg/kg bw per day	160 mg/kg bw per day	400 mg/kg bw per day
Fetuses	2/90 (2.2%)	0/82	8/73* (11%)	11/90** (12%)
Litters	2/15 (13%)	0/14	7/14* (50%)	5/15 (33%)

From Baeder & Hofmann (1994)
* $P < 0.05$; ** $P < 0.01$ (according to the Fisher exact test, one-sided)

Administration of NAG at dose levels of 160 and 400 mg/kg bw per day from day 6 to day 18 of pregnancy caused slight to moderate decreases in feed consumption of the dams. The fetuses in these groups showed statistically significant increases in the incidence of extra thoracic ribs. The NOAEL for maternal toxicity was 64 mg/kg bw per day, based on reduced feed consumption at 160 mg/kg bw per day. The NOAEL for developmental toxicity was 64 mg/kg bw per day, based on an increased incidence of extra thoracic ribs at 160 mg/kg bw per day (Baeder & Hofmann, 1994; Hofmann, Baeder & Mayer, 1995; Ernst & Leist, 1999b).

5.6 Special studies

(a) Neurotoxicity

Rats

An acute oral (dose range–finding) neurotoxicity study was performed in accordance with USEPA Guideline OPPTS 870.6200 (1998) in Wistar (SPF) rats treated with 0, 100, 1000 or 2000 mg/kg bw (purity 33.8%, doses refer to amount of active substance using a correction factor of 2.96). Rats (10 of each sex per group) were given a single dose by oral gavage at approximately 9 weeks of age. Mortality and clinical signs were observed hourly up to 10 hours after dosing and twice daily during test days 2–15. Feed consumption and body weights were recorded periodically pretest and during the observation period. All animals were killed on test day 15, necropsied and examined post mortem. A functional observational battery was performed, and locomotor activity, body temperature, landing foot splay and grip strength were measured pretest and 1, 7 and 14 days after application. Statements of adherence to QA and GLP were included.

No deaths occurred during the study. No treatment-related findings were observed in the functional observational battery or on locomotor activity, grip strength, body temperature, rearing or landing foot splay. Diarrhoea, ruffled fur and sedation were observed in almost all animals treated with 2000 mg/kg bw on day 1. These findings had fully reversed by day 2. No clinical signs were observed in the low- or mid-dose groups. Feed consumption and body weight gain were unaffected in the test groups when compared with the control animals. No macroscopic findings were observed at necropsy.

The NOAEL was 1000 mg/kg bw, based on clinical signs (diarrhoea, ruffled fur and sedation) observed at 2000 mg/kg bw (Hamann, 1999c).

An acute oral neurotoxicity study, including a water maze test to assess learning, memory and relearning, was performed on groups of Wistar (SPF) rats (10 of each sex per group) treated by gavage with NAG (purity 33.8%, doses refer to amount of active substance using a correction factor of 2.96) at a single dose of 0, 100, 1000 or 2000 mg/kg bw. All animals were tested pretreatment and again 1, 7 and 14 days after dosing. The study was not performed in accordance with any specific guideline. Histological examinations were performed on brain, spinal cord, sciatic nerve and tibial nerve from all animals. The general observations were performed as described above (Hamann, 1999c). Statements of adherence to QA and GLP were included.

No treatment-related findings were observed in any of the animals during the learning, memory and relearning phases of the water maze test. No treatment-related macroscopic or microscopic findings were observed in any of the organs or tissues examined. The single oral administration of NAG to rats at doses of 100, 1000 and 2000 mg/kg bw did not result in any deaths. Treatment-related findings were restricted to clinical signs at 2000 mg/kg bw and comprised sedation, ruffled fur and diarrhoea on day 1.

The NOAEL was 1000 mg/kg bw, based on clinical signs (diarrhoea, ruffled fur and sedation) observed at 2000 mg/kg bw (Hamann, 1999d).

Groups of 10 male and 10 female Wistar rats were given NAG (purity 33.8%) at a dietary level of 0, 20, 200 or 2000 ppm (equal to 0, 1.6, 16 and 159 mg/kg bw per day for males and 0, 1.8, 18 and 179 mg/kg bw per day for females, respectively) for up to 38 days. An additional five rats of each sex were used at each dose level for determining glutamine synthetase activity in the liver, kidney and brain at the end of the treatment period. The animals were observed daily for clinical signs, whereas body weight and feed consumption were recorded weekly. A functional observational battery, locomotor activity, body temperature, landing foot splay distance and grip strength were measured pretest and after 2 and 4 weeks of treatment. A water maze test was performed pretest and in week 5. A single dose of midazolam (2 mg/kg bw) given intraperitoneally before or 10 minutes after the water maze test was used as a positive control. At termination, all the animals were killed and examined macroscopically. The brain, heart, liver and kidneys were weighed, and histopathological examination was performed on the brain, spinal cord, and sciatic and tibial nerves of all the control and high-dose animals. Statements of adherence to QA and GLP were included.

No treatment-related mortality or clinical signs were observed. Body weight gain, feed consumption, functional observational battery parameters, locomotor activity, rearing, grip strength, body temperature, landing foot splay, or learning and memory tests (water maze) were unaffected by treatment with NAG.

In animals receiving NAG, a statistically significant inhibition of glutamine synthetase activity was observed in the liver of males at 200 and 2000 ppm and in females at 2000 ppm (Table 37). Glutamine synthetase activity in kidneys was decreased by NAG in high-dose males. The slight reduction in glutamine synthetase activity in males at 20 ppm was considered incidental, in view of the lack of dose dependency. No inhibition of glutamine synthetase activity was observed in the brain tissue of animals receiving NAG. In the absence of histopathological findings, the relatively small reductions in glutamine synthetase activity in liver and kidneys are considered not adverse.

No treatment-related macroscopic or microscopic abnormalities were seen in any of the animals.

The NOAEL for NAG was 2000 ppm (equal to 159 mg/kg bw per day), the highest dose tested (Hamann et al., 2000).

Table 37. Mean glutamine synthetase activity following 38 days of treatment with NAG in rats

Organ	% of control activity					
	Males			Females		
	20 ppm	200 ppm	2000 ppm	20 ppm	200 ppm	2000 ppm
Liver	100	79*	71**	104	95	79*
Kidney	88*	90	73**	107	97	101
Brain	98	100	95	97	95	98

From Hamann et al. (2000)
* $P < 0.05$; ** $P < 0.01$ (according to the parametric method of Dunnett)

The effect of subchronic treatment with NAG on the activity of glutamine synthetase was investigated in liver, kidneys and brain. NAG (supplied as an aqueous technical concentrate; concentration 33.8% w/w) was administered in the diet to groups of 10 male Wistar rats for 6, 13, 20 or 90 days at a dose of 1000 or 10 000 ppm. Additional groups of 10 male Wistar rats received NAG at a dose of 1000 or 10 000 ppm for 91 days followed by a 30-day recovery period. Control groups of 10 male Wistar rats received the untreated diet for the same period of time. Achieved nominal intakes of NAG were 63.2 and 658 mg/kg bw per day at 1000 and 10 000 ppm, respectively. Mortality and clinical signs were recorded daily, and feed consumption and body weights were measured weekly. All animals were necropsied at scheduled sacrifice. Organ weights (brain, liver and kidney) were measured, and all macroscopic findings were recorded. Samples of liver, kidney and brain were collected from all animals and processed for measurement of glutamine synthetase activity. Statements of adherence to QA and GLP were included.

No treatment-related effects were noted for clinical signs, feed consumption or body weight.

NAG induced a significant inhibition of glutamine synthetase activity in the liver and kidney (see Table 38). No toxicologically relevant effect of NAG on glutamine synthetase activity in the brain was observed. No cumulative effect over time was observed. The effects were (almost) completely reversed after a 30-day recovery period.

Table 38. Inhibition of glutamine synthetase activity in the rat by NAG

Tissue	Sacrifice after day:	% inhibition	
		1000 ppm	10 000 ppm
Liver	6	4	54**
	13	24**	60**
	20	26**	43**
	90	42**	46**
	Recovery	17*	6
Brain	6	6	7*
	13	0	0
	20	2	4
	90	1	2
	Recovery	3	3
Kidney	6	39**	46**
	13	41**	46**
	20	32**	45*
	90	45**	47**
	Recovery	5	13

From Schmid et al. (1999)
* $P < 0.05$; ** $P < 0.01$ (according to the Dunnett test based on pooled variance)

There were no treatment-related macroscopic findings or effects on liver and brain weights. Necropsy at 6, 13 or 20 days showed an increase in absolute and relative kidney weights in rats treated with NAG (up to 22%) that was fully reversible after 30 days of recovery.

The NOAEL for NAG was 1000 ppm (equal to 63.2 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver at 10 000 ppm (equal to 658 mg/kg bw per day) (Schmid et al., 1999).

The effect of NAG (concentration 33.8% w/w) at doses up to 10 000 µg/ml on the in vitro activity of glutamine synthetase was investigated in samples of liver, kidney and brain (neocortex, medulla oblongata and hypothalamic region) of Wistar rats. Statements of adherence to QA and GLP were included.

NAG induced no significant inhibition up to 1000 µg/ml. At 5000 and 10 000 µg/ml, a minimal level of inhibition was observed, reaching 9% and 15%, respectively, which can be attributed to the presence of glufosinate-ammonium as an impurity and the metabolic deacetylation of NAG to form glufosinate-ammonium. NAG induced no inhibition of glutamine synthetase in kidney over the whole range of concentrations tested.

In the brain (neocortex, medulla and hypothalamus), NAG did not induce any inhibition up to 1000 µg/ml, whereas at 5000 and 10 000 µg/ml, only a minimal inhibition was observed (up to 7%). This can be attributed to the presence of glufosinate-ammonium as an impurity and the metabolic deacetylation of NAG to form glufosinate-ammonium (Luetkemeier, 1999).

6. Observations in humans

No information is available.

C. 3-METHYLPHOSPHINICO-PROPIONIC ACID (MPP)

7. Biochemical aspects

7.1 Absorption, distribution and excretion

[^{14}C]MPP (radiochemical purity 98%) was administered either intravenously or orally by gavage at a single dose of 20 mg/kg bw to groups of five female SPF Wistar rats. The vehicle was an aqueous sodium solution. Urine and faeces were collected on a daily basis for 96 hours and radioassayed by LSC.

Total recovery of radioactivity was 96% and 93% for the oral and intravenous groups, respectively. After oral administration, 92% and 4% of the radiolabel was excreted in urine plus cage wash and faeces, respectively. Over the first 24 hours, 83% and 3% were excreted in urine and faeces, respectively. After intravenous administration, 93% and 0.6% were excreted in urine and faeces, respectively. After intravenous administration, 87% of the dose was excreted in urine over the first 24 hours (Kellner & Eckert, 1984).

7.2 Biotransformation

No information was available.

8. Toxicological studies

8.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with MPP are summarized in Table 39. Treatment-related mortality and signs of toxicity were observed during the acute oral studies.

Table 39. Results of studies of acute toxicity with MPP

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	NMRI	M/F	Oral	2% potato starch in deionized water	> 99.8	3050 (M) 3070 (F)	Diehl & Leist (1988a)[a]
Rat	Wistar	M/F	Oral	Water	> 99	2840 (M) 1900 (F)	Rupprich & Weigand (1984)[b]

F, female; LD_{50}, median lethal dose; M, male

[a] The following clinical signs were observed: reduced spontaneous activity, squatting position, contracted flanks, piloerection, high-legged gait, narrowed palpebral fissures, irregular breathing, reduced placing reflex, ataxic gait, prone or lateral position, gasping, increased respiratory rate, straddling of hind legs, hypersensitivity to touch, crawling locomotion and jerky breathing. Deaths occurred on the 1st day of treatment and up to the 10th day after treatment. Necropsy of animals that died revealed abnormalities in the stomach and intestine (inflated or fluid filled) and various pale-coloured organs. Dissection of the animals killed at the end of the study revealed no macroscopically visible abnormalities.

[b] The following clinical signs were observed: squatting position, contracted flanks, abdominal position, uncoordinated gait, piloerection, quiet behaviour, drowsiness, narrowed eye openings and jerky respiration. Some animals also showed skin pallor, noisy respiration and widening of the palpebral fissures. These clinical signs were more pronounced at the higher dose levels during the observation period. Lethally intoxicated animals died between 23 minutes and 3 days after administration. By day 7 after treatment, practically all of the animals were free of clinical signs. Macroscopic examination of the animals found dead showed petechial haemorrhages in the gastric mucosa, slight quantities of a blood-coloured mass in the stomach, stomach taut with brownish-yellow fluid and feed, a blackish-brown mass in the small intestine, clear to yellowish fluid in the small intestine, mucosa of small intestine whitish in colour, adrenals dark in colour, liver light-coloured in places, lungs congested with blood and lungs greenish-brown in colour. The animals killed at the end of the observation period were free of macroscopically visible changes.

(b) Dermal sensitization

In a dermal sensitization study using the Magnusson and Kligman maximization test, performed in accordance with OECD Test Guideline 406, MPP (purity > 99.8%) was tested in 20 female Pirbright-White guinea-pigs. A preliminary study established 1% and 50% test substance concentrations in saline as suitable for the intradermal induction and dermal induction and challenge phases, respectively. The control group consisted of 10 animals. In the first induction phase, the animals were subjected to two intradermal injections of 50% Freund's Complete Adjuvant, 1% MPP in isotonic saline and 1% MPP in 50% Freund's Complete Adjuvant. Seven days later, the same area of skin was treated by topical application of 0.5 ml of a 50% solution of MPP in saline and the test site covered with an occlusive dressing for 48 hours. The same induction procedure was carried out on control groups with vehicles only. On day 22, all animals were challenged by a 24-hour occluded topical application of 50% MPP in saline. The test sites were assessed 24 and 48 hours after removal of the occlusive bandages. Benzocaine was used as a positive control. Statements of adherence to QA and GLP were included.

The intradermal injections with Freund's Complete Adjuvant (with and without test substance) caused well-defined erythema and slight oedema. After the dermal challenge treatment on day 22, two treated animals showed very slight erythema. Under the conditions of this study, MPP was not a skin sensitizer (Diehl & Leist, 1988b).

8.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female NMRI mice received MPP for 13 weeks at a dietary concentration of 0, 320, 1600, 3200 or 8000 ppm (equal to 0, 55, 264, 522 and 1288 mg/kg bw per day for males and 0, 57, 279, 590 and 1540 mg/kg bw per day for females, respectively). Mortality and clinical signs were checked daily. Body weight and feed and water consumption were recorded weekly. Haematological examinations, clinical chemistry and urine analysis were performed at the end of the study. At termination, all animals were examined macroscopically, and selected organs were weighed. All major organs and tissues were examined microscopically. Statements of adherence to QA and GLP were included.

No mortalities or clinical signs were observed. Body weight gain, feed and water consumption and haematological and urine analysis parameters were not affected by treatment. In males of the high-dose group, a 41% reduction in serum uric acid was observed. At the high dose, relative kidney weights were increased in males (12%) and females (9%). As no treatment-related histopathological changes were found, these changes in relative organ weight were not considered adverse.

The NOAEL was 8000 ppm (equal to 1288 mg/kg bw per day), the highest dose tested (Ebert & Leist, 1989).

Rats

In a dietary range-finding study, Wistar rats (five of each sex per group) were fed diets containing MPP (purity > 99%) for 4 weeks at a concentration of 0, 50, 500, 2500 or 5000 ppm (equal 0, 5.6, 57, 286 and 554 mg/kg bw per day for males and 0, 5.5, 55, 282 and 561 mg/kg bw per day for females, respectively). Rats were examined daily for clinical signs of toxicity. Body weight and feed consumption were measured twice weekly during the study. Water consumption was measured weekly. Haematology, serum biochemical determinations, glutamine synthetase activity in the liver and urine analyses were performed after 4 weeks. At the end of the treatment period, the rats were necropsied, and selected organs were weighed. A wide range of organs of control and high-dose animals was examined microscopically. Statements of adherence to QA and GLP were included.

No deaths or clinical signs of systemic toxicity were observed. No toxicologically relevant effects on body weight gain, feed and water consumption, haematology, clinical chemistry, urine analysis or pathology were observed. Occasionally observed increases in haemoglobin, uric acid and triglyceride levels were within normal biological variation. In the high-dose females, there was a 9% increase in relative liver weight; however, it was not accompanied by histopathological changes, and it was therefore considered not to be toxicologically adverse (Ebert, Leist & Mayer, 1986).

In a 90-day dietary study, groups of 10 male and 10 female Wistar rats received MPP (purity 99.6%) at a concentration of 0, 400, 1600 or 6400 ppm (equal to 0, 34, 127 and 546 mg/kg bw per day for males and 0, 36, 141 and 570 mg/kg bw per day for females, respectively). Additional groups of 10 animals of each sex were maintained for a 4-week post-dosing recovery period. Animals were checked daily for mortality and clinical signs of toxicity. A detailed clinical examination, including a neurological assessment, was performed weekly. Body weights and feed and water consumption were measured weekly. Ophthalmological examinations were carried out pretest and at week 11. Haematology, clinical chemistry and urine analysis were performed at the end of treatment and at the end of the recovery period. At termination, the animals were examined macroscopically, and the major organs were weighed. A wide range of organs and tissues was examined histopathologically. Statements of adherence to QA and GLP were included.

No mortality occurred. No treatment-related effects on body weight gain, feed and water consumption or ophthalmoscopy were observed. Slight, but statistically significant, increases in

reticulocyte numbers in mid- and high-dose males and in lactate dehydrogenase levels in mid-dose females were found. Also, significant increases or decreases in α-, β- and γ-globulin were occasionally observed. However, as the effects were generally not dose dependent and were observed in one sex only, these changes were considered not to be toxicologically relevant. At the high dose, mild increases (up to 12%) in absolute or relative liver weights (males only) and kidney weights (both sexes) were observed at termination or in the recovery animals. In the absence of histopathological or clinical chemistry evidence for an effect of treatment on these organs, the organ weight changes were not considered adverse.

The NOAEL was 6400 ppm (equal to 546 mg/kg bw per day), the highest dose tested (Ebert & Mayer, 1987).

Dogs

In a 28-day oral range-finding study, groups of three male and three female Beagle dogs received MPP (purity 99.2%) in the diet at 0, 100, 320 or 1000 ppm (equal to 0, 7, 22 and 70 mg/kg bw per day for males and 0, 6, 19 and 58 mg/kg bw per day for females, respectively). After treatment, one animal of each sex per dose was kept for a 4-week recovery period. The dogs were observed daily for mortality and clinical signs. Body weight and feed and water consumption were measured weekly. The neurological status, ophthalmoscopy, a hearing test, haematology, clinical chemistry (including measurement of acetylcholinesterase activity in plasma, erythrocytes and brain) and urine analysis were assessed before dosing started and before termination of the study. At the end of the recovery period, haematology, clinical chemistry and urine analysis were also performed. At termination, all animals were necropsied, and major organs were weighed. A wide range of organs and tissues was examined histologically. Glutamine synthetase activity in the liver, kidney and brain was assessed. Statements of adherence to QA and GLP were included.

No mortality occurred. None of the dogs showed treatment-related effects on any of the parameters examined (Brunk & Mayer, 1987).

In a 90-day dietary study, MPP was administered to groups of Beagle dogs at a level of 0, 100, 400 or 1600 ppm (equal to 0, 7, 29 and 115 mg/kg bw per day for males and 0, 6, 26 and 103 mg/kg bw per day for females, respectively). All groups consisted of six animals of each sex, except for the low-dose group, which consisted of four dogs of each sex. Two males and two females each for the control, mid- and high-dose groups were kept under observation for a recovery period of about 4 weeks. Viability, clinical signs and feed consumption were assessed daily. Body weights were measured weekly. Neurological and hearing tests, ophthalmology, haematology, clinical chemistry and urine analysis were performed pretreatment, after approximately 6 weeks, before termination of the main phase of the study and towards the end of the recovery period. The hepatic and renal functions were tested with BSP and PSP at the same time points. At necropsy, organs were examined macroscopically and weighed, and selected organs and tissues were examined histopathologically. Glutamine synthetase activity in liver, kidney and brain was determined in all dogs at the time of sacrifice. Statements of adherence to QA and GLP were included.

No mortalities or signs of toxicity were noted. There was no effect on feed consumption or body weight. Neurological, ophthalmological and hearing tests and haematology and urine analysis revealed no effect of treatment. A number of clinical chemistry parameters showed small, but statistically significant, changes. However, often these findings were not dependent on dose, were not found consistently over the time points and were found in one sex only. These findings were considered to be incidental and not related to treatment. Glutamine synthetase activity and liver and kidney function were not affected by treatment. Macroscopic, histopathological and organ weight examination revealed no treatment-related effects.

The NOAEL was 1600 ppm (equal to 103 mg/kg bw per day), the highest dose tested (Brunk, 1988).

8.3 Long-term studies of toxicity and carcinogenicity

No information was available.

8.4 Genotoxicity

MPP was tested for genotoxicity in a limited range of guideline-compliant assays. No evidence for genotoxicity was observed.

The results of the genotoxicity tests are summarized in Table 40.

Table 40. Overview of genotoxicity tests with MPP[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 *uvrA*	4–5000 µg/plate (±S9)	> 99	Negative	Jung & Weigand (1984)[b,c]
Forward mutation	*Schizosaccharomyces pombe* strain P1	313–10 000 µg/ml (±S9)	99.8	Negative	Nunziata & Haroz, (1989)[b,d]
Mitotic recombination	*Saccharomyces cerevisiae* D4	625–10 000 µg/ml (±S9)	95.3	Negative	Forster (1989)[b,e]
Chromosomal aberrations	Human lymphocytes	100–1520 µg/ml (±S9)	99.6	Negative	Heidemann (1988)[f]
Gene mutation	V79 Chinese hamster cells, HPRT test	100–1000 µg/ml (±S9)	99.6	Negative	Müller (1988)[g]
Unscheduled DNA synthesis	Human cell line A 549	1–2000 µg/ml	99.6	Negative	Kramer & Müller (1987)[h]
In vivo					
Chromosomal aberrations	Chinese hamster bone marrow	0, 100, 333 or 1000 mg/kg bw (gavage)	99.8	Negative	Voelkner & Müller (1988)[i]
Micronucleus formation	NMRI mouse bone marrow	0, 200, 600 or 2000 mg/kg bw (gavage)	96.9	Negative	Müller (1989e)[j]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to QA and GLP were included.

[c] Toxicity was observed at doses of 500 µg/plate and higher. A small increase in number of revertant colonies of TA1535 at 5000 µg/plate was attributed to cytotoxicity observed at this dose level.

[d] Batch Ka 417/II.

[e] Batch Ka 417/II. Test design resembles OECD Test Guideline No. 481.

[f] Batch H404. Performed in accordance with OECD Test Guideline No. 473.

[g] Batch H404. Performed in accordance with OECD Test Guideline No. 476.

[h] Batch H404. Test design resembles OECD Test Guideline No. 482.

[i] Batch Ka 417/II. Performed in accordance with OECD Test Guideline No. 475. In a range-finding test, all animals at 1500, 2000 and 4000 mg/kg bw expressed signs of toxicity, such as reduction of spontaneous activity, eyelid closure and apathy. Mortalities were also observed. At 1000 mg/kg bw, all animals survived despite exhibiting signs of toxicity.

Table 40 (continued)

[j] Batch KA 417/II. Study design resembles OECD Test Guideline No. 474. At 2000 mg/kg bw, one female died 24 hours after treatment, and another animal was killed because of an unusual oedema in the right anterior leg. These animals displayed reduced spontaneous activity, narrowed palpebral fissures, forward movement in crawling posture and piloerection. The animals that survived were all free of clinical signs of toxicity. The ratio of polychromatic erythrocytes to normocytes remained essentially unaffected by the test compound; thus, there is no clear evidence that MPP reached the bone marrow. However, as sublethal doses were used, it is likely that the substance reached the systemic circulation and the target tissue.

8.5 Reproductive and developmental toxicity

(a) Multigeneration studies

No information is available.

(b) Developmental toxicity

Rats

In a developmental toxicity study, groups of 20 mated female Wistar rats were treated by gavage with MPP (purity 99.6%) in distilled water at a dose level of 0, 100, 300 or 900 mg/kg bw per day from days 7 through 16 of gestation (day 1 = day on which sperm were detected in the vaginal smear). Clinical signs were recorded daily. Body weight and feed consumption were measured weekly. All females were killed on day 21 of gestation and examined macroscopically, and selected organs were weighed. The uterus was examined, and the numbers of live and dead fetuses, resorptions, corpora lutea and implantations were counted. Body weight, crown–rump lengths and sex ratios of the fetuses as well as placental weight and diameter of fetuses undergoing resorption were recorded. About half of the fetuses from each litter were selected for skeletal examinations, and the other half for cross-sectional visceral examinations. Statements of adherence to QA and GLP were included.

At 900 mg/kg bw per day, one female died on GD 11 after having shown signs of flabbiness, disequilibrium, piloerection and increased urinary excretion. At this dose, feed consumption (6.75 g/100 g bw versus 8.62 g/100 g in controls) and body weight gain (18.9 g versus 32.3 g in controls) were reduced during the 1st week of treatment, and 10 females displayed piloerection persisting for several days. Two dams in this dose group had a bloody secretion in the vagina on days 13 and 15 of pregnancy, respectively. At 100 and 300 mg/kg bw per day, no evidence of treatment-related clinical signs or effects on feed consumption or body weight gain was found. At termination, no treatment-related macroscopic changes were found in any of the dose groups. Absolute kidney weight was increased by 19% in the high-dose females.

Three dams of the high-dose group and one dam of the mid-dose group lost all their conceptuses. No effects of treatment on fetal body weights and body lengths, sex ratio, placental weight, or incidence of skeletal and visceral abnormalities were found. At the high dose, an increased number of fetuses with wavy and/or thickened ribs was observed (15 versus 6 in the control group). However, the incidence of this finding was within the historical control range, and, in the absence of other developmental effects, it is considered unlikely to be related to treatment.

The NOAEL for maternal toxicity was 300 mg/kg bw per day, on the basis of one mortality, clinical signs of toxicity, reduced body weight gain and reduced feed consumption observed at 900 mg/kg bw per day. The NOAEL for fetal toxicity was 300 mg/kg bw per day, on the basis of three dams with total litter loss at 900 mg/kg bw per day. There was no evidence that MPP had a teratogenic effect (Albrecht & Baeder, 1994a).

Rabbits

Groups of 15 mated Himalayan rabbits received, by gavage, MPP at 0, 50, 100 or 200 mg/kg bw per day from days 6 to 18 of gestation. The vehicle was distilled water. The dose levels were

based on a range-finding study. Animals were examined daily for clinical signs and mortality. Body weight and feed consumption were recorded on GDs 0, 6, 13, 19 and 29. At GD 29, the dams were killed and examined macroscopically for abnormalities, and selected organs were weighed. The uterus was examined, and the numbers of live and dead fetuses and resorptions were determined. The live fetuses were removed and checked for viability for 24 hours. Body weights, crown–rump lengths, sex ratios of the fetuses and placental weights were recorded, and external, visceral and skeletal examinations of the fetuses were performed. Statements of adherence to QA and GLP were included.

Five dams from the high-dose group and one from the mid-dose group died during the study. In addition, four dams from the high-dose group and one from the 100 mg/kg bw per day group aborted and were killed. In these animals, signs of intoxication, such as disequilibrium, were observed, accompanied by reduced feed and water consumption and body weight reductions. In the surviving animals, body weight gains, feed consumption and the results of the macroscopic examination showed no effect of treatment. All surviving females in all groups carried live fetuses to full term. The number of corpora lutea, number of implantations and number of live fetuses in the treated dams that carried live fetuses to full term were not different from those for control dams. In the mid-dose dam that died and one high-dose dam that died, the conceptuses corresponded to the stage of gravidity at the time of death. Two of the five dams of the 200 mg/kg bw per day group that died had severely stunted fetuses, whereas two others had dead conceptuses undergoing resorption. In the high-dose dams killed due to suspected abortions, three dams had only 5–8 conceptuses, which perished at an early stage of gravidity and were undergoing resorption, whereas one dam had 9 conceptuses at the normal stage of development. In the 100 mg/kg bw per day group, the dam killed on day 22 due to suspected abortion had seven conceptuses undergoing resorption and a single live fetus that was normally developed. In all treatment groups, the dams that survived until termination showed no impairment of the intrauterine development of the conceptuses, the fetuses were normally developed and there was no increase in the number of dead conceptuses, except for one high-dose dam that had only one live fetus together with nine resorptions.

The live fetuses delivered in the treatment groups were normally developed. The body weights, body lengths, sex ratio, placental weight or placental gross appearance did not differ from controls. The survival rate of the fetuses 24 hours after delivery was high in all treated groups and showed no difference compared with the control group. There was no treatment-related increase in the incidence of external, skeletal and visceral malformations and variations. In the 50 mg/kg bw per day group, the number of fetuses with fewer than 13 ossified caudal vertebrae was increased. As a delay of skeletal ossification was not observed in the two higher-dose groups, this finding was considered not treatment related.

The NOAEL for maternal toxicity was 50 mg/kg bw per day, on the basis of one mortality, one abortion, clinical signs of toxicity, reduced body weight gain and reduced feed consumption observed at 100 mg/kg bw per day. The NOAEL for fetal toxicity was 50 mg/kg bw per day, on the basis of one dam with seven conceptuses undergoing resorption at 100 mg/kg bw per day. There was no evidence that MPP had a teratogenic effect (Albrecht & Baeder, 1994b).

8.6 Special studies

The capacity of MPP (purity > 99%) to inhibit glutamine synthetase activity was evaluated in addition to the other toxicology parameters. Groups of five male and five female Wistar rats received MPP in the diet at 0, 50, 500, 2500 or 5000 ppm (equal to 0, 5.6, 57, 286 and 554 mg/kg bw per day for males and 0, 5.5, 55, 282 and 561 mg/kg bw per day for females, respectively) for 28 days. The effect of MPP on glutamine synthetase activity in the liver was examined in the control and highest-dose groups. The effects on other toxicological parameters are described in the section on short-term toxicity (see above). Statements of adherence to QA and GLP were included.

No inhibition of glutamine synthetase activity in the liver was observed at 5000 ppm when compared with the control group. A slightly increased (approximately 10%) liver weight was

observed in females at 5000 ppm only. No change in kidney weight was observed (Ebert, Leist & Mayer, 1986).

9. Observations in humans

No information was available.

D. 2-METHYLPHOSPHINICO-ACETIC ACID (MPA)

10. Biochemical aspects

No information was available.

11. Toxicological studies

11.1 Acute toxicity

The results of studies of acute toxicity with MPA are summarized in Table 41. Treatment-related mortality and signs of toxicity were observed during the acute oral studies.

Table 41. Results of study of acute toxicity with MPA

Species	Strain	Sex	Route	Vehicle	Purity (%)	LD_{50} (mg/kg bw)	Reference
Rat	Sprague-Dawley	M/F	Oral	Deionized water	98.5	> 2000	Hammerl (1998)[a]

F, female; LD_{50}, median lethal dose; M, male

[a] Performed according to OECD Test Guideline No. 401. Batch 28688-92. Between 2 and 8 hours after administration, diarrhoea occurred in all treated rats. No other clinical signs were observed during the whole observation period.

11.2 Short-term studies of toxicity

Rats

In a 2-week dietary range-finding study, Wistar rats (six of each sex per group) were fed diets containing MPA (purity > 98.2%) at a concentration of 0, 2000, 5000 or 10 000 ppm (equal to 0, 243, 604 and 1231 mg/kg bw per day for males and 0, 248, 615 and 1128 mg/kg bw per day for females, respectively). Rats were examined daily for clinical signs of toxicity. Body weight and feed consumption were measured twice weekly. Haematology, serum biochemical determinations, glutamine synthetase activity in the liver and urine analyses were performed after 2 weeks. At the end of the treatment period, the rats were necropsied, and selected organs were weighed. The major organs of control and high-dose animals were examined microscopically.

No deaths or clinical signs of systemic toxicity were observed. No toxicologically relevant effects on body weight gain or feed consumption were observed. An increased body weight gain in mid- and high-dose females was attributed to a lower body weight of control females at the start of the test. Haematology, clinical chemistry and urine analysis showed no treatment-related effects. The few macroscopic or histological findings in control or treatment groups are considered to have occurred spontaneously and not to be treatment related (Syntin, 2001).

In a 90-day dietary study, groups of 10 male and 10 female Wistar rats received MPA (purity 98.3%) at a concentration of 0, 500, 2000 or 10 000 ppm (equal to 0, 34, 140 and 684 mg/kg bw per day for males and 0, 38, 156 and 772 mg/kg bw per day for females, respectively). Animals were checked daily for mortality and clinical signs of toxicity. A detailed clinical examination was performed weekly. Body weights and feed consumption were measured weekly. Ophthalmological

examinations was carried out pretest and at week 13. Haematology, clinical chemistry and urine analysis were performed at the end of treatment. The animals were tested in a functional observational battery during week 12. At termination, the animals were examined macroscopically, and the major organs were weighed. An extensive range of organs and tissues of the control and high-dose animals and all macroscopic lesions were examined histopathologically. Statements of adherence to QA and GLP were included.

No mortality or treatment-related clinical signs were found. Body weight gain, feed consumption and ophthalmoscopic, haematological, clinical chemistry and urine analysis parameters were not affected by treatment. The few differences that were occasionally noted were slight and within the historical control range. Postmortem examination revealed no treatment-related macroscopic, histological or organ weight changes.

The NOAEL was 10 000 ppm (equal to 684 mg/kg bw per day), the highest dose tested (Richard, 2001).

11.3 Long-term studies of toxicity and carcinogenicity

No information was available.

11.4 Genotoxicity

MPA was tested for genotoxicity in three guideline-compliant assays. No evidence for genotoxicity was observed in any test.

The results of the genotoxicity tests are summarized in Table 42.

Table 42. Overview of genotoxicity tests with MPA[a]

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Point mutations	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2 *uvrA*	1.6–5000 µg/plate (±S9)	98.2	Negative	Ballantyne (2001b)[b,c]
Chromosomal aberrations	Human lymphocytes	745.9–1821 µg/ml (−S9) 1024–1821 µg/ml (+S9)	98.2	Negative	Lloyd (2001a)[b,d]
Gene mutation	Mouse lymphoma L5178Y *Tk* locus	56.25–1821 µg/ml (±S9)	98.2	Negative	Lloyd (2001b)[b,e]

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] Positive and negative (solvent) controls were included in all studies.

[b] Statements of adherence to QA and GLP were included.

[c] Batch 28688-92. Performed in accordance with OECD Test Guideline No. 471.

[d] Batch 28688-92. Performed in accordance with OECD Test Guideline No. 476.

[e] Batch 28688-92. Performed in accordance with OECD Test Guideline No. 473.

11.5 Reproductive and developmental toxicity

No information was available.

12. Observations in humans

No information was available.

Comments

Glufosinate-ammonium

Biochemical aspects

After administration of single (2–500 mg/kg bw) or repeated oral doses (2 mg/kg bw), [^{14}C]glufosinate-ammonium was rapidly but incompletely absorbed (approximately 10%). Peak plasma concentrations were reached within 0.5–1 hour. The radiolabel was widely distributed, with highest concentrations in liver and kidneys. Radiolabel concentrations were low in the brain and fetus. The plasma half-life of the initial elimination phase was 4–5 hours. Excretion after single or repeated doses was rapid, with more than 90% excreted within 24 hours after administration of a low dose. Administration of higher doses resulted in slower absorption and excretion. In faeces, mainly glufosinate-ammonium and low concentrations (up to 10% of faecal radioactivity) of NAG were found, indicative of acetylation by microflora in the gut, as this metabolite is not found in urine. In urine, parent compound represented about 50% of the radioactivity, whereas MPB and MPP each represented 8–22% of the urinary radioactivity. Very low levels of MPA were found in urine. MPP represented 10–20% of residue found in liver. There were no marked sex differences in the kinetics and metabolism of glufosinate-ammonium.

Toxicological data

In 1999, the Meeting considered reports on the relevance of glutamine synthetase activity in the liver, kidney and brain of experimental animals and humans and concluded the following:

- A less than 50% inhibition of glutamine synthetase activity in rat liver was not associated with increased ammonia concentrations and thus was not considered to be adverse.
- Inhibition of kidney glutamine synthetase activity in the absence of pathological findings was not considered to be relevant to human risk assessment.
- Any statistically significant inhibition of glutamine synthetase activity in brain by more than 10% was considered a marker of potentially adverse effects on brain biochemistry and behaviour.

The present Meeting confirmed the conclusion of the 1999 JMPR, which is also supported by a recent published study on the essential role of glutamine synthetase in the implantation of mouse embryos.

The acute toxicity of glufosinate-ammonium is low in rats (LD_{50} > 1500 mg/kg bw; dermal LD_{50} > 2000 mg/kg bw; inhalation $LC_{50} \geq 1.26$ mg/l). Glufosinate-ammonium is not irritating to the skin or eyes of rabbits and is not a skin sensitizer (Magnusson and Kligman test and Buehler test in guinea-pigs; local lymph node assay in mice).

In acute toxicity studies in mice, clinical signs of neurotoxicity were observed at 231 mg/kg bw (the lowest dose tested) and above. Mortality was observed at doses greater than or equal to 300 mg/kg bw.

In a single-dose toxicity study in dogs, clinical signs of neurotoxicity were observed at 200 mg/kg bw (the lowest dose tested), and mortality was observed at 400 mg/kg bw.

In three 13-week dietary studies in mice (two of them being range-finding studies), the overall NOAEL was 1280 ppm (equal to 278 mg/kg bw per day), based on clinical signs (ruffled fur, sedation, ventral recumbence or hunched posture, and emaciation) observed at 3500 ppm (equal to 561 mg/kg bw per day).

In a 28-day dietary range-finding and two 13-week dietary studies in rats, the overall NOAEL was 4000 ppm (equal to 263 mg/kg bw per day), based on neurological effects in both sexes and reduced body weight gain and feed consumption, reductions in erythrocyte count and low reticulocyte

ratios in males at 7500 ppm (equal to 521 mg/kg bw per day). Glutamine synthetase activity was not measured in these studies.

In a 28-day range-finding capsule study in dogs, the NOAEL was 1 mg/kg bw per day, based on reductions in glutamine synthetase activity in the central nervous system (8–53%), a slight increase in spontaneous motor activity that occurred within a few days after the start of treatment and reductions in body weight gain and feed consumption observed during the 1st week of treatment at 8 mg/kg bw per day. In a 90-day dietary study in dogs, the NOAEL was 64 ppm (equal to 2.0 mg/kg bw per day), based on a reduction in body weight gain and feed consumption in females at 256 ppm (equal to 7.8 mg/kg bw per day).

In a 1-year dietary study in dogs, mortality on days 10 and 14 and severe clinical signs, starting on day 9 of treatment, were observed after treatment with 375 ppm (equal to 10.6–16.0 mg/kg bw per day). The two deaths out of 16 animals at the high dose were caused by heart and circulatory failure attributed to marked myocardial necrosis in one dog and to severe necrotizing aspiration pneumonia in the other dog. After lowering the dose to 250 ppm (equal to 8.4 mg/kg bw per day), no adverse effects were observed. The NOAEL was 150 ppm (equal to 4.5 mg/kg bw per day). The study indicates that glufosinate-ammonium has a steep dose–response curve in dogs. In the absence of glutamine synthetase measurements in the 90-day and 1-year studies in dogs, an overall NOAEL of 1 mg/kg bw per day was established for these studies.

In a 2-year feeding study in mice, the NOAEL was 80 ppm (equal to 10.8 mg/kg bw per day), based on increased mortality and reduced body weight gain in males and changes in clinical chemistry parameters in both sexes at the next higher dose (equal to 23 mg/kg bw per day in males and 64 mg/kg bw per day in females). No effect on tumour incidence was observed.

In a 130-week feeding study in rats, the NOAEL was 140 ppm (equal to 7.6 mg/kg bw per day), based on effects on haematology, glutathione levels in liver and blood, and a reduction of brain glutamine synthetase activity at 500 ppm (equal to 26.7 mg/kg bw per day). In this study and a 2-year carcinogenicity study in rats, no effect on tumour incidence was found.

The Meeting concluded that glufosinate-ammonium is not carcinogenic in mice or rats.

Glufosinate-ammonium was tested for genotoxicity in an adequate range of studies of genotoxicity in vitro and in vivo. No evidence for genotoxicity was observed in any test.

The Meeting concluded that glufosinate-ammonium is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that glufosinate-ammonium is unlikely to pose a carcinogenic risk to humans.

A range-finding one-generation study and a two-generation study of reproductive toxicity in rats were available. The overall NOAEL for parental toxicity was 500 ppm (equal to 44 mg/kg bw per day), based on reduced feed consumption in males at 2500 ppm (equal to 206 mg/kg bw per day). At 2500 ppm and above, the dams delivered no pups. The overall NOAEL for offspring toxicity was 500 ppm (equal to 44 mg/kg bw per day), the highest dose at which dams produced a litter. The overall NOAEL for reproductive toxicity was 120 ppm (equal to 8.7 mg/kg bw per day), based on reduced litter sizes in all litters at 360 ppm (equal to 18 mg/kg bw per day). The Meeting considered the possibility that the increased preimplantation loss observed in the range-finding one-generation study of reproductive toxicity at 2500 ppm (equal to 207 mg/kg bw per day) might be caused by an inhibition of glutamine synthetase activity prior to implantation; a published mechanistic study in mice indicates that glutamine synthetase activity in preimplantation embryonic cells is essential for the blastocyst to complete implantation. The Meeting concluded that the preimplantation loss and early deaths in the reproductive toxicity studies might be caused by a single exposure to glufosinate-ammonium.

In three developmental toxicity studies in rats, the overall NOAEL for maternal toxicity was 10 mg/kg bw per day, based on clinical signs and abortions at 50 mg/kg bw per day. The overall

NOAEL for developmental toxicity was 10 mg/kg bw per day, based on intrauterine deaths at 50 mg/kg bw per day.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 6.3 mg/kg bw per day, based on clinical signs, body weight loss and reduced feed consumption, an increased number of abortions and increased kidney weight at 20 mg/kg bw per day. The NOAEL for developmental toxicity was 6.3 mg/kg bw per day, based on an increased number of dead fetuses at 20 mg/kg bw per day.

The Meeting concluded that glufosinate-ammonium is not teratogenic in rats or rabbits.

In an acute gavage study of neurotoxicity in rats, the NOAEL was 100 mg/kg bw, based on clinical signs at 500 mg/kg bw.

In a dietary 38-day neurotoxicity study in rats and a 90-day dietary study investigating brain and liver glutamine synthetase inhibition in rats, the overall NOAEL was 100 ppm (equivalent to 6.2 mg/kg bw per day), based on a greater than 50% reduction in glutamine synthetase activity in the liver in males at 200 ppm (equal to 15 mg/kg bw per day).

In a dietary developmental neurotoxicity study in rats, the NOAEL for maternal toxicity was 1000 ppm (equal to 69 mg/kg bw per day), based on decreased body weight gain and feed consumption at 4500 ppm (equal to 292 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 14 mg/kg bw per day), based on reduced body weight gain during the preweaning period, effects on motor activity at postnatal days 17, 21 and 62, and hippocampal pathology in males at 1000 ppm (equal to 69 mg/kg bw per day).

Medical surveillance of plant production personnel did not find any effects related to the production of glufosinate-ammonium. Several human poisoning cases, sometimes leading to death, due to (suicidal) ingestion of glufosinate-ammonium have been reported in the literature. A variety of neurological symptoms have been described. It is not clear whether the toxicity was due to the active ingredient, to the surfactant contained in relatively high amounts in the formulation or to the combination of both.

Toxicological studies with the metabolites NAG, MPP and MPA, three metabolites that are found in plants, soil and livestock as well as in laboratory animals, were available. The toxicity of NAG, MPP and MPA is described separately below.

The Meeting concluded that the existing database on glufosinate-ammonium was adequate to characterize the potential hazards to fetuses, infants and children.

N-Acetyl-glufosinate (NAG)

Biochemical aspects

After administration of a single oral dose (3 mg/kg bw) of ^{14}C-labelled NAG to rats, NAG was rapidly but incompletely absorbed (approximately 5–10%). Peak plasma concentrations were reached within 1 hour. The highest residue levels were found in kidneys, followed by liver. Excretion after a single oral dose (3 mg/kg bw) was rapid, with approximately 95% of the absorbed dose excreted within 24 hours after administration. The absorbed NAG was predominantly excreted in urine. In faeces, mainly unchanged NAG was found, but about 10% was deacetylated to glufosinate by the intestinal microflora. In faeces, urine and tissues, minor amounts of MPP and MPA were found.

Toxicological data

The oral acute toxicity of NAG is low in rats and mice (LD_{50} > 2895 mg/kg bw). NAG is not a skin sensitizer (Magnusson and Kligman test in guinea-pigs).

In 4-week and 13-week dietary studies with NAG in mice, the overall NOAEL was 500 ppm (equal to 83 mg/kg bw per day), based on the inhibition of brain glutamine synthetase activity (11–13%) at 2000 ppm (equal to 233 mg/kg bw per day).

In a 4-week dietary range-finding study, two 13-week dietary studies and a 38-day dietary neurotoxicity study in rats, the overall NOAEL was 2000 ppm (equal to 159 mg/kg bw per day), based on statistically significant inhibition (11–12%) of liver glutamine synthetase activity at 10 000 ppm (equal to 658 mg/kg bw per day). Brain glutamine synthetase activity was reduced at 10 000 ppm (equal to 738 mg/kg bw per day). In the neurotoxicity study, no effects on glutamine synthetase and neurotoxicity parameters were observed at doses up to 2000 ppm (equal to 159 mg/kg bw per day), the highest dose tested.

In a 13-week dietary study in dogs, the NOAEL was 500 ppm (equal to 20 mg/kg bw per day), based on a reduction in brain glutamine synthetase activity (≥ 16%) at 2000 ppm (equal to 76 mg/kg bw per day).

In a 2-year dietary carcinogenicity study in mice, there were no toxicological findings and no increase in tumour incidence at the highest dose tested of 8000 ppm (equal to 1188 mg/kg bw per day). Glutamine synthetase activity was not measured.

In a 2-year dietary toxicity study in rats, the NOAEL was 2000 ppm (equal to 91 mg/kg bw per day), based on decreased body weight gain, increased incidence of soft faeces and increased incidences of polyarteritis nodosa in blood vessels and testes and urolithiasis at 20 000 ppm (equal to 998 mg/kg bw per day).

The Meeting concluded that NAG is not carcinogenic in mice or rats.

NAG was tested for genotoxicity in an adequate range of in vitro and in vivo studies. No evidence for genotoxicity was observed in any test.

The Meeting concluded that NAG is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that NAG is unlikely to pose a carcinogenic risk to humans.

In a range-finding one-generation study and a two-generation study of reproductive toxicity with NAG in rats, the NOAEL for parental, offspring and reproductive toxicity was 10 000 ppm (equal to 622 mg/kg bw per day), the highest dose tested.

In a developmental toxicity study in rats, the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the only dose tested.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 64 mg/kg bw per day, based on reduced feed consumption at 160 mg/kg bw per day. The NOAEL for developmental toxicity was 64 mg/kg bw per day, based on an increased incidence of extra thoracic ribs at 160 mg/kg bw per day.

The Meeting concluded that NAG is not teratogenic in rats or rabbits.

In two acute oral (gavage) studies of neurotoxicity in rats, the NOAEL was 1000 mg/kg bw, based on clinical signs (diarrhoea, ruffled fur and sedation) observed at 2000 mg/kg bw. No overt neurotoxicity was observed. Glutamine synthetase activity was not measured.

The Meeting concluded that the existing database on NAG was adequate to characterize the potential hazards to fetuses, infants and children.

3-Methylphosphinico-propionic acid (MPP)

Biochemical aspects

During the first 24 hours following administration of a single oral dose of ^{14}C-labelled MPP to rats, 83% and 3% of the radiolabel were excreted in urine and faeces, respectively.

Toxicological data

The acute oral toxicity of MPP is low in rats (oral LD_{50} = 1900 mg/kg bw). MPP is not a skin sensitizer (Magnusson and Kligman test in guinea-pigs).

In short-term dietary studies in mice (13 weeks, doses up to 8000 ppm, equal to 1288 mg/kg bw per day), rats (4 weeks and 13 weeks, doses up to 6400 ppm, equal to 546 mg/kg bw per day) and dogs (28 days and 90 days, doses up to 1600 ppm, equal to 103 mg/kg bw per day), no toxicity was observed. In the 4-week study in rats, glutamine synthetase activity in liver was not affected at doses up to 5000 ppm (equal to 554 mg/kg bw per day). In the two short-term studies in dogs, glutamine synthetase activity in liver, kidney and brain was not affected at doses up to 1600 ppm (equal to 103 mg/kg bw per day).

No long-term studies with MPP were available.

Glufosinate-ammonium was tested for genotoxicity in a limited range of studies in vitro. No evidence for genotoxicity was observed in any of these tests.

In a developmental toxicity study in rats, the NOAEL for maternal toxicity was 300 mg/kg bw per day, on the basis of one death out of 20 animals, clinical signs of toxicity and reduced body weight gain and feed consumption observed at 900 mg/kg bw per day. The NOAEL for fetal toxicity was 300 mg/kg bw per day, on the basis of 3 dams out of 20 with total litter loss at 900 mg/kg bw per day.

In a developmental toxicity study in rabbits, the NOAEL for maternal toxicity was 50 mg/kg bw per day, on the basis of one death out of 15 animals, one abortion, clinical signs of toxicity and reduced body weight gain and feed consumption observed at 100 mg/kg bw per day. The NOAEL for fetal toxicity was 50 mg/kg bw per day, on the basis of one dam with seven conceptuses undergoing resorption at 100 mg/kg bw per day.

The Meeting concluded that MPP is not teratogenic in rats or rabbits.

The Meeting concluded that the existing database on MPP was adequate to characterize the potential hazards to fetuses, infants and children.

2-Methylphosphinico-acetic acid (MPA)

The acute oral toxicity of MPA in rats was low (LD_{50} > 2000 mg/kg bw). In a 90-day dietary study in rats, the NOAEL was 10 000 ppm (equal to 684 mg/kg bw per day), the highest dose tested. Glutamine synthetase activity was not measured, but in view of the structural similarity between MPA and MPP, the Meeting considered it unlikely that MPA would inhibit this enzyme. MPA was not genotoxic in three genotoxicity tests in vitro.

Toxicological evaluation

The present Meeting compared the toxicity of NAG, MPP and MPA with that of glufosinate-ammonium and concluded that the toxicity of the metabolites was less than that of the parent compound. The Meeting established an ADI of 0–0.01 mg/kg bw for glufosinate-ammonium, on the basis of an overall NOAEL of 1 mg/kg bw per day, for reductions in glutamine synthetase activity in the brain of dogs. A safety factor of 100 was applied. This ADI also applies to its metabolites NAG, MPP and MPA. In view of the lower toxicity of NAG, MPP and MPA compared with glufosinate-ammonium, the Meeting noted that the application of the ADI to these metabolites is likely to be conservative. This ADI is considered to be adequately protective for any reproductive and developmental effects.

The Meeting established an acute reference dose (ARfD) for glufosinate-ammonium of 0.01 mg/kg bw, based on the NOAEL of 1 mg/kg bw per day in the 28-day capsule study in dogs for an increase in spontaneous motor activity that occurred within a few days after the start of treatment and reductions in body weight gain and feed consumption observed during the 1st week of treatment with 8 mg/kg bw per day and application of a safety factor of 100. This ARfD also applies to its

metabolites NAG, MPP and MPA. In view of the lower acute toxicity of NAG, MPP and MPA compared with glufosinate-ammonium, the Meeting noted that the application of the ARfD to these metabolites is likely to be conservative. This ARfD is considered to be adequately protective for any reproductive and developmental effects.

Levels relevant for risk assessment of glufosinate-ammonium

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	80 ppm, equal to 10.8 mg/kg bw per day	160 ppm, equal to 23 mg/kg bw per day
		Carcinogenicity	23 mg/kg bw per day[b]	—
Rat	Short-term studies of toxicity[c,d,e]	Toxicity	100 ppm, equal to 6.2 mg/kg bw per day	200 ppm, equal to 15 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	140 ppm, equal to 7.6 mg/kg bw per day	500 ppm, equal to 26.7 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 26.7 mg/kg bw per day[b]	—
	One- and two-generation studies of reproductive toxicity[a,d]	Parental toxicity	500 ppm, equal to 44 mg/kg bw per day	2500 ppm, equal to 206 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 44 mg/kg bw per day[b]	—
		Reproductive toxicity	120 ppm, equal to 8.7 mg/kg bw per day	360 ppm, equal to 18 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	10 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	50 mg/kg bw per day
	Developmental neurotoxicity study[c]	Maternal toxicity	1000 ppm, equal to 69 mg/kg bw per day	4500 ppm, equal to 292 mg/kg bw per day
		Embryo and fetal toxicity	200 ppm, equal to 14 mg/kg bw per day	1000 ppm, equal to 69 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	6.3 mg/kg bw per day	20 mg/kg bw per day
		Embryo and fetal toxicity	6.3 mg/kg bw per day	20 mg/kg bw per day
Dog	Short-term study of toxicity[f]	Toxicity	1 mg/kg bw per day	8 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.
[e] Based on inhibition of liver glutamine synthetase activity in a neurotoxicity study.
[f] Capsule administration.

Levels relevant for risk assessment of NAG

Species	Study	Effect	NOAEL	LOAEL
Mouse	Short-term studies of toxicity[a,b]	Toxicity	500 ppm, equal to 83 mg/kg bw per day	2000 ppm, equal to 233 mg/kg bw per day
	Two-year study of carcinogenicity[a]	Carcinogenicity	8000 ppm, equal to 1188 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
Rat	Short-term studies of toxicity[a,b]	Toxicity	2000 ppm, equal to 159 mg/kg bw per day	10 000 ppm, equal to 658 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	2000 ppm, equal to 91 mg/kg bw per day	20 000 ppm, equal to 998 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 998 mg/kg bw per day[c]	—
	One- and two-generation studies of reproductive toxicity[a,b]	Parental toxicity	10 000 ppm, equal to 622 mg/kg bw per day[c]	—
		Offspring toxicity	10 000 ppm, equal to 622 mg/kg bw per day[c]	—
		Reproductive toxicity	10 000 ppm, equal to 622 mg/kg bw per day[c]	—
	Developmental toxicity study[d]	Maternal toxicity	1000 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	1000 mg/kg bw per day[e]	—
Rabbit	Developmental toxicity study[d]	Maternal toxicity	64 mg/kg bw per day	160 mg/kg bw per day
		Embryo and fetal toxicity	64 mg/kg bw per day	160 mg/kg bw per day
Dog	Short-term study of toxicity[a]	Toxicity	500 ppm, equal to 20 mg/kg bw per day	2000 ppm, equal to 76 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.
[e] Only dose tested.

Levels relevant for risk assessment of MPP

Species	Study	Effect	NOAEL	LOAEL
Mouse	Short-term study of toxicity[a]	Toxicity	8000 ppm, equal to 1288 mg/kg bw per day[b]	—
Rat	Short-term study of toxicity[a]	Toxicity	6400 ppm, equal to 546 mg/kg bw per day[b]	—
	Developmental toxicity study[c]	Maternal toxicity	300 mg/kg bw per day	900 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day	900 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
Dog	Short-term study of toxicity[a]	Toxicity	1600 ppm, equal to 103 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Levels relevant for risk assessment of MPA

Species	Study	Effect	NOAEL	LOAEL
Rat	Short-term study of toxicity[a]	Toxicity	10 000 ppm, equal to 684 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw (ADI for glufosinate-ammonium, also applies to NAG, MPP and MPA)

Estimate of acute reference dose

0.01 mg/kg bw (ARfD for glufosinate-ammonium, also applies to NAG, MPP and MPA)

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of exposures in humans

Critical end-points for setting guidance values for exposure to glufosinate-ammonium and its metabolites NAG and MPP

	Glufosinate-ammonium	NAG	MPP
Absorption, distribution, excretion and metabolism in animals			
Rate and extent of absorption	Rapid, incomplete (~10%)	Rapid, incomplete (5–10%)	Rapid and complete (86%)
Distribution	Extensive; highest concentrations in liver and kidney	Extensive; highest concentrations in liver and kidney	No data
Potential for accumulation	Low	Low	Low
Rate and extent of excretion	> 90% within 24 h, primarily in faeces	> 95% within 24 h, primarily in faeces	86% within 24 h, primarily in urine
Metabolism in animals	Limited	Limited	No data
Toxicologically significant compounds in animals, plants and the environment	Glufosinate-ammonium, NAG, MPP, MPA	NAG, glufosinate-ammonium, MPP, MPA	MPP, MPA
Acute toxicity			
LD_{50}, oral, rat	> 1500 mg/kg bw	> 2895 mg/kg bw	1900 mg/kg bw
LD_{50}, dermal, rat	> 2000 mg/kg bw	No data	No data
LC_{50}, inhalation, rat	≥ 1.26 mg/l air	No data	No data
Rat, dermal irritation	Not an irritant	No data	No data
Rabbit, ocular irritation	Not an irritant	No data	No data
Dermal sensitization	Not a sensitizer (Magnusson & Kligman, Buehler, local lymph node assay)	Not a sensitizer (Magnusson & Kligman)	Not a sensitizer (Magnusson & Kligman)
Short-term studies of toxicity			
Target/critical effect	Brain (inhibition of glutamine synthetase) (dog)	Brain (inhibition of glutamine synthetase) (mouse, rat, dog)	None identified
Lowest relevant oral NOAEL	1 mg/kg bw per day (dog)	500 ppm, equal to 20 mg/kg bw per day (dog)	1600 ppm, equal to 103 mg/kg bw per day, highest dose tested (dog)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (rat)	No data	No data

	Glufosinate-ammonium	NAG	MPP
Lowest relevant inhalation NOAEC	0.012 mg/l air	No data	No data
Long-term studies of toxicity and carcinogenicity			
Target/critical effect	Mortality, body weight gain, clinical chemistry (mouse) Haematology, brain glutamine synthetase (rats)	Body weight gain, clinical signs, polyarteritis nodosa in blood vessels and testes, urolithiasis (rat)	No data
Lowest relevant NOAEL	80 ppm, equal to 10.8 mg/kg bw per day (mouse) 140 ppm, equal to 7.6 mg/kg bw per day (rat)	2000 ppm, equal to 91 mg/kg bw per day (rat)	—
Carcinogenicity	Not carcinogenic (mouse, rat)	Not carcinogenic (rat)	—
Genotoxicity			
	Not genotoxic	Not genotoxic	Not genotoxic in a limited range of studies
Reproductive toxicity			
Reproduction target/critical effect	Reduced litter size (rat)	No reproductive target	No data
Lowest relevant parental NOAEL	500 ppm, equal to 44 mg/kg bw per day (rat)	10 000 ppm, equal to 622 mg/kg bw per day (rat)	—
Lowest relevant reproductive NOAEL	120 ppm, equal to 8.7 mg/kg bw per day (rat)	10 000 ppm, equal to 622 mg/kg bw per day (rat)	—
Lowest relevant offspring NOAEL	500 ppm, equal to 44 mg/kg bw per day (rat)	10 000 ppm, equal to 622 mg/kg bw per day (rat)	—
Developmental toxicity			
Developmental target	Intrauterine deaths (rat, rabbit)	Increased incidence of extra thoracic ribs (rabbit)	Intrauterine deaths (rat, rabbit)
Lowest relevant maternal NOAEL	10 mg/kg bw per day (rat) 6.3 mg/kg bw per day (rabbit)	64 mg/kg bw per day	50 mg/kg bw per day (rabbit)
Lowest relevant developmental NOAEL	10 mg/kg bw per day (rat) 6.3 mg/kg bw per day (rabbit)	64 mg/kg bw per day	50 mg/kg bw per day (rabbit)
Neurotoxicity			
Acute oral neurotoxicity NOAEL	1 mg/kg bw per day (dog); increased motor activity	Not neurotoxic (2000 mg/kg bw)	No data
Short-term neurotoxicity NOAEL	1 mg/kg bw per day (dog); inhibition of brain glutamine synthetase, increased motor activity	500 ppm, equal to 45 mg/kg bw per day (dog); inhibition of brain glutamine synthetase	Not neurotoxic Brain glutamine synthetase was not inhibited in dogs at 1000 ppm, equal to 58 mg/kg bw per day
Developmental neurotoxicity NOAEL	200 ppm, equal to 14 mg/kg bw per day (rat); increased motor activity, hippocampal pathology	No data	No data
Medical data			
	(Suicidal) poisonings producing several neurological effects and deaths. No adverse effects reported in plant production personnel.	No data	No data

Summary for glufosinate-ammonium

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Short-term studies (dog)	100
ARfD	0.01 mg/kg bw	Short-term studies (dog)	100

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