GLYPHOSATE

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Explanation

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective systemic herbicide that was last evaluated by the JMPR in 1986, when an acceptable daily intake (ADI) of 0–0.3 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 31 mg/kg bw per day, the highest dose tested in a 26-month study of toxicity in rats. In 1997, the Joint Meeting evaluated aminomethylphosphonic acid (AMPA), the major metabolite of glyphosate, and concluded that AMPA was of no greater toxicological concern than its parent compound. A group ADI of 0–0.3 mg/kg bw was established for AMPA alone or in combination with glyphosate. Glyphosate was re-evaluated by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting reviewed new data on glyphosate that had not been reviewed previously and relevant data from the previous evaluations.

Evaluation for acceptable daily intake

Several of the studies performed with glyphosate or AMPA were finalized before the OECD guidelines for testing of chemicals and the regulations for good laboratory practice were enacted. Nevertheless, all the relevant studies were subjected to quality assurance and, with few exceptions, their protocols complied with present guideline requirements.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution and excretion of glyphosate has been studied in a number of animal species (rats, rabbits, monkey, goats, chickens) treated with single or repeated doses (6.7–1000 mg/kg bw) and by different routes of application (oral, intramuscular, intraperitoneal, intravenous). The results of studies relating to urinary and faecal excretion and residues in tissues are summarized in Table 1.

Rats

Concentrations of radiolabel in the plasma and bone marrow were studied in nine male and nine female Crl:CD BR rats given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.7%; radiochemical purity, 98%) as a single intraperitoneal dose at 1150 mg/kg bw. The rats were housed individually in metabolism cages and blood samples were collected from three to six rats after 0.25, 0.5, 1, 2, 4, 6 and 10 h. At

Table 1. Excretion and residues of radioactivity after oral or parenteral administration of ^{14}C-labelled glyphosate, expressed as a percentage of the administered dose

Dose	Species	Urine		Faeces		Tissues		Reference
		Males	Females	Males	Females	Males	Females	
Single dose, oral administration								
6.7 mg/kg bw, 120 h[a]	Rat	14–16	35–43	81–85	49–55	0.14–0.65	0.83–1.02	Colvin & Miller (1973a)[d]
10 mg/kg bw, 24/48 h	Rat	17.9/34.0	12.8/12.5	59.3/60.5	80.3/91.2	ND	ND	Davies (1996d)
10 mg/kg bw, 72 h	Rat	13.0	10.6	88.5	88.7	0.59	0.49	Davies (1996a)
10 mg/kg bw, 168 h	Rat	28.6	22.5	62.4	69.4	0.44	0.31	Ridley & Mirly (1988)
30 mg/kg bw, 168 h	Rat	29.04	30.71	58.84	56.53	0.62	0.64	Powles (1992b)
1000 mg/kg bw, 72 h	Rat	16.7	17.5	89.6	84.5	0.52	0.58	Davies (1996b)
1000 mg/kg bw, 168 h	Rat	30.55	22.41	53.27	60.37	0.47	0.40	Powles (1992b)
1000 mg/kg bw, 168 h	Rat	17.8	14.3	68.9	69.4	0.28	0.24	Ridley & Mirly (1988)
5.7–8.8 mg/kg bw, 120 h	Rabbit	7–11	ND	80–97	ND	0.1–1.2	ND	Colvin & Miller (1973b)[d]
Single dose, intraperitoneal administration								
2.3–3.6 mg/kg bw, 120 h	Rat	82–90	ND	6–14	ND	0.53–1.00	ND	Colvin & Miller (1973a)[d]
Single dose, intravenous administration								
10 mg/kg bw, 168 h	Rat	79.0	74.5	4.65	8.3	1.27	1.09	Ridley & Mirly (1988)
30 mg/kg bw, 168 h	Rat	85.98	84.18	3.42	1.48	1.35	1.09	Powles (1992b)
Single dose, intramuscular administration								
4 mg/animal, 168 h	Monkey	89.9	ND	ND	ND	ND	ND	Maibach (1983)
Repeated doses, oral administration								
10 mg/kg bw, 72 h[b]	Rat	10.6	10.7	86.6	90.7	0.46	0.41	Davies (1996c)
10 mg/kg bw, 168 h[b]	Rat	30.9	23.1	61.0	70.9	0.54	0.35	Ridley & Mirly (1988)
30 mg/kg bw, 168 h[b]	Rat	34.28	34.63	49.64	46.73	0.96	0.83	Powles (1992b)
400 mg/animal per day, 120 h	Goat	ND	9.44	ND	78.16	ND	ND	Powles (1994a)
30 mg/hen per day, 168 h	Hen	ND	ND	ND	76.45c	ND	ND	Powles (1994b)

IM, intramuscular; IP, intraperitoneal; IV, intravenous; ND, not determined

[a] Glyphosate labelled with ^{14}C at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon.

[b] 14 daily doses of unlabelled glyphosate at 10 mg/kg bw, followed by a single dose of ^{14}C-labelled glyphosate at 10 mg/kg bw.

[c] Reported as excreta

[d] Cited in Annex 1, reference 47

0.5, 4 and 10 h after dosing, three animals of each sex were killed and the femoral bone marrow was isolated. The plasma and bone marrow samples were analysed for radioactivity by liquid scintillation counting.

Peak levels of radioactivity were observed at 0.5 h after dosing in plasma (males, 1867 mg/kg; females, 2019 mg/kg) and bone marrow (males, 267 mg/kg; females, 413 mg/kg). The amount of radioactivity in the plasma decreased rapidly, while it remained more constant in bone marrow over the experimental period of 10 h. The analysis of the first order elimination rates indicated a half-life time of 1 h (males and females) for plasma and 4.2 h (females) or 7.6 h (males) for bone marrow (Ridley, 1983).

In a study of absorption, distribution and excretion which was considered concisely by the 1997 JMPR for the evaluation of AMPA, groups of five male and five female Crl:CD(SD)BR rats received [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, >99.8%; radiochemical purity, >99%) orally by gavage as a single dose at 10 or 1000 mg/kg bw, or intravenously at a single dose at 10 mg/kg bw. A further group of five male and five female rats received unlabelled glyphosate as 14 consecutive oral doses at 10 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 10 mg/kg bw. For measurement of radioactivity in expired air, an additional test group of three male and three female rats received a single oral dose at 10 mg/kg bw, and expired gases were removed at 6, 12 and 24 h after dosing. For determination of pharmacokinetic parameters, additional test groups of three male and three female rats received a single dose at 10 mg/kg bw orally or intravenously, and blood samples were taken from the tail vein at various times between 0.25 and 168 h after dosing. The animals were housed individually in metabolism cages from which urine and faeces were collected at regular intervals. Animals used for detection of radioactivity in expired air were sacrificed at 24 h and all remaining animals at 7 days after dosing, and selected tissues were removed. Radioactivity in urine, faeces, blood, expired air and tissues was determined by liquid scintillation counting.

After a single oral dose of 10 mg/kg bw, <0.2% of the administered radioactivity was found in the expired air at 24 h after dosing, and therefore expired gases were not collected for the other test groups.

After a single intravenous dose at 10 mg/kg bw, 74.5–79.0% of the administered dose was eliminated in urine and 4.7–8.3% in the faeces (Table 2). Less than 0.1% of the administered dose was found in the organs taken at necropsy, with approximately 1% of the administered dose remaining in the residual carcass.

For the groups treated orally, most of the administered dose was eliminated in the faeces at both 10 mg/kg bw (62.4–69.4%) and 1000 mg/kg bw (68.9–69.4%), with the urine accounting for 22.5–28.6% and 14.3–17.8% of the administered dose at the lower and higher doses, respectively (Table 1.1-6). Less than 0.05% of the administered dose appeared in the organs after oral dosing and <0.5% remained in the residual carcass. Repeated dosing at 10 mg/kg bw had no significant effect on the routes of excretion of ^{14}C-labelled glyphosate nor on the percentage of the administered dose remaining in the organs, tissues and residual carcass at sacrifice.

Analysis of individual tissues demonstrated that bone contained the highest concentration of [^{14}C]glyphosate equivalents (0.3–31 ppm). The remaining tissues contained

Table 2. Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C-labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)							
	10 (single dose, intravenous)		10 (single dose, oral)		10 (repeated doses, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	79.0	74.5	28.6	22.5	30.9	23.1	17.8	14.3
Faeces	4.65	8.30	62.4	69.4	61.0	70.9	68.9	69.4
Organs/tissues	0.0941	0.0521	0.0460	0.0194	0.0473	0.0313	0.0355	0.0266
Residual carcass	1.18	1.04	0.395	0.286	0.497	0.315	0.248	0.208
Gastrointestinal tract contents	0.0394	0.0388	0.0226	0.0145	0.0138	0.0095	0.0258	0.0429
Cage wash	0.890	1.30	1.30	1.96	0.820	1.96	3.86	8.00
Total recovery[a]	86.0	85.3	92.8	94.2	93.3	96.3	90.9	92.1

From Ridley & Mirly (1988)
[a] Total recovery is the mean of values for individual animals

Table 3. Mean tissue concentration of radioactivity (ppm) at 168 h in rats given ^{14}C-labelled glyphosate as single or repeated doses

Tissue	Dose (mg/kg bw)							
	10 (single dose, intravenous)		10 (single dose, oral)		10 (repeated doses, oral)		1000 (single doses, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Whole blood	0.0185	0.00996	0.00454	0.00269	0.00476	0.00288	0.328	0.166
Liver	0.104	0.0498	0.0298	0.0135	0.0407	0.0257	1.91	1.37
Brain	0.0414	0.0360	0.00705	0.00551	0.0144	0.0110	0.750	0.556
Kidney	0.106	0.0714	0.0216	0.0132	0.0327	0.0196	1.94	1.35
Spleen	0.0439	0.0320	0.0119	0.00727	0.0155	0.0130	2.61	2.98
Lung	0.103	0.0785	0.0148	0.0120	0.0211	0.0167	1.54	1.13
Heart	0.0263	0.0170	0.00622	0.00398	0.00804	0.00632	0.590	0.518
Testes/ovaries	0.0182	0.0223	0.00276	0.00326	0.00529	0.00813	0.363	0.572
Stomach	0.0237	0.0182	0.00795	0.00367	0.0377	0.0239	2.38	2.36
Small intestine	0.0262	0.0164	0.0216	0.0183	0.0441	0.0257	1.90	1.55
Colon	0.0348	0.0178	0.0342	0.0159	0.0429	0.0298	11.0	9.2
Bone	1.48	1.59	0.552	0.313	0.748	0.462	30.6	19.7
Bone marrow	0.0692	0.0303	0.0290	0.00639	0.0245	0.0231	4.1	12.5
Abdominal muscle	0.00766	0.00605	0.00232	0.0016	0.00278	0.00216	0.262	0.214
Shoulder muscle	0.0106	0.0327	0.00388	0.00667	0.00783	0.00590	0.419	0.423
Abdominal fat	0.00535	0.00366	0.00364	0.00324	0.00557	0.00576	0.418	0.457
Residual carcass	0.344	0.337	0.106	0.087	0.157	0.101	8.27	7.74
Tail	0.699	0.611	ND	ND	ND	ND	ND	ND

From Ridley & Mirly (1988)
ND, not determined

glyphosate equivalents at a concentration of between 0.0003 and 11 ppm (Table 3). In the bone and some highly perfused tissues, levels were statistically higher in males than in females.

The estimated half-life for whole body elimination of radioactivity was 2.11–7.52 h for the alpha phase and 69–337 h for the beta phase. The half-life in males at the higher dose was found to be significantly longer than those given the lower dose. Pre-treatment at the lower dose had no significant effect on the whole body elimination.

Based on the area under the curve for the blood concentration of radioactivity after oral or intravenous administration, the oral absorption of glyphosate was found to be 30.3–35.4%. This compared favorably with the absorption (30.2–36.2%) calculated from the data on excretion in the urine after oral and intravenous administration. The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated after a single oral dose at 10 or 1000 mg/kg bw (Ridley & Mirly, 1988).

In a preliminary study of absorption and distribution, male Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.6%; radiochemical purity, 94.3–97.4%) as a single oral dose at 30 mg/kg bw orally by gavage in 0.9% saline. Blood samples were taken from the tail vein of three animals at various times between 0.5 and 48 h after dosing. Additional animals were killed 4, 10 and 24 h after dosing and the tissue distribution of radioactivity was investigated by whole body autoradiography.

The plasma concentrations of radioactivity reached a maximum 3–4 h after dosing and were in the range of 0.705 to 1.769 µg equivalents/ml. Thereafter the concentration declined rapidly and radioactivity could not be detected 12 h after dosing. The elimination half-life and area under the plasma concentration–time curve were 6.2–12.35 h and 18.62–23.09 µg equivalents.h/ml in two of the rats. Pharmacokinetic parameters could not be calculated for the third animal. Autoradiography showed that the highest concentration of radioactivity was present at 10 h in bone, bone marrow, cartilage, parts of the gastrointestinal tract, kidney, urinary tract and nasal mucosa. At termination 24 h after dosing, the concentation of radioactivity was negligible in all tissues except bone, bone marrow, parts of the gastrointestinal tract, bladder and kidney cortex (Powles, 1992a).

In a study of absorption, distribution and excretion, groups of five male and five female Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 96.8%; radiochemical purity, >98%) as a single dose at 30 or 1000 mg/kg bw orally by gavage in saline, or intravenously as a single dose at 30 mg/kg bw. A further group of five male and five female rats received unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 30 mg/kg bw. The animals were housed individually in metabolism cages from which urine, faeces and expired air were collected at regular intervals. Animals were sacrificed after 90% of the dose had been eliminated or 7 days after dosing, whichever was sooner. At necropsy, a blood sample was taken and selected tissues were removed. Radioactivity in urine, faeces, blood, expired air and tissues was determined by liquid scintillation counting.

After administration of a single intravenous dose at 30 mg/kg bw, >84% of the dose was eliminated in the urine (Table 4), mostly within 8 h after dosing (Tables 5 and 6). Faecal elimination accounted for <3.5% of the administered radioactivity. Only a very small proportion of the radioactivity was eliminated in exhaled air and <1.4% was present in tissues and the residual carcass when the animals were sacrificed.

In contrast, faeces was the major route of elimination when ^{14}C-labelled glyphosate was given by the oral route. About 57–59% of a single oral dose of 30 mg/kg bw was excreted in the faeces (Table 4); most of this was eliminated in the 12–36 h after dosing (Tables 5 and 6). Urinary elimination was slower for the oral dose at 30 mg/kg bw than for the intravenous dose; 29–31% was eliminated, mainly within 36 h of dosing.

Table 4. Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C-labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	85.98	84.18	29.04	30.71	34.28	34.63	30.55	22.41
Faeces	3.422	1.484	58.84	56.53	49.64	46.73	53.27	60.37
CO_2	0.024	0.023	0.075	0.065	0.085	0.055	0.064	0.067
Tissues	1.353	1.093	0.619	0.635	0.955	0.825	0.469	0.400
Total[a]	97.75	100.0	96.63	96.71	90.14	89.95	99.70	100.4

From Powles (1992b)
[a] Including cage wash and debris

Table 5. Excretion of radioactivity in the urine and faeces (% of administered dose) by male rats given ^{14}C-labelled glyphosate

Time (h)	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
0–4	72.10	NS	3.215	NS	8.992	NS	5.561	NS
4–8	7.344	NS	9.638	NS	11.79	NS	14.21	NS
8–12	2.422	1.193	4.239	NS	4.950	24.61	2.918	NS
12–24	1.694	1.035	7.137	45.84	5.091	15.58	3.819	38.63
24–36	0.813	0.530	3.163	8.304	2.387	7.404	1.991	9.788
36–48	0.369	0.258	0.752	3.126	0.622	1.046	0.806	2.385
48–72	0.458	0.191	0.588	1.409	0.447	1.007	0.687	1.612
72–96	0.320	0.158	0.197	0.121	NS	NS	0.247	0.549
96–120	0.187	0.073	0.115	0.040	NS	NS	0.172	0.081
120–144	0.141	0.023	NS	NS	NS	NS	0.096	0.165
144–168	0.112	0.029	NS	NS	NS	NS	0.043	0.058
Total	85.98	3.422	29.04	58.84	34.28	49.64	30.55	53.27

From Powles (1992b)
NS, no sample, either because no faeces were voided during the collection period or because collection had ceased before that time-point.

Excretion was unaffected by administration of unlabelled glyphosate for 14 days prior to the administration of ^{14}C-labelled glyphosate and the routes and rates of excretion of a higher dose of ^{14}C-labelled glyphosate (1000 mg/kg bw) were almost identical to those at the lower dose (Tables 4, 5, 6). There was no significant sex difference in the elimination of glyphosate for any dose regimen. Irrespective of the dose, route or frequency of duration <1.4% of the administered dose was retained in tissues. The highest concentration of radioactivity was present in bone, with lower concentrations in bone marrow, kidney, liver, lungs and the residual carcass (Table 7) (Powles, 1992b).

In a study of absorption, distribution and excretion, groups of five male and five female Alpk:AP$_f$SD rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 99.2–99.5%; radiochemical purity, >98%) as a single dose at 10 or 1000 mg/kg bw orally by gavage in deionized water. An additional group of five male

Table 6. Excretion of radioactivity in the urine and faeces (% of administered dose) by female rats given ^{14}C-labelled glyphosate

Time (h)	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
0–4	71.89	NS	3.150	NS	5.515	NS	2.078	NS
4–8	6.397	NS	11.91	NS	15.21	NS	13.32	NS
8–12	2.056	0.502	4.489	NS	5.006	17.44	2.386	NS
12–24	1.429	0.631	7.202	40.68	6.184	18.25	2.622	48.27
24–36	0.778	0.145[a]	2.266	10.94	1.520	8.613	0.966	8.524
36–48	0.364	0.388	0.775	2.455	0.427	1.498	0.378	1.945
48–72	0.497	0.178	0.551	2.158	0.766	0.925	0.384	1.086
72–96	0.309	0.047	0.238	0.235	NS	NS	0.165	0.505
96–120	0.206	0.020	0.134	0.059	NS	NS	0.096	0.029
120–144	0.145	0.000	NS	NS	NS	NS	0.012	0.006
144–168	0.109	0.000	NS	NS	NS	NS	0.000	0.009
Total	84.18	1.484	30.71	56.53	34.63	46.73	22.41	60.37

From Powles (1992b)
NS, no sample, either because no faeces were voided during the collection period or because collection had ceased before that time-point.
[a]Only one sample was analysed for this time-point.

Table 7. Mean tissue concentration of radioactivity (ppm) at 168 h in rats given ^{14}C-labelled glyphosate as a single dose or as repeated doses

Tissue	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Blood	0.050	0.084	0.011	0.000	0.000	0.000	0.000	0.000
Bone	4.195	4.355	2.246	2.562	3.096	2.505	56.32	40.66
Bone marrow	0.255	1.264	0.322	0.545	0.325	0.144	3.080	0.000
Brain	0.118	0.120	0.056	0.056	0.019	0.000	0.000	0.000
Abdominal fat	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
Carcass	0.423	0.335	0.197	0.214	0.339	0.284	5.628	4.476
Heart	0.051	0.025	0.051	0.045	0.000	0.000	0.000	0.000
Kidney	0.304	0.298	0.278	0.205	0.515	0.317	5.170	3.968
Liver	0.241	0.222	0.251	0.254	0.615	0.425	6.144	0.000
Lungs	0.264	0.279	0.124	0.126	0.183	0.173	2.904	1.216
Muscle	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ovaries	NA	0.034	NA	0.068	NA	0.028	NA	0.000
Plasma	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
Salivary gland	0.082	0.068	0.053	0.079	0.084	0.100	0.000	0.000
Spleen	0.117	0.117	0.140	0.091	0.164	0.153	0.000	0.000
Testes	0.000	NA	0.000	NA	0.000	NA	0.000	NA
Uterus	NA	0.248	NA	0.143	NA	0.239	NA	0.000

From Powles (1992b)
NA, not applicable

and five female rats received unlabelled glyphosate as consecutive oral doses at 10 mg/kg bw per day for 14 days followed by ^{14}C-labelled glyphosate as a single oral dose at 10 mg/kg bw. The animals were housed individually in metabolism cages from which urine and faeces were collected at regular intervals. At termination 72 h after dosing, representative samples

of tissues were removed. Radioactivity in urine, faeces and tissues was determined by liquid scintillation counting.

After a single oral dose, the rate of excretion was rapid, with >87% of the dose being excreted within 24 h of dosing by both sexes, and total excretion was effectively complete after 72 h. No pronounced sex difference was apparent in either the routes or rates of excretion. Faeces was the predominant route of excretion, accounting for 88.5–89.6% and 84.5–88.7% of the administered dose in males and females, respectively, while excretion via urine accounted for 13.0–16.6% and 10.6–17.5% in males and females, respectively. At termination, tissue concentration of radioactivity was very low and accounted for <0.6% of the administered dose in both sexes (Table 8). The highest concentrations were present in bone (Table 9).

After repeated dosing, the rate of excretion was rapid, with >90% of the administered dose being excreted in both sexes within 24 h of dosing, and total excretion was effectively complete after 72 h. No pronounced sex difference was apparent in either the routes or rates of excretion. Faeces was the predominant route of excretion, accounting for 86.6% and 90.7% of the administered dose in males and females, respectively, while excretion via urine accounted for 10.6% and 10.7% in males and females, respectively. At termination, the tissue concentration of radioactivity was very low and accounted for <0.5% of the administered dose in both sexes (Table 8). The highest concentrations were present in bone (Table 9) (Davies, 1996a, 1996b, 1996c).

In a study of absorption, distribution and excretion, groups of two male and two female Alpk:AP$_f$SD rats received [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 99.2%; radiochemical purity, >98%) orally by gavage in deionized water at a single dose of 10 mg/kg bw. The animals were housed individually in metabolism cages from which urine and faeces were collected at regular intervals. The study was terminated 24 h or 48 h after dosing. Radioactivity in urine and faeces was determined by liquid scintillation counting, and in tissues by whole body autoradiography.

The rate of excretion was rapid, with >77% of the dose excreted within 24 h of dosing by both sexes, and total excretion was practically complete after 48 h. Faeces was the predominant route of excretion, accounting for 59.3–60.5% and 80.3–91.2% in males and females, respectively, while excretion via urine accounted for 17.9–34.0% and 12.5–12.8%

Table 8. Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C-labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)					
	10 (single dose)		10 (repeated doses)		1000 (single dose)	
	Males	Females	Males	Females	Males	Females
Urine	13.0	10.6	10.6	10.7	16.7	17.5
Faeces	88.5	88.7	86.6	90.7	89.6	84.5
Gastrointestinal tract and contents	0.19	0.17	0.1	0.1	0.2	0.22
Cage wash	0.3	0.4	0.2	0.2	0.1	0.2
Tissues (including carcass)	0.59	0.49	0.46	0.41	0.52	0.58
Total	102.6	100.3	98.0	102.2	107.1	103.1

From Davies (1996a, 1996b, 1996c)

Table 9. Mean tissue concentration of radioactivity (ppm) at 72 h in rats given ^{14}C-labelled glyphosate as a single oral dose or as repeated oral doses

Tissue	Dose (mg/kg bw)					
	10 (single dose)		10 (repeated doses)		1000 (single dose)	
	Males	Females	Males	Females	Males	Females
Brain	0.011	0.009	0.010	0.010	1.23	1.16
Gonads	0.007	0.024	0.007	0.026	0.91	2.94
Heart	0.012	0.011	0.011	0.012	1.11	1.25
Kidneys	0.068	0.049	0.061	0.049	6.51	6.05
Liver	0.059	0.044	0.055	0.045	5.48	5.23
Lungs	0.031	0.026	0.026	0.029	2.87	3.54
Spleen	0.026	0.024	0.022	0.025	2.44	3.11
Salivary glands	0.017	0.018	0.019	0.027	1.81	2.09
Abdominal fat	0.007	<0.004	0.008	0.006	0.54	0.50
Bone (femur)	0.511	0.395	0.358	0.345	49.78	44.93
Muscle	0.007	0.006	0.008	0.007	0.82	0.83
Blood	0.011	0.009	0.014	0.010	0.89	0.80
Plasma	<0.004	<0.004	<0.004	<0.005	<0.40	<0.40
Residual carcass	0.062	0.056	0.050	0.046	4.77	5.86

From Davies (1996a, 1996b, 1996c)
Limit of detection was 0.004 or 0.35 µg equivalents/g (at 10 or 1000 mg of ^{14}C-labelled glyphosate/kg bw, respectively)

in males and females, respectively. At termination, the greatest intensity of tissue radioactivity was present in bone at 24 h and 48 h. Some radioactivity was also apparent in the kidneys after 24 h, but had declined to negligible amounts after 48 h (Davies, 1996d).

Goats

In a study of absorption, distribution, metabolism and excretion, two lactating goats (strain, British Saanen; age, approximately 3 years of age; body weight, approximately 46.5 and 62 kg) were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 97.5%; radiochemical purity, >97%) as repeated doses at a mean dose of 355 or 400 mg/animal per day (equivalent to a nominal dietary concentration of 200 ppm) by oral gavage for 5 or 3 consecutive days. Excreta were collected from both animals at 24-h intervals after the first dose. The goats were milked twice daily and the milk was pooled to provide a daily sample for each animal. The first goat, which was given five consecutive doses, was killed approximately 23.5 h after the last dose, and the liver, kidneys and samples of muscle and fat were removed at necropsy. From the second goat, given three consecutive doses, blood samples were taken at 1, 2, 3, 4, 6, 8, 12 and 24 h after the initial dose. The second goat was killed when the plasma concentration of radiolabel was at a maximum (approximately 8 h after the final dose). Excreta, milk and tissues from this animal were used for identification of metabolites.

For the first goat, the overall recovery of administered radioactivity was 89.9%, most of which was present in faeces (78.16%), urine (9.44%) and cage debris/cage wash (2.22%). Negligible radioactivity was recovered from milk (0.03%) and tissues (0.05%). The transfer coefficient for milk was low (approximately 0.07%) with peak concentration (0.072 ppm) achieved on day 4 of dosing. At necropsy, residues were highest in kidney (3.852 ppm), liver (0.404 ppm) and skeletal muscle (0.035 ppm), and below the limit of detection (0.028 ppm) in fat.

For the second goat, at 8 h after the final dose, 57.6% of administered radioactivity was recovered in the excreta, with 52.6, 4.7 and 0.03% present in the faeces, urine and milk, respectively. The concentration of radioactivity in the plasma peaked at 6–8 h after the initial dose (0.102–0.101 ppm), while the concentration in milk was highest on day 3 of dosing (0.086 ppm). At necropsy, residues were highest in kidney (12.15 ppm), liver (0.225 ppm) and skeletal muscle (0.061 ppm), and below the limit of detection (0.036 ppm) in fat. Unchanged glyphosate was the major component detected in both urine and faeces (94–96%) by high-performance liquid chromatography (HPLC) and confirmed by Fourier-transform infrared (FT-IR) spectroscopy. Small amounts of AMPA were tentatively identified in the urine and faeces by thin-layer chromatography (TLC), but not confirmed by HPLC. The main residues in tissues and milk were glyphosate, with only low levels of AMPA tentatively identified in kidney (Powles, 1994a).

Chickens

In a study of absorption, distribution, metabolism and excretion, two groups of five laying hens (strain, ISA; age, 20–22 weeks; body weight, approximately 1.5 kg) were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 97.5%; radiochemical purity, >97%) as repeated doses at a mean dose of approximately 30 mg/animal per day (equivalent to a nominal dietary concentration of 200 ppm) by oral gavage for 7 or 5 consecutive days (group A and B, respectively). Excreta and eggs were collected at 24-h intervals after the initial dose. From each animal in group B, blood samples were collected at 1, 2, 3, 4, 6, 8 and 12 h after the first dose. The hens were sacrificed 23.5 h after the last dose (group A) or 1 h after the last dose when the plasma concentration of radioactivity was at a maximum (group B). At necropsy, selected tissues were removed from each bird and radioactivity was determined in all excreta, egg yolk, egg white, liver, muscle, fat and skin samples. Tissues from hens in group B were used for identification of metabolites.

In hens in group A, the overall recovery of administered radioactivity was 80.34% at termination, with 76.45% being present in excreta and 3.86% in cage wash/cage debris, and negligible amounts in tissues (0.02 ppm) and eggs (<0.01 ppm). Radioactivity in egg white reached a plateau (0.059 ppm) at day 6, while the concentration in yolks increased throughout the dosing period and reached 0.484 ppm at day 7. At study termination, radioactivity was detected in liver, skin and fat (1.242, 0.212 and 0.153 ppm, respectively), but was below the limit of detection (0.043 ppm) in muscle.

In hens in group B, the concentration of radioactivity in the plasma attained a maximum (0.475 ppm) 1 h after the initial dose, then declined slowly and was still measurable in one hen 12 h after dosing. At termination, radioactivity was present in liver, skin, fat and muscle (1.080, 0.359, 0.083 and 0.041 ppm, respectively). The residues in egg white and yolk were highest at day 5 (0.072 and 0.228 ppm, respectively). Unchanged glyphosate was the major component detected in excreta, liver, skin, fat, muscle, egg white and egg yolk by TLC (and confirmed by FT-IR spectroscopy). Small amounts of AMPA were detected in excreta, liver and skin by TLC but, in each case, confirmation of the presence of that metabolite could not be confirmed by HPLC (Powles, 1994b).

1.2 Biotransformation

In a study of biotransformation that was considered concisely by the 1997 JMPR for the evaluation of AMPA, groups of five male and five female Crl:CD(SD)BR rats received

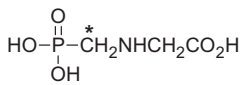
[^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, >99.8%; radiochemical purity, >99%) as a single oral dose at 10 or 1000 mg/kg bw, as repeated oral doses at 10 mg/kg bw per day, or as a single intravenous dose at 10 mg/kg bw (for details of the study design, see Ridley & Mirly, 1988). For identification and quantification of parent compound and metabolites in urine and faecal samples, chromatographic (cation-exchange HPLC, ion-pair HPLC) and spectroscopic (nuclear magnetic resonance [NMR]; mass spectrometry [MS]) techniques were used.

Glyphosate was isolated as the predominant radioactive fraction in the urine (overall recovery, 81.3%) and faeces (overall recovery, 99.2%) and was positively identified in each case by ^{1}H-NMR, ^{13}P-NMR and by MS with and without derivatization. The minimum content of glyphosate in either urine or faecal samples from the individual rats was 97.46%. HPLC analyses further indicated that glyphosate in the excreta accounted for 98.50–99.33% of the administered [^{14}C]glyphosate. In rats dosed orally at 10 mg/kg bw, either as single or multiple doses, there was evidence for formation of 0.2–0.3% and 0.4% AMPA, respectively, from metabolism of glyphosate in vivo. Since AMPA was not formed after intravenous administration, it seems likely that the formation of AMPA after oral administration of glyphostae at low doses is due to a very minor amount of gastrointestinal metabolism of glyphosate, possibly by the gastrointestinal microflora (Howe et al., 1988).

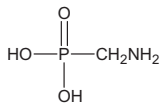
In a study of excretion and biotransformation, two male and two female bile-duct cannulated Alpk: AP$_f$SD rats received [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 99.5%; radiochemical purity, 97.8%) as a single dose at 1000 mg/kg bw given orally by gavage. The rats were housed individually in metabolism cages, and urine, faeces and bile were collected at regular intervals between 2 h and 48 h after dosing. The samples were analysed for radioactivity by liquid scintillation counting. For identification and quantification of parent compound and metabolites in urine and faecal samples from previous studies performed by Davies (1996a, 1996b, 1996c), chromatographic (TLC, HPLC) and spectroscopic (NMR) techniques were used.

For male and female bile-duct cannulated rats, excretion of administered radioactivity over 48 h was 20.8% and 16.3 % in the urine, 39.1% and 30.5% in the faeces, and 0.06% and 0.06% in bile, respectively. In faecal samples from previous studies, it was confirmed that all extracted radioactivity was glyphosate. In urine samples, the major radioactive component was unchanged glyphosate (10.5–16.7% of administered dose), while only

Figure 1. Structural formulae of glyphosate and AMPA



Glyphosate (* Denotes position of radiolabel)



Aminomethylphosphonic acid (AMPA)

trace amounts of aminomethyl phosphonic acid (AMPA, 0.07–0.66%) were detected (Macpherson, 1996).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of tests for the acute oral, dermal or inhalation toxicity of glyphosate are summarized in Table 10.

Groups of 10 ICR mice of each sex were given glyphosate as a single dose at 1000, 5000 or 10 000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Two out of 10 males and one out of 10 females at 10 000 mg/kg bw died. Reduced activity was observed at 5000 mg/kg bw and greater. No treatment-related gross necropsy changes were found at sacrifice (Shirasu & Takahashi, 1975).

Groups of five male and five female Bom:NMRI mice were given glyphosate as a single dose at 2000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs were observed in all mice on day 1 and included piloerection and sedation. No treatment-related gross necropsy changes were found at sacrifice (Dideriksen, 1991).

Groups of five male and five female fasted Wistar rats were given glyphosate as a single dose at 2500, 3500, 5000, 7000 or 9900 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Mortality occurred in 1 out of 10, 1 out of 10, 3 out of 10, 8

Table 10. Acute toxicity of glyphosate

Species	Strain	Sex	Route	Vehicle (particle size)	Purity (%)	LD_{50} (mg/kg bw) LC_{50} (mg/l)	Reference
Mouse	ICR	10F, 10M per dose	Oral	0.2% carboxy-methylcellulose	96.7	>10 000	Shirasu & Takahashi (1975)
Mouse	NMRI	5F, 5M	Oral	Water	98.6	>2 000	Dideriksen (1991)
Rat	Wistar	5F, 5M per dose	Oral	Water	99.0	5 600 (4 900–6 300)	Heenehan (1979a)
Rat	Sprague-Dawley	5F, 5M	Oral	1% Methocel	85.5	>5 000	Blaszcak (1988a)
Rat	Sprague-Dawley	5F, 5M	Oral	Water	97.76	>5 000	Reagan (1988a)
Rat	Sprague-Dawley	5F, 5M	Oral	0.5% carboxy-methylcellulose	98.6	>5 000	Cuthbert & Jackson (1989a)
Rat	Wistar	5F, 5M	Oral	Water	95.6	>5 000	Doyle (1996a)
Goat	(Spanish)	5F	Oral	Water	98.7	3 530 (2 950–4 220)	Rowe et al. (1987)
Rat	Wistar	5F, 5M	Dermal	Water	95.6	>2 000	Doyle (1996b)
Rabbit	New Zealand White	2F, 2M	Dermal	0.9% saline	99	>5 000	Heenehan (1979b)
Rabbit	New Zealand White	5F, 5M	Dermal	0.9% saline	85.5	>5 000	Blaszcak (1988b)
Rabbit	New Zealand White	5F, 5M	Dermal	0.9% saline	97.76	>5 000	Reagan (1988b)
Rat	Sprague-Dawley	5F, 5M	Inhalation (4 h, nose-only)	None (22.5 µm)	98.6	>4.98	McDonald & Anderson (1989)
Rat	Wistar	5F, 5M	Inhalation (4 h, nose-only)	None (2.9–3.6 µm)	95.6	>4.43	Rattray (1996)

F, female; M, male

out of 10 and 10 of 10 rats at 2500, 3500, 5000, 7000 and 9900 mg/kg bw, respectively. Treatment-related clinical signs were observed at all doses and included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and faecal staining of the abdomen. Treatment-related gross necropsy changes were usually observed in animals that died and included brown/white fluid in the stomach or the intestine and coloured spots in the lungs, the liver and the kidneys (Heenehan et al., 1979a).

Groups of five male and five female fasted CD (Sprague-Dawley derived) rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs included wet rales, faecal staining, urinary staining and soft stools. Some animals had decreased food consumption after dosing; and one of the five females exhibited weight loss on day 7, but gained weight between days 7 and 14. No treatment-related gross necropsy changes were found at sacrifice (Blaszcak, 1988a).

Groups of five male and five female fasted Sprague-Dawley rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 15 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs included diarrhoea, apparent urinary incontinence and hair loss on the abdomen. There were no effects on body weights, and no treatment-related changes were found at gross necropsy after sacrifice (Reagan, 1988a).

Groups of five male and five female fasted Sprague-Dawley rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs included piloerection, reduced activity and ataxia through day 9. No treatment-related gross necropsy changes were found at sacrifice (Cuthbert & Jackson, 1989a).

Groups of five male and five female fasted Alpk:AP$_f$SD (Wistar-derived) rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination, and there were no treatment-related clinical signs. All animals lost weight initially because they had been fasted before dosing, but all exceeded their initial weight by day 3 and, apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study. No treatment-related gross necropsy changes were found at sacrifice (Doyle, 1996a).

Groups of five female Spanish goats were given glyphosate as a single dose at 1980, 3090, 4620 or 10 000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Mortality was none out of five, one out of five, five out of five and five out of five at 1980, 3090, 4620 or 10 000 mg/kg bw, respectively. Treatment-related clinical signs included diarrhoea (at all doses) and colic, depression, and ataxia at 3090 mg/kg bw and greater. All surviving animals appeared to be normal at the end of the experiment. At gross necropsy, no treatment-related changes were found. Microscopic examination revealed moderate to severe tubular nephrosis in animals that died prior to terminal sacrifice. This lesion may have contributed to the observed elevations in blood urea nitrogen and creatinine (Rowe et al., 1987).

Groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received glyphosate as a single dermal application at 2000 mg/kg bw and were observed for 14 days

before sacrifice. All animals survived until study termination. There were no treatment-related signs of systemic toxicity and practically no signs of skin irritation, with the exception of one male with slight erythema on days 2 and 3 and one female with scabs from day 3 to 8. Most animals had exceeded their initial weight by the end of the study. No treatment-related gross necropsy changes were found at sacrifice (Doyle, 1996b).

Groups of two male and two female New Zealand White rabbits received glyphosate as a single dermal application at 5000 mg/kg bw and were observed for 14 days before sacrifice. All animals survived until study termination. Very slight erythema was observed in two animals and well-defined erythema was observed in the remaining two animals. Clinical signs included clear nasal discharge in each animal (up to day 6) and weight loss in one animal. No treatment-related gross necropsy changes were found at sacrifice (Heenehan et al., 1979b).

Groups of five male and five female New Zealand White rabbits received glyphosate as a single dermal application at 5000 mg/kg bw and were observed for 14 days before sacrifice. All animals survived until study termination, and no clinical signs or dermal effects were seen throughout the study. All animals exhibited slight body-weight losses or no weight change on day 7, but most had slight weight gains between days 7 and 14. No treatment-related gross necropsy changes were found at sacrifice (Blaszcak, 1988b).

Groups of five male and five female New Zealand White rabbits received glyphosate as a single dermal application at 5000 mg/kg bw and were observed for 14 days before sacrifice. One female rabbit exhibited diarrhoea and/or anorexia on days 9–13 and died on day 14. This finding was considered to be consistent with mucoid enteropathy, a condition occasionally encountered in control rabbits. Therefore, this death was considered to be spontaneous and unrelated to treatment. Anorexia, diarrhoea and soft stools were also noted in two males and one female rabbit that survived to study termination. No treatment-related gross necropsy changes were found at sacrifice (Reagan, 1988b).

Groups of five male and five female Sprague-Dawley rats were exposed to glyphosate at a mean aerial concentration of 4.98 mg/l (mean measured particle size, 22.5 µm) for 4 h in a snout-only system and were observed for 14 days before sacrifice. There was no mortality during the study. Animals were slightly subdued after dosing at day 1 only. There were no effects on body-weight gain, and no treatment-related gross necropsy changes were found at sacrifice (McDonald & Anderson, 1989).

Groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats were exposed to glyphosate at a mean aerial concentration of 2.47 or 4.43 mg/l (mean measured particle size, 2.9–3.6 µm) for 4 h in a nose-only system and were observed for 14 days before sacrifice. At 4.43 mg/l, two out of five males and two out of five females were found dead or were killed in extremis on days 5, 6 or 9 of the study. There was no mortality at 2.47 mg/l. Treatment-related clinical signs during exposure were salivation, irregular breathing and auditory hypoaesthesia, while irregular breathing, reduced righting reflex, shaking and splayed gait were observed in both groups immediately after exposure. At necropsy, the two males found dead had dark lungs. No treatment-related gross necropsy changes were found in the other animals (Rattray, 1996).

(b) Dermal and ocular irritation and dermal sensitization

The results of tests for dermal and ocular irritation and dermal sensitization with glyphosate (glyphosate acid and glyphosate salts) are summarized in Table 11.

The potential of glyphosate acid to irritate the skin was evaluated in five studies in male and/or female New Zealand White rabbits. The studies were performed in compliance with the principles of good laboratory practice (GLP) and according to the test guidelines of the United States Environmental Protection Agency (US EPA) or the OECD (TG 404). In the first study, animals with intact skin and abraded skin were exposed for 24 h and the

Table 11. Irritation and sensitization potential of glyphosate

Species	Strain	Sex	End-point	Form of glyphosate, vehicle	Purity (%)	Result	Reference
Rabbit	New Zealand White	3F, 3M	Skin irritation (24 h)[a]	Glyphosate, water	99	Not irritating	Heenehan (1979c)
Rabbit	New Zealand White	2F, 4M	Skin irritation (4 h)	Glyphosate, 0.9% saline	85.5	Minimally irritating	Blaszcak (1988c)
Rabbit	New Zealand White	3F, 3M	Skin irritation (4 h)	Glyphosate, 0.9% saline	97.76	Not irritating	Reagan (1988c)
Rabbit	New Zealand White	4F, 2M	Skin irritation (4 h)	Glyphosate, water	98.6	Not irritating	Cuthbert & Jackson (1989b)
Rabbit	New Zealand White	6F	Skin irritation (4 h)	Glyphosate, water	95.6	Not irritating	Doyle (1996c)
Rabbit	New Zealand White	6NS (3NS[b])	Eye irritation	Glyphosate, 25% w/v in water	99.0	Severely irritating	Heenehan (1979d)
Rabbit	New Zealand White	3F, 3M	Eye irritation	Glyphosate	85.5	Severely irritating	Blaszcak (1988d)
Rabbit	New Zealand White	6NS	Eye irritation	Glyphosate	97.76	Severely irritating	Reagan (1988d)
Rabbit	New Zealand White	1 (NS)	Eye irritation	Glyphosate	98.6	Severely irritating	Cuthbert & Jackson (1989c)
Rabbit	New Zealand White	3F, 3M (3M[b])	Eye irritation	Glyphosate	98.2	Severely irritating	Kuhn (1996)
Rabbit	New Zealand White	6F	Eye irritation	Glyphosate	95.6	Moderately irritating	Johnson (1997)
Rabbit	New Zealand White	3F, 3M (2F, 1M[b])	Eye irritation	Glyphosate IPA salt	65.0	Not irritating	Branch (1981)
Rabbit	New Zealand White	6NS	Eye irritation	Glyphosate ammonium salt	90.8	Slightly irritating	Busch (1987a)
Rabbit	New Zealand White	6NS	Eye irritation	Glyphosate sodium salt	70.7	Slightly irritating	Busch (1987b)
Rabbit	New Zealand White	3F, 3M	Eye irritation	Glyphosate MEA salt	62.0 (46.6[c])	Slightly irritating	Blaszcak (1998)
Rabbit	New Zealand White	2F, 1M	Eye irritation	Glyphosate potassium salt	57.8 (47.13[c])	Slightly irritating	Bonnette (2001)
Guinea-pig	Hartley	5F, 5M	Skin sensitization (Buehler test)	Glyphosate	97.7	Not sensitizing	Auletta (1983)
Guinea-pig	Dunkin-Hartley	20F	Skin sensitization (M & K test)	Glyphosate	98.6	Not sensitizing	Cuthbert & Jackson (1989d)
Guinea-pig	Crl(HA)BR	20F	Skin sensitization (M & K test)	Glyphosate	95.6	Not sensitizing	Doyle (1996d)

F, females; IPA, isopropylamine; M, males; MEA, monoethanolamine; M & K, Magnusson & Kligman; NS, not stated;
[a] Two abraded and two intact sites
[b] Eyes were washed 20 or 30 s after treatment
[c] Purity expressed as glyphosate acid

responses scored at 24 h and 72 h. There were no signs of dermal irritation or systemic toxicity in any animal (Heenehan, 1979c). In the remaining four studies, animals with intact skin were exposed for 4 h and the responses scored at 30–60 min and 24, 48 and 72 h. In three studies, glyphosate did not produce dermal irritation (Reagan, 1988c; Cuthbert & Jackson, 1989b; Doyle, 1996b), while in one study, glyphosate produced very mild, transient dermal irritation, i.e. very slight erythema at one or both sites in five of six animals (Blaszcak, 1988c).

The potential of glyphosate acid to irritate the eye was evaluated in six studies in male and/or female New Zealand White rabbits. The studies were performed in compliance with the principles of GLP and according to the test guidelines of the US EPA or the OECD (TG 405). A volume of 0.1 ml (or 65–100 mg) of the test material was applied to one eye of each of the animals, the contralateral eye serving as the control. In one study, the test material was applied as a 25% w/v solution in distilled water (Heenehan, 1979d). In the remaining five studies, the test material was applied undiluted as wet cake (Blaszcak, 1988d) or as powder (Reagan, 1988d; Cuthbert & Jackson, 1989c; Kuhn, 1996; Johnson, 1997). The eyes were examined and scored for ocular reactions for up to 21 days after treatment.

In the first study, in the unwashed eyes there were positive scores for corneal opacity and ulceration (one out of six), for conjunctival redness (five out of six), for chemosis (one out of six) and for conjunctival necrosis (one out of six), while two out of three of the washed eyes had positive scores for corneal opacity and ulceration, conjunctival redness and chemosis. All eyes were clear of signs of irritation by day 7 (Heenehan, 1979d).

In the second study, all six animals showed moderate to severe conjunctival irritation (redness, chemosis, discharge, necrosis) and corneal ulceration, five had iritis and corneal opacities. All animals were free of ocular irritation within 7 to 14 days (Blaszcak, 1988d).

In the third study, ocular responses were corneal opacity and conjunctival irritation with blistering (six out of six), pannus of the cornea (three out of six), prominent vascularization of the conjunctiva (one out of six) and blood like discharge (one out of six). Ocular irritation persisted through study termination (day 21) in three out of five animals (Reagan, 1988d).

The fourth study was conducted in a single animal since the ocular effects (slight corneal opacity, moderate iridial responses, slight to severe conjunctival responses, slight to moderate discharge) suggested that glyphosate is severely irritant. Iridial and conjunctival responses were reversible by 96 h; however, slight cornea1 responses persisted until 96 h after instillation when the study was terminated (Cuthbert & Jackson, 1989c).

In the fifth study, the observed ocular effects (corneal opacity, conjunctival redness, chemosis) indicated severe irritation in all six unwashed eyes and moderate irritation in two out of three washed eyes. Irritation persisted through day 21 in two out of six unwashed eyes and in none of the washed eyes (Kuhn, 1996).

In the sixth study, five animals were pre-treated with a local anaesthetic before dosing since a moderate initial pain reaction was observed in the first animal dosed. Slight corneal opacity and iritis and slight to moderate conjunctival effects (redness, chemosis, discharge) were seen in all animals for up to 4 days. Irritation had completely regressed by day 7 in five animals and by day 8 in the remaining animal (Johnson, 1997).

The potential of glyphosate salts (isopropylamine, ammonium, sodium, monoethanolamine, potassium) to irritate the eye was evaluated in five studies in male and/or female New Zealand White rabbits and using test procedures as described above. The test material was applied undiluted as a liquid (Branch, 1981; Blaszcak, 1998; Bonnette, 2001) or as powder (Busch, 1987a; Busch, 1987b).

In the study with the glyphosate isopropylamine (IPA) salt, there were no signs of ocular irritation in the six animals with washed eyes as well as in the three animals with unwashed eyes up to 72 h after dosing (Branch, 1981).

In the study with the glyphosate ammonium (NH_4^+) salt, all six animals exhibited conjunctival redness, swelling, blistering and discharge within 1 h. The effects were reversible by 48 h (Busch, 1987a).

In the study with the glyphosate sodium (Na^+) salt, all six animals exhibited conjunctival redness, swelling, blistering and discharge within 1 h. The effects were reversible by 72 h (Busch, 1987b).

In the study with the glyphosate monoethanolamine (MEA) salt, one out of six animals had moderate conjunctival irritation (redness, chemosis, discharge) after 1 h, the remaining five animals had only slight conjunctival irritation. The effects were reversible by 24 h (Blaszcak, 1998).

In the study with the glyphosate potassium (K^+) salt, all three animals exhibited iritis and moderate conjunctival irritation (redness, chemosis, discharge) within 1 h. The effects were reversible by 48 h (Bonnette, 2001).

The dermal sensitization potential of glyphosate was evaluated in a Buehler test that complied with the principles of GLP. On the basis of the results of a preliminary test, groups of five male and five female Hartley Albino guinea-pigs received glyphosate (undiluted) or the positive control 2,4-dinitrochlorobenzene (DCNB) at 0.3–0.5% for both the induction phase and the challenge; additional groups of three male and three female animals were used as controls for irritation. In the induction phase, each animal received the test material three times per week for 3 weeks at a volume of 0.2 ml. Two weeks after the final induction dose, the challenge treatment was administered in the same manner. The skin reactions were scored 24 h and 48 h after removal of the patches. Beginning with the sixth induction exposure to glyphosate, mild irritation was apparent in several animals and severe irritation was seen in a few animals. No dermal responses were observed in the animals exposed to glyphosate at challenge (Auletta, 1983).

The dermal sensitization potential of glyphosate was also evaluated in a Magnusson-Kligman maximization test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 406). On the basis of the results of a preliminary test, groups of 10 male and 10 female Dunkin-Hartley guinea pigs received glyphosate by an intradermal injection (10% w/v in distilled water) and 6 days later by topical application (25% w/v in distilled water). Slight skin irritation was observed at the treated sites. Two weeks after the topical induction, the animals were challenged with glyphosate (25% w/v in distilled water). The skin reactions were scored 24 h and 48 h after removal of the patches. None of the animals showed a positive response at challenge (Cuthbert & Jackson, 1989d).

The dermal sensitization potential of glyphosate was evaluated in another Magnusson-Kligman maximization test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 406). On the basis of the results of a preliminary test, groups of 10 male and 10 female albino Crl(HA)BR guinea-pigs received glyphosate by an intradermal injection (0.1% w/v in deionized water) and 1 week later by topical application (75% w/v in deionized water). Slight skin irritation was observed at the treated sites. Two weeks after the topical induction, the animals were challenged with glyphosate (75% and 30% w/v in deionized water). The skin reactions were scored 24 h and 48 h after removal of the patches. Scattered mild redness was seen after challenge with the 75% w/v preparation of glyphosate in 3 out of 20 test animals and 1 out of 10 control animals; however, this response was considered to be due to skin irritation. Challenge with a 30% w/v preparation of glyphosate did not elicit a skin reaction (Doyle, 1996d).

2.2 Short-term studies of toxicity

Mice

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 10 male and 10 female CD-1 mice were fed diets containing glyphosate (purity, 99.5%) at a concentration that was adjusted weekly to give doses of 200, 1000 or 4500 mg/kg bw per day for 13 weeks. Animals were observed daily for symptoms of ill health and mortality. Body weights and food consumption were recorded weekly, and water consumption was monitored throughout the study. Ophthalmoscopy examinations were performed during week 12 of treatment. Blood samples were collected from the orbital sinus for haematology (seven parameters) and from the dorsal aorta at necropsy for clinical chemistry analysis (16 parameters). However, small sample volumes precluded analysis of total protein, albumin and cholesterol. At study termination, all animals were killed and necropsied, 13 organs were isolated and weighed and some 35 separate tissues were fixed for microscopy. All tissues from animals in the control group and that receiving the highest dose, in addition to the kidneys, liver and lungs of animals in the groups receiving the lowest and intermediate doses underwent a full histopathological examination.

No mortalities, clinical signs, haematological or biochemical findings and no organ weight changes were observed that could be attributed to treatment. Gross or histopathological examination did not reveal effects of glyphosate administration. Taking into account the limited range of clinical chemistry parameters evaluated, the the NOAEL was 4500 mg/kg bw per day, the highest dose tested in this study (Perry et al., 1991b).

In a study performed by the NTP, groups of 10 male and 10 female B6C3F_1 mice were fed diets containing glyphosate (purity, 99%) at a concentration of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm for approximately 13 weeks. The calculated mean time-weighted intakes were equal to 507, 1065, 2273, 4776 and 10 780 mg/kg bw per day for males and 753, 1411, 2707, 5846 and 11 977 mg/kg bw per day for females. Food and water were available ad libitum. Analyses of stability of glyphosate in the diet were performed before the start of the study. All animals were observed twice daily for mortality and morbidity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. At study termination, the standard haematology and clinical chemistry parameters were measured. At sacrifice, all animals were given a gross necropsy, and liver, thymus, right kidney, right testis heart and lung were

Table 12. Incidence and severity[a] of cytoplasmic alteration of the parotid salivary gland in mice fed diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)					
	0	3125	6250	12 500	25 000	50 000
Males	0/10	0/10	5/10 (1.0)	9/10 (1.6)	10/10 (2.8)	10/10 (4.0)
Females	0/10	0/10	2/10 (1.0)	9/10 (1.3)	10/10 (2.4)	10/10 (3.1)

From Chan & Mahler (1992)

[a] Average severity score (given in parentheses) was based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

weighed. Organs and tissues were collected and preserved for histopathology. All tissues from animals in the control group and in that receiving the highest dose were examined microscopically. Salivary glands were also examined in all groups receiving lower doses.

Reduced body-weight gain was observed at 25 000 and 50 000 ppm in males and females. There were no differences in food consumption between control and treated mice. The only significant gross finding in the study was a "dark" salivary gland in a male at the highest dose; no other gross abnormalities were observed at necropsy. Histological changes were observed only in the parotid salivary gland (Table 12).

The cytoplasmic alterations consisted of a diffuse increase in the basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared to be enlarged with an appearance of reduced numbers of ducts. No histological changes were observed in the submandibular and sublingual glands.

The NOAEL was 3125 ppm (equal to 507 mg/kg bw per day) on the basis of parotid salivary gland lesions at 6250 ppm and greater, and reduced body-weight gain at 25 000 ppm and greater (Chan & Mahler, 1992).

Rats

In a range-finding study, groups of five male and five female Sprague-Dawley rats were fed diets containing glyphosate (purity, 97.7%) at a concentration of 0, 30 000, 40 000 or 50 000 ppm (equivalent to approximately 1500, 2000 and 2500 mg/kg bw per day) for 4 weeks. All animals were observed twice daily for mortality and morbidity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. All animals were sacrificed and given a gross necropsy at the end of the study. The liver and kidneys from each animal were preserved; for animals in the control group and at the highest dose, these organs were examined microscopically.

No animals died during the study. Slightly reduced body-weight gains were noted in both sexes at all three doses, although significant reductions consistently occurred only in males and females at the highest dose (9.6% and 9.0%, respectively, after 4 weeks). Daily food consumption was reduced for males at the intermediate and highest dose during the first week of the study. Food intake for treated females was comparable to that of controls throughout the study. The only clinical signs of toxicity were soft stools and/or diarrhoea, which occurred in both sexes at all doses with diarrhoea being the predominant sign in animals at the highest dose during the last 3 weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions. Because of frequent

occurrence of soft stools and/or diarrhoea at all doses, no NOAEL could be derived from this study (Reyna & Thake, 1989).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the OECD (TG 407), groups of five male and five female Sprague-Dawley rats were fed diets containing glyphosate (purity, 99.5%) at a concentration that was adjusted weekly to give doses of 0, 50, 250, 1000 or 2500 mg/kg bw per day for 4 weeks. The animals were observed daily for mortality and symptoms of ill health and once a week received a detailed clinical examination. Body weights and food consumption were calculated weekly, water consumption was monitored by visual inspection throughout the study. Blood samples were collected from the orbital sinus for haematology (seven parameters) and clinical chemistry (17 parameters) analysis. At study termination, all animals were sacrificed and necropsied. The liver, heart, kidney, spleen and adrenals were processed and examined histopathologically for all animals in the control group and at a doses of 2500 mg/kg bw per day. Examination was subsequently extended to kidneys from all females in the groups receiving the lowest and intermediate doses.

A single unscheduled death during the study occurred when a male rat from the group receiving a dose of 250 mg/kg bw per day died during blood sampling and could not be attributed to treatment with glyphosate. Soft faeces were noted in three males from the group receiving the highest dose during weeks 3–4 of the study, but were not seen in any other group. A slight but consistent body-weight reduction was observed in males and females at the highest dose of 2500 mg/kg bw per day, although statistical significance was not reached. There were no notable intergroup differences with regard to food and water consumption or haematology parameters. In males, equivocal increases in plasma alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were seen at the three higher doses. In females, plasma ALT activity was significantly increased at the highest dose, as was total bilirubin. In addition, increased plasma concentrations of phosphate were noted in males at the two higher doses. There were neither notable intergroup differences in organ weights nor gross pathological findings. However, an increase in the incidence of very mild to slight nephrocalcinosis was observed by means of histopathology in female rats dosed at 250 mg/kg bw per day and greater (Table 13).

On the basis of the histopathological findings in kidneys and supported by changes in clinical chemistry parameters, the NOAEL was 50 mg/kg bw per day, i.e. the lowest dose tested (Atkinson et al., 1989).

Table 13. Incidence and severity of nephrocalcinosis in female rats given diets containing glyphosate for 4 weeks

	Dose (mg/kg bw per day)									
	Males					Females				
	0	50	250	1000	2500	0	50	250	1000	2500
Mineral deposits (nephrocalcinosis)	0/5	NI	NI	NI	0/5	0/5	0/5	2/5	2/5	4/5
Severity:										
Very mild/minimal	0	NI	NI	NI	0	0	0	1	1	2
Mild/slight	0	NI	NI	NI	0	0	0	1	1	2

From Atkinson et al. (1989)
NI, not investigated

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, groups of 12 male and female Sprague-Dawley rats were fed diets containing glyphosate (purity, 95.2%) at a concentration of 0, 1000, 5000 or 20 000 ppm for 90 days. The calculated mean intakes were equal to 63, 317 and 1267 mg/kg bw per day for males and 84, 404 and 1623 mg/kg bw per day for females. Clinical signs, body weight, food consumption, haematology and clinical chemistry parameters were monitored routinely. Gross examinations were performed for all groups, and kidneys, liver, and testes were weighed. A standard range of tissues from animals in the control group and at the highest dose was examined microscopically, as well as kidneys, livers, and lungs from animals at the lowest and intermediate doses.

No treatment-related effects were observed at up to the highest dose. However, parotid salivary glands were not included in the histopathological examination. The NOAEL was 20 000 ppm (equal to 1267 and 1623 mg/kg bw per day for males and females, respectively), the highest dose tested (Stout & Johnson, 1987).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly to give doses of 0, 30, 300 or 1000 mg/kg bw per day for 13 weeks. Animals were observed daily for symptoms of ill health and mortality. Body weights and food consumption were recorded weekly and water consumption was measured gravimetrically on a weekly basis. Ophthalmoscopy examinations were performed before the start of the study and again at week 13 of treatment. Blood samples were collected from the orbital sinus during week 13. The collected blood was analysed by haematology (eight parameters) and clinical chemistry (19 parameters). Urine samples were collected over a 4-h period of food and water deprivation, and the samples were analysed for 10 parameters. At study termination all animals were sacrificed and necropsied, 14 organs removed and weighed, and some 40 separate tissues fixed for microscopy. All tissues from animals in the control group and at the highest dose, in addition to kidneys, liver, lungs and parotid salivary glands of all the other test animals, underwent a full histopathological examination.

There were no mortalities or clinical signs throughout the 13-week study that could be attributed to the administration of glyphosate. Body-weight gain tended to be lower in males at the highest dose, but statistical significance was not attained. No such effect was seen in any other group. There were no notable intergroup differences in either sex with regard to food and water consumption or ophthalmoscopy. Haematological examinations did not reveal findings that could be related to substance administration. Females at the highest dose showed slight but statistically significant increases in concentrations of glucose (11%), total protein (9%), albumin (9%) and creatinine (8%) compared with those in the control group. Urine analysis revealed a reduction in pH in males at the highest dose. There were no intergroup differences in organ weights and no gross pathological findings that could be attributed to treatment with glyphosate.

In contrast to a 4-week study in rats conducted at the same testing facility (Atkinson et al., 1989, see above), the incidence of nephrocalcinosis in this 13-week study was evenly distributed among dose groups and sexes and did not follow a dose–response relationship, and is therefore clearly not treatment-related. Thus, the previous finding was not confirmed.

An increase in the incidence of cellular alterations (deep basophilic staining and enlargement of cytoplasm) was noted in the parotid salivary glands of both sexes in all treated groups. In addition, the severity (graded as very mild, mild, moderate, severe, and very severe) of these findings showed a dose-related increase, but reached statistical significance in males at the highest dose only (Table 14).

In conclusion, rats treated with glyphosate for 13 weeks showed dose-related histopathological changes in the parotid salivary gland. However, at the lower doses of 30 and 300 mg/kg bw per day, these changes were only minimal with respect to severity and incidence and are considered to be of equivocal toxicological significance. The NOAEL was 300 mg/kg bw per day on the basis of the more pronounced severity of cellular alterations in the parotid salivary gland at 1000 mg/kg bw per day (Perry et al., 1991a).

In a study of toxicity performed by the United States NTP, groups of 10 male and 10 female F344/N rats were fed diets containing glyphosate (purity, 99%) at a concentration of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm for approximately 13 weeks. Ten additional animals of each sex were included at each dietary concentration for evaluation of haematologic and clinical pathology parameters. The calculated mean intakes were equal to 205, 410, 811, 1678 and 3393 mg/kg bw per day for males and 213, 421, 844, 1690 and 3393 mg/kg bw per day for females. Food and water were available ad libitum. Analyses of the stability of glyphosate in the diet were performed before the start of the study. All animals were observed twice daily for mortality and morbidity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. At study termination, standard haematology and clinical chemistry parameters were measured. At sacrifice, all animals were given a gross necropsy, and liver, thymus, right kidney, right testis heart and lung were weighed. Organs and tissues were collected and preserved for histopathology. All tissues from animals in the control group and at the highest dose were examined microscopically. Salivary glands were also examined for animals at all lower doses.

All animals survived until the end of the study. Diarrhoea was observed in males at the highest dose and in females for the first 50 days of the study. Weight gain was reduced in males at 50 000 and 25 000 ppm, and the final mean body weight was approximately 18% and 6% less than that of controls, respectively. Females at the highest dose exhibited a 5% decrease in body-weight gain compared with the controls. Small increases in several

Table 14. Incidence and severity of cytoplasmic alteration of the parotid salivary gland in rats given diets containing glyphosate for 13 weeks

	Dose (mg/kg bw per day)							
	Males				Females			
	0	30	300	1000	0	30	300	1000
No. of animals examined	10	10	10	10	10	10	10	10
Severity:								
Very mild	3	7	6	0	2	7	7	1
Mild	0	0	3	2	0	1	2	4
Moderate	0	0	1	3	0	0	0	3
Severe	0	0	0	5*	0	0	0	1
Total incidence	3	7	10**	10**	2	8*	9**	9**

From Perry et al. (1991a)
*$p < 0.05$, **$p < 0.01$

erythrocyte parameters were noted in males at doses of 12 500 ppm and greater. These changes were unremarkable and generally consistent with a mild dehydration. Plasma ALP and ALT activities were slightly increased in males at 6250 ppm and greater and in females at 12 500 ppm and greater. In the absence of histopathological findings in the liver, these increases are considered to be of no toxicological significance.

No treatment-related gross abnormalities or organ weight changes were observed at necropsy. Histopathological changes were observed only in the parotid and submandibular glands of male and female rats. The study authors combined the findings for these two glands (Table 15). The findings for each gland individually or for individual animals were not reported. No histological alterations were observed in the sublingual gland. The changes were described as cytoplasmic alterations and consisted of basophilic changes and hypertrophy of the acinar cells. Considering the 16-fold difference between the lowest dose of 3125 ppm and the highest dose of 50 000 ppm, the incidence–response curve appears to be relatively flat and the degree of change is slight, progressing from only minimal to moderate.

In conclusion, the administration of glyphosate to rats for 13 weeks produced dose-related histopathological changes in the parotid and submandibular salivary glands. However, at the lower doses of 3125 and 6250 ppm, these changes were only minimal with respect to severity and are considered to be of equivocal toxicological significance. The NOAEL was 6250 ppm (equal to 410 mg/kg bw per day) on the basis of the more pronounced severity of cellular alterations in the salivary glands at 12 500 ppm and greater (Chan & Mahler, 1992).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 12 male and 12 female Alpk:AP Wistar-derived rats were fed diets containing glyphosate (purity, 97.4%) at a concentration of 0, 1000, 5000 or 20 000 ppm for 90 days. The calculated mean intakes were equal to 81, 414 and 1693 mg/kg bw per day for males and 90, 447 and 1821 mg/kg bw per day for females. Clinical observations, body weights and food consumption were measured and all animals subjected to a full examination post mortem. Cardiac blood samples were taken and urine samples were collected for clinical pathology. Selected organs were weighed and specified tissues taken for subsequent histopathological examination. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study.

All animals survived the study in good clinical condition. A low incidence of diarrhoea and light coloured faeces were seen in both sexes at 20 000 ppm in the second week of the study. Males at the highest dose showed statistically significant reductions in

Table 15. Incidence and severity[a] of cytoplasmic alteration of the parotid and submandibular salivary glands (combined) in rats given diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)					
	0	3125	6250	12 500	25 000	50 000
Males	0/10	6/10 (1.0)	10/10 (1.0)	10/10 (1.8)	10/10 (2.7)	10/10 (2.9)
Females	0/10	8/10 (1.0)	10/10 (1.0)	10/10 (2.1)	10/10 (2.4)	10/10 (3.0)

From Chan & Mahler (1992)

[a] Average severity score (in parentheses) was based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Table 16. Selected haematological and clinical chemistry findings at study termination (week 13) in rats given diets containing glyphosate

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	1000	5000	20 000	0	1000	5000	20 000
Platelet count $\times 10^9$/l	708	668	638**	625	695	673	635*	625**
Prothrombin time	12.4	13.0*	13.3**	13.5**	NE	NE	NE	NE
ALT (mU/ml)	51.9	52.3	62.3*	65.2**	45.0	45.2	46.2	55.0**
ALP (mU/ml)	148	159	176*	215**	91	94	99	140**
Urea (mg/100 ml)	41.9	39.9	40.0	37.7*	40.6	40.1	42.1	35.9**
Triglycerides (mg/100 ml)	153	157	144	120**	NE	NE	NE	NE
Albumin (g/100 ml)	4.81	4.60*	4.82	4.62*	NE	NE	NE	NE
Total protein (g/100 ml)	6.53	6.22*	6.43	6.06**	NE	NE	NE	NE
Glucose (mg/100 ml)	NE	NE	NE	NE	182	183	183	208**

From Botham (1996)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; NE, no treatment-related effect observed
*$p < 0.05$, **$p < 0.01$; Student's *t*-test, two-sided

body-weight gain and food utilization efficiency when compared with controls. There was some evidence for a reduction in platelet count in males and females fed diets containing glyphosate at 5000 or 20 000 ppm. Also, a marginal dose-related increase in prothrombin time was observed in males at all doses. The differences, however, were small and considered not to be of haematological significance. Plasma ALP and ALT activities were increased in both sexes at 20 000 ppm and, to a lesser extent, in males at 5000 ppm. Further minor changes that did not always follow a clear dose–response relationship are detailed in Table 16. A few of these effects had already become apparent at week 4. In addition, plasma AST activity was increased in females at the highest dose at this early time-point, but not at study termination. There were no treatment-related effects on urine biochemistry.

The statistically significant reductions in absolute heart and liver weights in males at the highest dose were associated with lower body weights at termination. The only notable histopathological finding was a uterine leiomyosarcoma in one female at 5000 ppm. Although rare, the finding of such a tumour in an animal receiving the intermediate dose was considered to be incidental to treatment.

In the absence of other findings at 5000 ppm, the increases in plasma ALT and ALP activities in males at this dose were not considered to be of toxicological significance. The NOAEL was 5000 ppm, equal to 414 mg/kg bw per day, on the basis of reduced growth (males only) and clinical chemistry changes that may be associated with an altered liver metabolism and/or slight liver damage at 20 000 ppm (Botham, 1996).

Dogs

In a dose range-finding study performed in compliance with the principles of GLP, one male and one female beagle dogs were fed gelatin capsules containing glyphosate (purity, 99.5%) at increasing doses of 100, 300 or 1000 mg/kg bw per day, each dose being administered for 7 consecutive days. A second group of one male and one female dog received gelatin capsules containing glyphosate at a dose of 1000 mg/kg per day for 14 consecutive days. Animals were observed daily for clinical signs, body weights were recorded twice weekly, and food consumption was recorded daily. Blood, urine and faecal samples were taken before dosing and at termination. Terminal studies comprised gross examination and the weighing of heart, liver, kidneys and spleen. Specimens of these organs

plus tissue from adrenals, gonads and thymus were preserved but not evaluated microscopically.

In the first group, no treatment-related clinical signs were observed. Body weights and food consumption were considered to be satisfactory throughout the treatment period. There were no treatment-related haematological findings. A mild increase in plasma ALT activity was found in the male dog and cholesterol concentrations were slightly reduced in both animals. Studies at termination found no lesions attributable to treatment. In the second group, no treatment-related clinical signs were observed. However, loose faeces were recorded for the male dog throughout the study. Body weights and food consumption were considered to be satisfactory throughout the treatment period. There were no treatment-related haematological findings. A mild increase in plasma ALT activity was recorded in the male dog. Studies at termination found no lesions attributable to treatment (Goburdhun & Oshodi, 1989).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 409), groups of four male and four female beagle dogs were fed diets containing glyphosate (purity, 99.1%) at a concentration of 0, 2000, 10 000 or 50 000 ppm for 90 days. The calculated mean intakes were equal to 68, 323 and 1680 mg/kg bw per day for males and 68, 334 and 1750 mg/kg bw per day for females. Clinical signs including faecal consistency were recorded daily and body weights weekly. A more detailed examination including cardiac and pulmonary auscultation and indirect ophthalmoscopy was made before the start of the experiment and before termination. Food residues were recorded daily. A full range of haematology and biochemistry analyses were performed before the start of treatment and in weeks 4, 8 and 13. Urine samples were collected and analysed once before the start of the experiment and in week 13. On completion of the 90-day dosing period, all animals were killed and a full macroscopic examination carried out. Selected organs were weighed and specified tissues taken from all groups for histopathological examination. The achieved dietary concentrations of glyphosate were all within ± 9% of the target concentrations. The homogeneity of the diets was considered to be satisfactory and glyphosate was shown to be stable over 39 days.

There were no mortalities during the study. All the dogs ate all the diet presented during the dosing period. Body-weight gain of males and females at 50 000 ppm showed a slight depression throughout the study, but the differences were occasionally statistically significant only in females. There were no changes in the haematological profile attributable to treatment. In male dogs, plasma concentrations of albumin, total protein and calcium were slightly (statistically significantly) decreased at 50 000 ppm. In female dogs, plasma ALP activities were statistically significantly increased (119–125% of controls) throughout the study at 50 000 ppm. Urine analysis did not reveal indications of treatment-related findings. No adverse effects were seen at examination post mortem and no histopathological changes attributable to compound were found. Kidney weights (adjusted for body weight) of males given diets containing glyphosate at 10 000 or 50 000 ppm were statistically significantly increased (111 and 113% of controls, respectively). There was also a statistically significantly increase in liver weight (adjusted) at these doses in male dogs (111% and 113% of controls, respectively). These weight increases were not associated with any histopathological lesion.

The NOAEL was 10 000 ppm (equal to 323 mg/kg bw per day), on the basis of reduced body-weight gains and changes in clinical chemistry parameters at 50 000 ppm (Hodge, 1996).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 452), groups of four male and four female beagle dogs were fed gelatine capsules containing glyphosate (purity, 98.6–99.5%) at a dose of 0, 30, 300 or 1000 mg/kg bw per day once daily for 52 weeks. Administration of multiple capsules was necessary at the highest dose; control animals received the same number of capsules as did the group given the highest dose. The accuracy of dosing was acceptable (<5% deviation from nominal weight) and there was no indication of degradation of encapsulated glyphosate over 7 days under the storage conditions employed. The animals were observed daily for signs of ill health or reaction to the test material; observations were recorded with regard to the nature, time of onset, severity and duration. Dogs were weighed weekly, and food consumption was recorded daily. Ophthalmoscopy examinations were performed on both eyes prior to the start of dosing and again during weeks 13, 29, 39 and 51 of treatment. Laboratory investigations of haematology, clinical chemistry and urine analysis were performed on all dogs before the start of dosing and again during weeks 13, 26, 39 and 51 of treatment. Blood samples were taken from the jugular vein after the dogs had been fasted over night. Urine and faecal samples were collected over the final 17 h of a 21-h period of water deprivation while the animals were housed in metabolism cages for the conduct of kinetic investigations (determination of concentrations of glyphosate in the plasma). After completion of dosing, all the animals were sacrificed and subjected to a gross pathological examination. Seventeen organs were removed and weighed and approximately 37 tissues were processed for histopathological examination.

There were no mortalities throughout the test period. Changes in faecal consistency (soft/loose/liquid) were recorded frequently for animals in the group receiving glyphosate at a dose at 1000 mg/kg bw. This finding was observed 4–6 h after dosing and was also recorded on isolated occasions for a few animals at 300 mg/kg bw. It was considered to be related to the administration of glyphosate. There were no other clinical signs related to treatment with glyphosate. Food consumption was maximal or near maximal for all test groups. Mean body-weight gain showed a non-statistically significant reduction in males at all doses (approximately 83, 75 or 75% of that of the control group for the groups receiving the lowest, intermediate, and highest dose respectively) and in females at the highest dose (81% of that of the control group). Ophthalmoscopy and laboratory examinations revealed no treatment-related abnormalities. Plasma concentrations of glyphosate suggested that absorption was dose-related and remained constant throughout the duration of the study. Mean values detected were 0.36, 1.82 and 6.08 µg/ml for the groups receiving the lowest, intermediate and highest doses, respectively. At necropsy, no abnormal gross findings and no significant intergroup organ weight differences attributable to treatment with glyphosate were noted. In males, absolute and relative weights of the liver were slightly increased (4%, 8% and 10% above that of the control group, and 10%, 17% and 19% above that of the control group for the groups receiving the lowest, intermediate and highest doses, respectively), but the differences did not achieve statistical significance. There were no significant histopathological findings at any dose.

The faecal inconsistencies seen a few hours after dosing were most likely to be related to high local concentrations of glyphosate in the gastrointestinal tract that were attributable to the administration of the test substance in capsules. The NOAEL was 30 mg/kg bw per day on the basis of the changes in faecal consistency and the reduced body-weight gain in males at 300 mg/kg bw per day and greater (Goburdhun, 1991).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 452), groups of four male and four female beagle dogs were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0, 3000, 15 000 or 30 000 ppm for 1 year. The calculated mean intakes were equal to 91, 440 and 907 mg/kg bw per day for males and 92, 448 and 926 mg/kg bw per day for females. Analysis of the diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. Clinical signs were recorded daily and each dog was weighed and given a more thorough examination weekly. All dogs were given a full clinical examination (including cardiac and pulmonary auscultation and indirect ophthalmoscopy) by a veterinarian before the study, during weeks 13, 26, 39, and before termination. Food residues were recorded daily. A comprehensive range of haematology and biochemistry analyses were performed in weeks −1, 4, 13, 26 and before termination. Urine samples were collected before the start of the experiment, mid-term and during the week before termination. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem. Selected organs were weighed and specified tissues taken from all groups for histopathological examination.

There were no mortalities during the study. There was no effect on food consumption; only three dogs left small amounts of food intermittently during the study. Body weight was slightly reduced in females at 30 000 ppm, with a maximum reduction of 11% (compared with that of controls) in week 51. These dogs showed a gradual reduction in growth rate, compared with that of controls, which was consistently significant from week 23 onwards. A similar change in body-weight gain in females receiving glyphosate at the lowest dose of 3000 ppm, although occasionally reaching statistical significance, was not regarded as treatment-related since a dose–response relationship was lacking. There was no effect on body weight in males at any dose tested. There were no toxicologically significant effects on any of the haematological parameters measured. Plasma concentrations of cholesterol was slightly increased in both sexes in the treated groups at weeks 26 and 52, but there was no evidence of a dose–response relationship. Plasma concentrations of phosphorus were significantly lower in groups of treated males at week 52, which was due, in part, to slightly higher concentrations for individual control animals. The significantly reduced plasma concentration of sodium in males at the highest dose at week 52 was solely attributable to one animal. There were no treatment-related effects in any of the clinical chemical parameters measured in urine. No adverse effects of glyphosate were seen at examination post mortem and there were no treatment-related effects on organ weights. No histopathological changes attributable to administration of glyphosate were found.

The NOAEL for females was 15 000 ppm (equal to 448 mg/kg bw per day) on the basis of a reduction in body weight at 30 000 ppm. The NOAEL for males was 30 000 ppm (equal to 907 mg/kg bw per day), the highest dose tested (Brammer, 1996).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 451), groups of 50 male and 50 female CD-1 mice were fed diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly for the first 13 weeks and every 4 weeks thereafter to give doses of 0, 100, 300 and 1000 mg/kg bw per day for 104 weeks. Routine analysis of the diet was

performed at regular intervals throughout the study providing satisfactory results. Test animals were examined daily for mortalities and clinical signs. Once per week, animals received a detailed clinical examination with particular regard to palpable masses. Body weight and food consumption were recorded weekly until week 13 of dosing and thereafter every 4 weeks. Blood samples were collected during weeks 52, 77 and 102 and analysed for differential leukocyte counts. At study termination all surviving animals were sacrificed and necropsied, all premature decedents were also necropsied. Fifteen organs were removed and weighed and some 35 tissues were evaluated histologically for all surviving animals in the control group and at the highest dose, premature decedents were also examined. The kidneys, liver, lungs and any abnormal tissue from animals at the intermediate dose were also examined.

There were no unscheduled deaths during the course of the study that were attributable to the administration of glyphosate. Clinical signs were distributed equally throughout all the groups, and included emaciation, a hunched posture, subdued behaviour and exophthalmic eyes. There were no notable intergroup differences in the incidences of externally palpable masses. All groups receiving glyphosate showed comparable food consumption and weight gains when compared with the controls. There were no remarkable intergroup differences in differential blood counts in either sex at any of the time-points tested. The increased thymus weight in males at the intermediate and highest doses was not associated with any findings at necropsy or after histological evaluation. Owing to the slight magnitude of the increase seen, the lack of a dose–response relationship, and the lack of an effect in females, the increases were considered to be chance effects. During necropsy examinations, the incidence of lung masses was slightly higher in males at the highest dose (18/50) than in the control group (10/50); however, histopathology failed to reveal adverse lung findings. The occurrence of mineral deposits in the brain was significantly increased in males at the highest dose when compared with the control group (13/50 compared with 4/49). It should be noted that this is a common finding in mice of this age and strain. There were no other findings in the males, and no findings at all in the females that could be attributed to treatment with glyphosate.

There were no statistically significant increases in the incidence of any tumours, either benign and malignant, in either sex when compared with the control groups. However, the number of animals with multiple types of tumour was slightly higher in both sexes at the highest dose (males, 16/50; females, 11/50) than in the controls (males, 11/50; females, 6/50). This led to a slight increase in the total number of tumours in the at the highest dose for both sexes (males, 60; females, 43) compared with the controls (males, 49; females, 36). Haemangiosarcoma was evident in 4/50 males at the highest dose, in 2/50 females at the lowest dose, and in 1/50 females at the highest dose, but in none of the 50 animals of the control group. Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue was evident in 2/50 males at the lowest and highest doses, and in 3/50 females at the lowest and intermediate doses and 1/50 females at the highest dose when compared with the respective controls (0/50). Owing to the lack of a dose–response relationship, the lack of statistical significance and the fact that the incidences recorded in this study fell within the historical ranges for controls, these changes are not considered to be caused by administration of glyphosate.

In conclusion, administration of glyphosate to CD-1 mice for 104 weeks produced no signs of carcinogenic potential at any dose. The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Atkinson et al., 1993a).

Rats

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 452), groups of 24 male and 24 female Alpk:AP$_f$SD (Wistar-derived) rats were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000 or 20 000 ppm for 1 year. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. The calculated mean intakes were equal to 141, 560 and 1409 mg/kg bw per day for males and 167, 671 and 1664 mg/kg bw per day for females. The animals were monitored daily for mortality and clinical observations. Body weights and food consumption were measured and, at the end of the scheduled treatment period, the rats were killed and subjected to a full examination post mortem. Blood and urine samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no unscheduled deaths during the course of the study that could be attributed to the administration of glyphosate. Apart from a small increase in the number of male and female animals in the group receiving gyphostae at 20 000 ppm that showed wet or dry urinary staining, there were no other treatment-related clinical observations and no treatment-related ophthalmological findings. At the two higher doses, body weights were lower than those of the concurrent controls, with the difference reaching statistical significance at 20 000 ppm in both sexes and at 8000 ppm only in females towards study termination. There was no effect on body weight in animals at 2000 ppm. Food consumption was lower and food utilization was slightly less efficient at 20 000 ppm, the reductions being most marked at the start of the study. There was a trend for reduced food intake for females at 8000 ppm, which correlates with the reduction in body-weight gain at this dose in the latter stages of the study.

Deviations in some clinical chemistry parameters, such as reductions in plasma concentration of cholesterol and triglycerides or a dose-related increase in plasma ALP activity throughout the study as well as occasional increases in the activities of plasma AST, ALT and creatine kinase, were mostly confined to groups receiving the high and intermediate doses and were probably treatment-related (Table 17). In the absence of any histopathological findings, these changes are considered to be of marginal toxicological relevance. There was no evidence of any effect of glyphosate on urine parameters.

At necropsy, there were no gross pathological findings that could be attributed to treatment and no consistent organ weight changes. Histopathology revealed an increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland in both sexes at 20 000 ppm (Table 18). At 8000 ppm, this finding was of minimal severity and its incidence was only slightly above that in the control animals. No other microscopic findings could be ascribed to administration of glyphosate.

Similar numbers and types of neoplasms were diagnosed in the control group and in the group receiving glyphosate at 20 000 ppm, but the duration of the study was not sufficiently long to enable final conclusions to be made with regard to carcinogenicity. The NOAEL was 2000 ppm, equal to 141 mg/kg bw per day, on the basis of a reduction in body weight and clinical chemistry findings at dietary concentrations of 8000 ppm and greater (Milburn, 1996).

Table 17. Selected clinical chemistry findings in rats given diets containing glyphosate for 1 year

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20 000	0	2000	8000	20 000
Cholesterol:								
Week 14	2.46	2.53	2.31	2.28*	2.13	2.28	2.26	2.21
Week 27	3.09	3.05	2.75*	2.70**	2.62	2.67	2.76	2.78
Triglycerides:								
Week 14	1.56	1.63	1.28**	1.28**	0.94	0.92	0.89	0.95
Week 27	1.51	1.43	1.15**	0.97**	1.07	1.10	1.13	1.10
ALP:								
Week 14	248	281	342**	429**	161	201*	227**	292**
Week 27	221	250	306**	412**	135	171	200**	254**
Week 53	232	258	291**	379**	87	100	114	160**
ALT:								
Week 14	84	93	111**	110**	66	79	88**	91**
Creatine kinase:								
Week 14	118	124	127	144**	97	108	107	124**

From Milburn (1996)
ALP, alkaline phosphatase; ALT, alanine aminotransferase
*$p < 0.05$, **$p < 0.01$; Student's *t*-test, two-sided

Table 18. Incidence of focal basophilia of parotid acinar cells in rats given diets containing glyphosate for 1 year

	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20 000	0	2000	8000	20 000
Severity:								
Minimal	2	0	3	10	2	0	6	8
Slight	0	0	0	3	0	0	0	5
Moderate	0	0	0	0	0	0	0	2

From Milburn (1996)

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, groups of 60 male and 60 female Charles River CD®(SD)BR rats were fed diets containing glyphosate (purity, 96.5%) at a concentration of 0, 2000, 8000 or 20 000 ppm for 2 years. In principle, the study was also compliant with OECD TG 453, although satellite groups comprised only 10 animals of each sex and group and survival had fallen below 50% (varying between 29% and 44%) in all test groups at scheduled termination. Regular dietary analyses gave satisfactory actual concentration, homogeneity and stability. The calculated mean intakes were equal to 89, 362 and 940 mg/kg bw per day in males and 113, 457 and 1183 mg/kg bw per day in females. All animals were observed twice daily for mortality and moribundity and detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week for the first 13 weeks and then every fourth week thereafter. Ophthalmic examinations were performed before the test and just before terminal sacrifice. Comprehensive determinations of haematological, blood biochemistry and urine analysis parameters were conducted on 10 animals of each sex per dose each at 6, 12 (interim sacrifice), 18, and 24 (study termination) months. Ten animals of each sex per dose were sacrificed at month 12. All animals were given a complete gross necropsy. Brain, kidneys, liver and testes with epididymides were weighed. Approximately 40 tissues were preserved and examined microscopically.

There were no statistically significant differences in mortality during the study. No evidence of treatment-related clinical signs was recorded except the ophthalmological findings mentioned below. Statistically significant reductions in body weight were noted in females at the highest dose from week 7 to approximately month 20. During this time, absolute body weights gradually decreased to 14% below the control value owing to a reduction in body-weight gain by up to 23%. In contrast, body-weight gain in all treated male groups was comparable to that of controls. Food consumption was not adversely affected by treatment in any sex despite an increase in males at the highest dose.

The ophthalmic examination before study termination revealed a statistically significant difference ($p < 0.05$) between the incidences of cataractous lens changes in males in the control group and in the group receiving the highest dose (none out of 15 compared with five out of 20). The occurrence of cataractous lens changes in males at the lowest and intermediate doses, as well as in all treated groups of females, were comparable to that of their respective controls. The observed incidence for this finding of 25% for male CD rats at the highest dose was within the range (0–33%) observed in previously conducted studies at this laboratory, but a treatment-related impact could not be excluded. An independent pathologist's examination confirmed a statistically significant increase ($p < 0.05$) in the incidence of cataractous lens changes in males at the highest dose (one out of 14 compared with eight out of 19) and concluded that there appeared to be a treatment-related occurrence of lens changes affecting males at the highest dose. Histological examination of the eyes at study termination revealed the incidences of cataract and/or lens fibre degeneration (Table 19). The results of histopathology also suggested that there was an increase in cataractous lesions in male rats at 20 000 ppm, although the difference in incidences in the control group and at the lowest and intermediate doses was less pronounced than suggested by ophthalmoscopy.

This outcome was essentially confirmed by re-evaluation by an independent laboratory. It was concluded that there was a slight, statistically significant (as indicated in the Cochrane-Armitage linear trend test) increased incidence of basophilic degeneration of the posterior subcapsular lens fibres in males at the highest dose, but not in those at the intermediate or lowest dose, nor in any treated group of females.

There were various changes in haematology and serum chemistry parameters, but these changes were not consistently noted at more than one time-point, were within ranges for historical controls, were small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically not significant. However, the statistically significant increase in alkaline phosphatase activity in females at the highest dose at study termination is in line with observations made in other long-term studies in rats, although it was partly attributable to one animal with an

Table 19. Incidences of cataract and lens fibre degeneration determined by histological examination in male rats given diets containing glyphosate for 1 year

	Dietary concentration (ppm)			
	0	2000	8000	20 000
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

From Stout & Ruecker (1990)

outstandingly high value. Statistically significant reductions in urine pH were noted in males at the highest dose at months 6, 18, and 24, reflecting the renal excretion of glyphosate, which is an acid.

Statistically significant increases in liver weight were noted in males at the highest dose. There were no other statistically significant changes in organ weights that occcurred in a dose-related manner. Gross abnormalities observed at necropsy were not considered to be related to administration of glyphosate.

Regarding neoplastic lesions, the only statistically significant difference between control and treated animals was an increase in the incidence of pancreatic islet cell adenomas in males at the lowest dose. The incidences of this lesion were 1 out of 58 (2%), 8 out of 57 (14%), 5 out of 60 (8%), and 7 out of 59 (12%) in males in the control group and at the lowest, intermediate and highest dose, respectively. The historical-control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of studies reported recently in the literature revealed a prevalence of 0–17% in control males with several values being ≥ 8%. More importantly, the incidences of islet cell adenomas clearly did not follow a dose-related trend in the treated groups of males, as indicated by the lack of statistical significance in the Peto trend test. It should be noted that there was also considerable inter-group variability in the numbers of females with this tumour (5 out of 60, 1 out of 60, 4 out of 60 and 0 out of 59 in the control group and at the lowest, intermediate and highest doses, respectively). There was no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a male in the control group, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support the conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to administration of glyphosate.

With regard to non-neoplastic changes (apart from the findings in the eye, described above), histopathological examination revealed an increase in the number of animals displaying inflammation of the stomach squamous mucosa at 8000 and 20 000 ppm, achieving statistical significance for females only at the intermediate dose. The incidences of this lesion in all groups of animals are shown in Table 20.

Although the incidence of this lesion in females at the intermediate dose (15%) was slightly outside the range for historical controls (0–13.3%) for the laboratory, there was no

Table 20. Incidence of inflammation and hyperplasia of the stomach squamous mucosa in rats given diets containing glyphosate for 24 months

	Dietary concentration (ppm)			
	0	2000	8000	20 000
Males				
Inflammation	2/58	3/58	5/59	7/59
Hyperplasia	3/58	3/58	4/59	7/59
Females				
Inflammation	0/59	3/60	9/60**	6/59
Hyperplasia	2/59	3/60	7/60	6/59

From Stout & Ruecker (1990)
** $p \leq 0.01$; Fisher's exact test with Bonferroni inequality

dose-related trend across all groups of treated females and there was no significant difference in any group of males. Therefore, it is equivocal whether this finding was treatment-related. However, a weak irritation potential of the test material may be assumed at high doses. In contrast to other long-term studies, no histological changes in salivary glands were reported. However, it must be mentioned that in this study only the mandibular (submaxillary) salivary glands were evaluated microscopically, and not the parotid glands.

In conclusion, administration of glyphosate to Sprague-Dawley rats for 24 months produced no signs of carcinogenic potential. The NOAEL was 8000 ppm, equal to 362 mg/kg bw per day, on the basis of a reduction in body weight in females and cataractous lens changes in males at 20 000 ppm (Stout & Ruecker, 1990).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, groups of 85 male and 85 female Sprague-Dawley rats were fed diets containing glyphosate (purity, 98.7–98.9%) at a concentration that was adjusted weekly for the first 12 weeks and every 2 months thereafter to give doses of 0, 100, 300 and 1000 mg/kg bw per day for 104 weeks. The doses were selected on the basis of results from a 13-week dietary study of toxicity. Routine analysis of the diet was performed at regular intervals throughout the study, giving acceptable results. Fifty rats of each sex per dose were allocated to the 104-week study of oncogenicity, and 35 rats of each sex per dose were allocated for long-term testing for toxicity. Fifteen rats of each sex per dose from every group testing for toxicity in the long term were killed after 52 weeks, all remaining rats were dosed until scheduled termination after 104 weeks. Test animals were examined daily for mortalities and clinical signs. Once per week, animals received a detailed clinical examination, with particular regard to palpable masses. Body weight and food consumption were recorded weekly until week 13 of dosing and thereafter every 4 weeks. Ophthalmoscopy examinations were performed on 20 males and 20 females from the control groups and the group receiving the highest dose before initiation of dosing and again during weeks 24, 50 and 102. Blood samples were collected from the orbital sinus of 10 males and 10 females from each group after approximately 14, 25, 51, 78 and 102 weeks. Blood samples were analysed for eight haematology parameters and nineteen clinical chemistry parameters. At study termination, all surviving animals (and the premature decedents, if possible) were sacrificed and necropsied. Fifteen organs were removed and weighed and some 35 tissues were evaluated histologically from all surviving animals in the control group and at the highest dose, premature decedents being also examined in this way. The kidneys, liver, lungs, sublingual, submaxillary and parotid salivary glands and any abnormal tissue from the groups receiving the intermediate dose were also examined.

Survival was not affected by treatment and there were no clinical signs of toxicity that were thought to be related to administration of glyphosate. Ophthalmoscopy did not reveal any indications of adverse effects. Body-weight gain was reduced in males and females at the highest dose. At the lower doses, no consistent and clearly dose-related body-weight change was to be seen. Food consumption and water intake were not affected.

Haematological changes were not considered to be treatment-related, although erythrocyte volume fraction and haemoglobin were occasionally increased in males and females at the highest dose. However, a similar increase was also observed at other doses, in particular in males receiving a dose of 100 mg/kg bw per day, and a clear dose–response relationship was lacking. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In contrast, clinical chemistry

investigations and urine analysis elucidated some changes that could be attributed to administration of the compound. An increase in plasma ALP activity became most apparent in males and females at the highest dose, but was also noted and occasionally reached statistical significance at the intermediate doses of 300 and 100 mg/kg bw per day (Table 21). All other changes in clinical chemistry were not considered to be unequivocally treatment-related. Urine pH was consistently decreased in males at the highest dose and tended to be lower from a dose of 100 mg/kg bw per day onwards. However, a similar effect was not observed in females.

At interim sacrifice after 52 weeks, absolute weight of the liver was reduced at doses of 1000, 300 and 100 mg/kg bw per day. For males, however, this finding was not confirmed by the sensitive means of covariance analysis, i.e. with correction for final body weight. At terminal sacrifice, no statistically significant decrease in liver weight was noted. In contrast, mean weight of the kidney was reduced in groups of males at 100 and 1000 mg/kg bw after 104 weeks, but a clear dose–response relationship was lacking. A probably treatment-related impact on weight of the salivary gland was noted in both sexes at interim kill. At study termination, weight of the submaxillary (mandibular)/sublingual glands in both sexes and of the parotid salivary gland (females only) still tended to be higher at the two higher doses. However, when compared with these values in the controls, the difference was rather small, statistical significance was not achieved and there was no clear dose–response relationship.

Gross necropsy did not reveal indications of treatment-related non-neoplastic changes. The only remarkable histopathological finding attributed to administration of glyphosate was a dose-related increase in the number of animals exhibiting cellular alteration of the parotid and mandibular (submaxillary) salivary glands at the highest dose and at both intermediate doses. The changes were seen after 52 weeks. This alteration was described as the occurrence of hypertrophic and weakly (mandibular gland) or more deeply (parotid gland) basophilic acinar cells without any evidence of degeneration or other toxic damage. The severity of alteration was graded by the study pathologist on a scale ranging from "slight" to "very severe" (slight/very mild, mild, moderate, severe, very severe). The changes graded as "moderate" or "severe" were seen more frequently at 300 and 1000 mg/kg bw per day (Table 22). The sublingual salivary gland was not affected.

Neoplasia was present in all groups, but there was no relationship with dose in the incidence of any individual tumour or in the total incidence of animals with tumours.

Table 21. Plasma alkaline phosphatase (ALP) activity (IU/l) in rats given diets containing glyphosate for 104 weeks

Time-point	Dose (mg/kg bw per day)									
	Males					Females				
	0	10	100	300	1000	0	10	100	300	1000
Week 14	287	229	320	334	461***	182	158	213	223	244*
Week 25	251	272	267	306	367***	148	152	201*	227**	225**
Week 51	308	293	310	353	403	144	143	190*	195*	221**
Week 78	258	286	284	351*	414***	124	139	172	207**	186*
Week 102	212	265	287*	267	365***	190	161	193	228	286*

From Atkinson et al. (1993b)
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Table 22. Incidence and severity of cellular alteration of salivary glands in rats given diets containing glyphosate for 104 weeks

	Dose (mg/kg bw per day)									
	Males					Females				
	0	10	100	300	1000	0	10	100	300	1000
No. of animals examined	50	46	49	50	49	50	50	50	50	48
Parotid										
Severity:										
Slight	4	4	8	3	4	1	2	2	2	5
Mild	3	5	9	21***	14**	0	5	9**	9**	13***
Moderate	0	0	4	17***	18***	1	1	1	9*	18***
Severe	0	0	0	0	0	0	0	0	1	2
Total incidence	7	9	21**	41***	36***	2	8	12**	21***	18***
Mandibular										
Severity:										
Slight	7	5	10	14	9	2	0	3	1	6
Mild	0	0	12***	28***	22***	9	8	9	15	19**
Moderate	0	0	0	0	0	0	0	0	2	1
Total incidence	7	5	22***	42***	31***	11	8	12	18	26**

From Atkinson et al. (1993b)
** $p < 0.01$, *** $p < 0.001$

In conclusion, administration of glyphosate to rats for 104 weeks produced no evidence of a carcinogenic response. The liver and the salivary glands were identified as the main target organs of glyphosate-related toxicity in the long term. At 100 mg/kg bw per day, the changes in salivary glands were only minimal with respect to severity and were not considered to be of toxicological significance. Thus, the NOAEL was 100 mg/kg bw per day on the basis of the more pronounced cellular alteration of salivary glands at 300 mg/kg bw per day and greater (Atkinson et al., 1993b).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 453), groups of 52 male and 52 female Alpk:AP$_f$SD (Wistar-derived) rats were fed diets containing glyphosate (purity, 97.6%) at a concentration of 0, 2000, 6000 or 20 000 ppm for 2 years. A further 12 males and 12 females were added to each group and were designated for interim kill after 1 year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in the diet were determined and found to be satisfactory. The calculated mean intakes were equal to 121, 361 and 1214 mg/kg bw per day in males and 145, 437 and 1498 mg/kg bw per day in females. Clinical observations (including ophthalmoscopy), body weights, food consumption, haematology and clinical biochemistry (blood and urine), were measured throughout the study. In addition, a functional observational battery (FOB), including motor activity, was conducted in week 52 in animals allocated to the long-term assessment of toxicity part of the study. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues taken for subsequent histopathological examination.

Survival in males in the control group and in groups receiving the lowest and intermediate doses approached 25% by week 104 of the study, although survival at the highest dose was significantly better. Survival in females was similar across all groups and better than in males in the group receiving the lower dose. There was a treatment-related increase in the incidence of red-brown staining of tray papers (particularly in males), and isolated

observations of red-brown coloured urine noted in three males and one female at 20 000 ppm. No other treatment-related clinical signs of toxicity (including ophthalmoscopic findings and FOB) occurred. The body weights of males and females at 20 000 ppm were statistically significantly lower than those of the controls throughout the study; however, the difference was rather small with a maximum reduction compared with control values of approximately 5% for males and 8% for females. There were no dose-related and/or statistically significant effects on body weight in males or females at 2000 or 6000 ppm. The decrease in body weight at the highest dose was paralleled by lower food consumption throughout the first year of the study in males and females and an impaired food utilization in these groups during weeks 1–4.

Minor variations from mean values for the controls were obtained for most haematological parameters, but showed no consistency and were confined to intermediate time-points and/or doses and these changes were thus considered not to be treatment-related. In contrast, some clinical chemistry findings were assumed to be caused by administration of glyphosate, at least at the highest and intermediate doses. There was a clear dose-related increase in plasma ALP activity in both sexes throughout the study reaching statistical significance at the two higher doses (Table 23). In the groups at 2000 ppm, the mean values also tended to be higher; however, the increase was marginal and only occasionally achieved statistical significance (in males at week 79 and in females at week 53). In addition, there was evidence of increases in plasma ALT and AST activities, and in total bilirubin concentration at one or more time-points. These findings were confined to the groups receiving the intermediate and highest doses and frequently occurred in one sex only. In males at the highest dose, plasma concentrations of triglycerides and cholesterol were consistently decreased throughout the study. Plasma concentrations of creatinine were lower in all treated female groups at week 27 and in females receiving 6000 and 20 000 ppm at week 14, but in the absence of any effects later in the study, this was considered as having occurred by chance rather than suggesting an adverse effect. In males at the highest dose of 20 000 ppm,

Table 23. Plasma alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities in rats given diets containing glyphosate for 2 years

	Dietary concentration (ppm)							
	Males				Females			
	0	2000	6000	20 000	0	2000	6000	20 000
ALP (IU/l):								
Week 14	234	246	284**	387**	156	177	245**	266**
Week 27	196	219	239**	327**	121	136	166**	203**
Interim kill	230	244	269	306**	82	102	123*	144**
Week 53	231	249	277**	357**	92	117*	152**	172**
Week 79	208	254*	244	353**	114	131	181**	178**
Week 105	184	205	218	280	144	129	158	173
ALT (IU/l):								
Week 14	94.9	103.5	121.8**	143.4**	81.9	95.2	103.9*	94.9
Week 27	91.8	95.9	116.8	125.9*	99.5	113.8	132.7*	101.8
Interim kill	77.6	84.0	97.7	123.3**	83.4	82.8	113.2*	95.9
Week 53	84.2	99.8	103.5	133.8**	90.1	108.2	121.5*	114.0
Week 79	69.2	81.2	102.4**	105.9**	90.0	97.2	110.6	116.0*
Week 105	64.1	58.6	63.9	82.7	83.5	78.6	78.9	108.2**

From Brammer (2001)
ALP, alkaline phosphatase; ALT, alanine aminotransferase
*$p < 0.05$, **$p < 0.01$; Student's *t*-test, two-sided

urinary pH was lower than in controls throughout the study, and the incidence and number of erythrocytes in the urine was increased in males and, to a lesser extent, in females.

No increase in tumour incidence was observed. A number of probably treatment-related macroscopic findings were seen in males at 6000 ppm and/or 20 000 ppm, consisting of a minor increase in the incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes. However, there were no consistent, dose-related and/or statistical significant organ-weight changes that could be considered to indicate an adverse effect of glyphosate.

In contrast to the previous 1-year feeding study in rats (Milburn, 1996, see above) that was performed in the same laboratory and on the same rat strain, microscopic changes were seen in the liver and kidneys, but not the salivary glands of rats at 20 000 ppm (Table 24). Changes in the liver comprised a weak and rather equivocal increase in the incidence of hepatitis (evidence obtained in male only) and proliferative cholangitis, but the severity of the latter finding was not altered. There were a number of changes in the kidneys of both sexes, notably renal papillary necrosis, with or without papillary mineralization, and transitional cell hyperplasia. The incidence was greater in males than females. These renal findings were considered to be related to treatment but are consistent with the feeding of high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation observed. A decrease in the incidence of tubular degeneration of the testis in males at 20 000 ppm was considered to be without adverse consequence.

In conclusion, dietary administration of glyphosate at up to the highest dietary concentration of 20 000 ppm for up to 2 years produced little evidence of toxicity in the long term, with the kidney, the prostate and possibly the liver being the target organs. A number of findings (e.g. renal papillary necrosis, prostatitis, periodontal inflammation and urinary acidosis) might be attributed to the acidity of the test substance. No indications of neurotoxicity were obtained. The improved survival in males at the highest dose was likely to be associated with lower food consumption, lower body weights and a decreased severity of renal glomerular nephropathy. In the absence of treatment-related histopathological findings at 2000 and 6000 ppm, the marginal changes in some clinical chemistry parameters at these doses were considered to be of no toxicological significance.

Table 24. Selected microscopic findings in rats given diets containing glyphosate for 2 years

Finding	Dietary concentration (ppm)							
	Males (n = 64)				Females (n = 64)			
	0	2000	6000	20 000	0	2000	6000	20 000
Liver:								
Proliferative cholangitis	56	57	55	64	55	58	59	61
Hepatitis	8	6	9	13	6	7	4	6
Kidney:								
Papillary necrosis	0	1	0	14	0	1	2	5
Transitional cell hyperplasia	2	3	0	5	3	1	0	1
Prostate: prostatitis	13	22	23	37	—	—	—	—
Testis: unilateral tubular degeneration	18	13	18	5	—	—	—	—
Periodontal inflammation	25	27	23	42	18	24	32	28

From Brammer (2001)

Administration of glyphosate for 2 years produced no evidence of a carcinogenic response to treatment in rats. The NOAEL was 6000 ppm (equal to 361 mg/kg bw per day), on the basis of a reduction in body weight and food consumption, and indications for kidney, prostate and liver toxicity at 20 000 ppm (Brammer, 2001).

2.4 Genotoxicity

Glyphosate has been extensively tested for genotoxicity in a wide range of assays both in vitro and in vivo, including end-points for gene mutation, chromosomal damage and DNA damage and repair. Numerous studies of genotoxicity have been reported, including many publications with limited experimental details, partly contradictory results and the technical specification of test material often being unknown. Data of very different quality are available for glyphosate as the acid but also for the salts (e.g. the isopropylamine salt) and also for different plant protection products (formulations). However, in this review the focus is on mutagenic properties of the active substance. The results of the available regulatory studies with the active ingredient using test material corresponding to the Food and Agriculture Organization of the United Nations (FAO) specification were uniformly negative. The few published data (mostly obtained in vitro) suggesting positive results in validated and widely accepted test systems are contradicted by the vast majority of studies with clearly negative outcomes. More important, the studies in standard test systems in vivo clearly proved the lack of mutagenic effects. Thus, it may be concluded that glyphosate active ingredient is devoid of a relevant genotoxic potential. The experimental data on which this assessment relies are summarized in Tables 25 and 26.

In vitro, glyphosate gave negative results in a number of assays for gene mutation in bacteria across a wide range of concentrations in the tester strains of *S. typhimurium* and *E. coli*. The compound also gave negative results in assays for gene mutation in mammalian cell systems at both the *Tk* and *Hgprt* loci. Glyphosate was non-clastogenic in standard guideline assays for chromosome aberrations and damage. These negative results for gene mutation and clastogenicity have been generated in both the absence and presence of metabolic activation (S9).

In contrast, investigations designed to examine the effects of long-term exposure to glyphosate on cells in culture have reported positive findings for the induction of chromosomal aberrations and sister chromatid exchange (SCE), but these have been ascribed as a likely consequence of the perturbation of the homeostasis of the cells. Some reports of increases in SCE frequencies have involved very small numerical increases, albeit attaining statistical significance. This is frequently seen with SCE as an end-point. Overall, the available data show that glyphosate was non-clastogenic when evaluated in appropriate assays for chromosomal damage. Glyphosate did not induce DNA repair as measured in both bacterial (Rec assay) and mammalian systems (UDS assay). The in-vitro studies submitted for this review are summarized in Table 25.

Glyphosate has been extensively investigated for clastogenic activity in vivo, in mice mainly by assay for micronucleus formation in the bone marrow. These data show that glyphosate is non-clastogenic after both single and repeated administration. An isolated weakly positive finding after intraperitoneal injection is contradicted by other results including all studies using the more relevant oral route of administration. Likewise, glyphosate proved non-clastogenic in the assay for cytogenetic damage (metaphase) in bone marrow in rats. Glyphosate can also be considered to be non-mutagenic to germ cells, giving a

Table 25. Results of studies of genotoxicity with glyphosate in vitro

End-point	Test object	Concentration	Purity (%)	Results	Reference
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E. coli* WP2P and WP2P*uvrA* ± S9	100, 200, 500, 1000, 2500, 5000 µg/plate in DMSO	95.6	Negative	Callander (1996)
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA98, TA100	310–5000 µg/plate (+S9); 160–2500 µg/plate (−S9)	98.6	Negative	Jensen (1991a)
Reverse mutation[b]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, and *E. coli* WP2 ± S9	10–5000 µg/plate	98.4	Negative	Shirasu et al. (1978) (published by Li & Long, 1988)
Reverse mutation[c]	*S. typhimurium* TA98, TA100, TA1535, TA1537 ±S9	≤1000 µg/plate	98.4	Negative	Kier (1978)
Reverse mutation	*S. typhimurium* TA97a, TA98, TA100 and TA102 ±S9	25–2000 µg in aqueous solution	Not specified ("technical concentrate")	Negative	Chruscielska et al. (2000)
Reverse mutation	*S. typhimurium* TA97, TA98, TA100 and TA1535 ±S9	0–10 000 µg/plate	99	Negative	Chan & Mahler (1992)
Point mutation[a]	Mouse lymphoma L5178Y $Tk^{+/-}$ cells ±S9	444, 667, 1000 µg/ml	95.6	Negative	Clay (1996)
Point mutation[d]	Mouse lymphoma L5178Y $Tk^{+/-}$ cells ±S9	0.52–4.2 mg/ml (+S9); 0.61–5.0 mg/ml (−S9)	98.6	Negative	Jensen (1991b)
Point mutation[c]	Chinese hamster ovary cells, HGPRT locus, ±S9	5–25 mg/ml (+S9); 5–22.5 mg/ml (−S9)	98.7	Negative	Li (1983a) (published by Li & Long, 1988)
Cytogenetic damage	Human lymphocyte cultures from male and female donors ±S9; two harvest times	100, 750, 1250 µg/ml	95.6	Negative	Fox (1998)
Cytogenetic damage	Human lymphocyte cultures; exposure time 24 & 48 h (−S9) or 3 h (+S9); harvest after 24 or 48 h	237–562 µg/ml (+S9); 33–333 µg/ml (−S9)	96	Negative	Van de Waart (1995)
Sister chromatid exchange	Human lymphocytes	0–6 mg/ml	99.9	Positive at ≥1 mg/ml Insufficient data for adequate assessment.	Bolognesi et al. (1997)
Chromosomal aberration and sister chromatid exchange	Bovine lymphocyte cultures, 72-h treatment	17, 85, 170 µmol/l	≥98	Positive at all three concentrations	Lioi et al. (1998a)[f]
Chromosomal aberration and sister chromatid exchange	Human peripheral lymphocytes, 72-h treatment	5.0, 8.5, 17.0, 51.0 µmol/l	≥98	Positive at 8.5, 17.0 and 51.0 µmol/l	Lioi et al. (1998b)
DNA damage (Rec assay)[g]	*B. subtilis* strains H17 (rec+) and M45 (rec-)	20–2000 µg/disc	98.4	Negative	Shirasu et al. (1978) (published by Li & Long, 1988)
Unscheduled DNA synthesis[c]	Hepatocytes from F344 rats	≤125 µg/ml	98.7	Negative	Williams (1983) (published by Li & Long, 1988)

[a] A positive control was employed; GLP and QA statements included. Complied with current regulatory guidelines
[b] This study was performed before the publication of current guidelines and before GLP, however the protocol generally adhered to these guidelines and is considered acceptable
[c] The study was performed before GLP but is considered acceptable
[d] Study complied with GLP and is considered acceptable, QA statements are included
[e] The study was conducted to GLP (self-certification of the laboratory). The study is considered acceptable
[f] Non-standard test system; effects ascribed to a likely alteration in the oxidative state of the treated cells after long exposure
[g] The study was performed before GLP and was not conducted in the presence of metabolic activation

negative result in an assay for dominant lethal mutation in mice. Tests for DNA damage using alkaline elution in the liver and kidney of mice are of limited value on account of the use of the intraperitoneal route of administration and the lack of appropriate data on toxicity. Similar concerns apply to studies on DNA binding from the same laboratory, although no adducts were identified after administration of glyphosate isopropylamine salt. The results of the studies of genotoxicity provided are summarized in Table 26.

Table 26. Results of studies of genotoxicity with glyphosate in vivo

End-point	Test object	Concentration	Purity (%)	Result	Reference
Micronucleus formation[a]	Charles River CD-1 mice (males and females), bone marrow; sampling at 24 h and 48 h after dosing	5000 mg/kg bw (single oral dose)	95.6	Negative	Fox & Mackay (1996)
Micronucleus formation[b]	NMRI mice (males and females, bone dose) marrow; sampling after 24 h, 48 h and 72 h	0–5000 mg/kg bw (single oral)	98.6	Negative	Jensen (1991c)
Micronucleus formation	B6C3F1 mice (males/females), peripheral (normochromatic, blood erythrocytes	0–50 000 ppm (examination after dietary administration for) 13 weeks)	Not stated	Negative	Chan & Mahler (1992)
Micronucleus formation	Swiss CD-1 mice (males only), bone marrow. Sampling 6 h and 24 h after final dose.	300 mg/kg bw (2 × 150 mg/kg bw); intraperitoneal administration	99.9	Weakly positive after 24 h	Bolognesi et al. (1997)
Micronucleus formation	Mice (strain not specified, males only), bone marrow; Sampling time 24, 48 and 72 h after dosing	300 mg/kg bw (single intraperitoneal injection)	Not stated ("technical concentrate")	Negative	Chruscielska et al. (2000)
Cytogenetic damage[c]	Sprague-Dawley rats, bone marrow. Sampling after 6 h, 12 h and 24 h	0–1000 mg/kg bw (single intraperitoneal injection)	98.7	Negative	Li (1983b, 1983c) (published by Li & Long, 1988)
Dominant lethal mutation[c]	Charles River CD-1 mice, males treated and paired with a total of 16 untreated dams over a period of 8 weeks	0, 200, 800 or 2000 mg/kg bw (single oral dose)	98.7	Negative	Rodwell (1980)
Alkaline elution assay for DNA single-strand breaks and formation of alkali-labile sites[d]	Liver and kidney of male Swiss CD-1 mice. Sampling 4 h and 24 h after administration	0 and 300 mg/kg bw (single intraperitoneal administration)	99.9	Weakly positive[d] after 4 h in both organs, suggesting possible transient DNA damage. Biological significance equivocal, effects might also be due to toxicity.	Bolognesi et al. (1997)

Table 26. Continued

End-point	Test object	Concentration	Purity (%)	Result	Reference
Oxidative DNA damage measured by quantification of 8-hydroxydes-oxyguanosine (8-OhdG) adducts	Liver and kidney of Swiss CD-1 mice. Sampling after 8 h and 24 h after dosing.	0 and 300 mg/kg, (single intraperitoneal administration)	99.9	Positive (increase in 8-OhdG adducts in the liver after 24 h). The promutagenic DNA lesion 8-OhdG is a biomarker for oxidative stress.	Bolognesi et al. (1997)
Measurement of DNA adducts using ^{32}P-postlabelling technique	Liver and kidney of Swiss CD-1 mice	0, 130, 270 mg/kg bw, (single intraperitoneal administration)	Glyphosate IPA salt, no details of purity given	Negative (no increase in relative level of adducts)	Peluso et al. (1998)
Wing-spot[e]	*Drosophila melanogaster* larvae	0.1, 0.5, 1, 2, 5, 10 mmol/l in distilled water	96	Weakly positive only in the standard crosses	Kaya et al. (2000)

[a] A positive control was included; GLP and QA statements were included. Complied with current regulatory guidelines
[b] Study complied with GLP and is considered to be acceptable, QA statements were included
[c] The study was performed before GLP but is considered to be acceptable
[d] The use of the intraperitoneal route of administration when the liver is to be sampled is inappropriate when an assessment of the in-vivo status is required, since the deposition of test material into the intraperitoneal cavity gives, in effect, an in-vitro exposure. This assay is a non-selective measurement of the migration of DNA through a filter and is a measurement of DNA size. Any factor that affects the DNA size is therefore detectable. One of the most important elements for control is that of toxicity. If the materials (or the manipulative procedures) induce cytotoxicity in the population under investigation, then the result will be an increase in the elution rate constant. Therefore, this assay cannot distinguish between toxicity-induced DNA damage and genotoxicity-induced DNA damage. The lack of reported controls, with the choice of administration route, makes it difficult to draw any conclusions from these data
[e] No information was provided on toxicity after treatment

2.5 Reproductive toxicity

(a) Multigeneration studies

In a two-generation study conducted in compliance with the principles of GLP and according to the guidelines of the US EPA and the OECD (TG 416), groups of 28 male and 28 female Crl:CD(SD)BR VAF/Plus rats (aged 6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity, 99.2%) at a concentration of 0, 1000, 3000 or 10 000 ppm for 70 days before their first mating and until termination. The highest dietary concentraion was set at 10 000 ppm since administration of diets containing glyphosate at 30 000 ppm in a preliminary study was associated with signs of maternal toxicity. The F_1 generation (24 males and 24 females per group) was selected from the F_{1A} litters and treated from 1 week after weaning for at least 84 days before first mating. Each generation was mated twice, changing partners for the second mating and avoiding sister/brother matings throughout. Treatment was continued for both sexes until the day 21 of weaning of the second litter when animals were sacrificed for organ weighing, gross pathological examination and microscopy of reproductive tissues parents of both generations in the control group and at the highest dose. On postnatal day 4, litters were adjusted (as far as possible) to four male and four female pups. Fresh diets were prepared weekly and were appropriately controlled for concentration, homogeneity and stability on several occasions throughout the study. The overall calculated mean daily intake of glyphosate during the pre-mating phase was 0, 66, 197 and 668 mg/kg bw per day for F_0 males; 0, 75, 226 and 752 mg/kg bw per day for F_0 females; 0, 76, 230 and 771 mg/kg bw per day for F_1 males; and 0, 82, 245 and 841 mg/kg bw per day for F_1 females.

In adults, parameters studied were signs of reaction to treatment, mortality, food and water consumption, body-weight changes, mating performance and pregnancy rate, length of gestation, weighing of relevant organs (approximately eight), preservation of tissues (approximately 40) after macroscopic examination of respective organs, including microscopy of salivary glands in all surviving F_0 and F_1 animals. Litter data comprised number and state of pups at parturition, sexing, weighing and examination for external abnormalities. Internal abnormalities were studied in pups culled by postnatal day 4. Also recorded were the onset of vaginal opening and cleavage of the balanopreputial skinfold (F_1 generation only).

No treatment-related clinical signs were noted in the parents of either generation. There was a total of four mortalities in each parent generation; however, none of the mortalities were considered to be treatment-related. The highest dose caused a slight increase in food and water consumption of F_1 females, a slightly lower mean body weight of F_1 males at selection for the second generation, but a weight gain comparable to that of controls from this point. There were no adverse effects of treatment on mating performance, pregnancy rate or duration of pregnancy in either generation. There were no effects on the total number of litters being born within groups, total litter loss, litter size, pup mortality or sex ratio. Litter weights in all treated groups were lower at the first F_0 mating; however, this was not seen at the second F_0 mating or in either F_1 mating, so it is not considered to be an adverse effect of treatment with glyphosate. There was no effect on sexual maturation in either sex as evaluated by mean age at vaginal opening or attainment of balanopreputial skin-fold cleavage in female or males respectively.

Treatment-related histopathological changes were apparent in the parotid salivary gland of both F_0 and F_1 males and females at 3000 ppm and at 10 000 ppm, and in the submaxillary salivary gland of F_0 females at 3000 ppm and at 10 000 ppm, and F_1 females at 10 000 ppm (Table 27). The changes manifested as hypertrophy of acinar glands with prominent granular cytoplasm, the morphology severity was classified as "minimal" (grade 2) on a scale from "trace" (grade 1) to "severe" (grade 5). There were no other treatment-related macroscopic or histopathological findings in adult rats or offspring, no effects on any organ weights (including reproductive organs).

In conclusion, administration of glyphosate at a dietary concentration of up to 10 000 ppm and over two successive generations had no effect on sexuality and fertility of

Table 27. Incidence of cellular alteration of salivary glands in a multigeneration study in rats fed diets containing glyphosate

Alteration	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10 000	0	1000	3000	10 000
F_0 generation:								
Parotid gland	2/27	2/28	3/28	12/26	0/28	2/27	5/28	17/28
Submaxillary gland	0/27	—	—	0/26	0/28	1/27	4/28	14/28
F_1 generation:								
Parotid gland	1/24	0/24	4/23	10/23	0/24	0/27	4/24	9/23
Submaxillary gland	0/24	—	—	0/23	0/24	0/27	0/24	3/23

From Brooker et al. (1992)
—, not examined
No statistical analysis was done

males or females. The NOAEL for parental and offspring toxicity was 3000 ppm, equal to 197 mg/kg bw per day, on the basis of increased food and water consumption of F_1 females, lower body weight of F_1 males, and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females only) salivary glands in both F_0 and F_1 adults at 10 000 ppm (Brooker et al., 1992).

In a two-generation study conducted in compliance with the principles of GLP and according to the guidelines of the US EPA and the OECD (TG 416), groups of 26 male and 26 female Wistar-derived Alpk:AP_fSD rats (aged 5–6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity, 97.6%) at a concentration of 0, 1000, 3000 or 10 000 ppm. After 10 weeks, the animals were mated and allowed to rear the ensuing F_{1A} litters to weaning. The breeding programme was repeated with the F_1 parents selected from the F_{1A} offspring to produce the F_{2A} litters after a 10-week pre-mating period. Diets were appropriately controlled for concentration, homogeneity and stability on several occasions throughout the study. The overall calculated mean daily intake of glyphosate during the pre-mating phase was 0, 99, 293 and 985 mg/kg bw per day for F_0 males; 0, 104, 323 and 1054 mg/kg bw per day for F_0 females; 0, 117, 352 and 1161 mg/kg bw per day for F_1 males; and 0, 123, 371 and 1218 mg/kg bw per day for F_1 females.

Observations and measurements in adults comprised clinical observations, food and water consumption, body-weight changes, reproductive performance, estrous cycle, developmental landmarks (F_1 only), and post-mortem examinations, including uterine assessment, organ weights, sperm analysis, histopathology and quantification of oocytes (F_1 only). Observations and measurements for pups comprised number at birth until day 29, survival, individual and litter weight, clinical condition, sex distribution, and post-mortem examination including organ weights of selected pups.

There were no treatment-related mortalities or clinical findings in parents of either generation. The effects of glyphosate on body weight and food consumption were confined to the F1 males given 10 000 ppm, with a statistically significantly lower body weight from week 2 to week 8 and a statistically significantly lower food consumption throughout the pre-mating period (Table 28). Food utilization values over the duration of the study were not statistically significantly different from those of the controls.

Table 28. Body weights (adjusted for initial weight) and food consumption during the pre-mating period for F_1 males fed diets containing glyphosate

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10 000
Body weight(g)				
Week 1	80.2	81.1	78.1	75.3
Week 4	246.2	247.6	242.8	237.3**
Week 8	403.6	410.1	395.3	387.0*
Week 11	461.7	471.3	455.5	449.7
Food consumption (g/rat per day)				
Week 1	19.3	19.7	19.0	18.1*
Week 4	34.6	35.5	33.9	32.6**
Week 8	35.5	36.1	34.1	33.0**
Week 10	35.5	35.7	34.1	33.0**

* $p < 0.05$, ** $p < 0.01$; Student's t-test, two-sided
From Moxon (2000)

Table 29. Adjusted mean body weights (g) of F_{1A} and F_{2A} pups

Litter	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
F_{1A} pups								
Day 1	5.8	6.1	6.0	6.1	5.4	5.8	5.6	5.7
Day 5	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
Day 8	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
Day 15	26.8	26.1	25.8	24.6*	26.1	25.2	24.5	23.8*
Day 22	43.4	42.4	41.4	39.2*	41.9	40.3	39.4	37.7*
Day 29	81.7	79.5	79.6	74.6*	77.1	74.0	74.1	69.9**
F_{2A} pups								
Day 1	6.3	6.3	6.3	6.2	6.1	5.9	5.9	5.8
Day 5	9.7	9.9	9.3	9.5	9.3	9.6	9.1	9.1
Day 8	14.3	14.7	13.8	14.2	13.8	14.2	13.4	13.7
Day 15	27.4	28.3	26.4	27.5	26.7	27.5	25.8	26.5
Day 22	44.5	46.2	43.1	44.9	42.7	44.8	41.8	42.9
Day 29	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4

*$p < 0.05$, **$p < 0.01$; Student's t-test, two-sided
From Moxon (2000)

Glyphosate did not have an adverse effect on the estrous cycle in females, on the number of primordial follicles in F_1 females, or on the number of sperm, sperm motility parameters or morphology in males, or on reproductive performance in either sex in either generation. There was no adverse effect of glyphosate on developmental landmarks (time to preputial separation or vaginal opening) or pup survival, on litter size during lactation, on the clinical condition of the pups or on the proportion of male pups in either the F_{1A} or F_{2A} litters. The body weights of F_{1A} pups were lower in comparison to those in the control group from day 8 onwards, but a similar effect was not seen in the F_{2A} pups (Table 29). There was no treatment-related effect on total litter weight.

At sacrifice, liver and kidney weights adjusted for body weight of F_0 males at 10000 ppm were slightly but statistically significantly higher (about 5 and 4%, respectively) than concurrent control values. Similar changes were not observed in the F_1 males or in adult females of either generation. No histopathological changes were observed in any tissue from the F_0 or F_1 animals that could be attributed to treatment.

In conclusion, administration of glyphosate at a dietary concentration of up to 10000 ppm and over two successive generations had no effect on the sexuality or fertility of males and females. The NOAEL for parental and offspring toxicity was considered to be 3000 ppm, equal to 293 mg/kg bw per day, on the basis of a reduction in body weight of F_{1A} pups and a subsequent reduction in body weight of F_1 parent males at 10000 ppm (Moxon, 2000).

(b) Developmental toxicity

Rats

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the guidelines of the US EPA and the OECD (TG 414), groups of 25 time-mated female Crl:CD(SD)BR VAF/Plus rats were given glyphosate (purity, 98.6%; in aqueous solution/suspension with 1% methylcellulose) at a dose of 0, 300, 1000 or 3500 mg/kg bw per day by gavage on days 6–15 of gestation (day 0 being the day of mating).

All animals were observed daily for clinical signs and mortality, and body weight and food consumption were measured on days 1, 3, 8, 10, 12, 14, 16, 18 and 20 of gestation. Water consumption was measured daily. On day 20 of gestation, the dams were killed, and a macroscopic examination was carried out post mortem. Pregnancy status was determined and numbers of corpora lutea, live fetuses and intrauterine deaths were recorded. All live fetuses were weighed, examined for external abnormalities, and sexed by gonadal inspection. Approximately half the fetuses in each litter were prepared and examined for skeletal alterations (modified Dawson technique), and the remainder were prepared and examined for soft tissue alterations (Wilson technique).

There were two maternal deaths at the highest dose after signs of respiratory distress on day 7 and 13, respectively, and another dam at the highest dose was sacrificed on day 10 after a probable intubation error. At the highest dose, clinical abnormalities included salivation, loose stools and noisy respiration. The latter was also observed in two animals at the intermediate dose on one occasion. Body-weight gain was markedly reduced at the highest dose (by 16–81% of control values, days 6–20 of gestation) and marginally reduced at the intermediate dose (by 86–97% of control values, days 6–20 of gestation). Food consumption was slightly decreased at the highest dose during the dosing period (75–94% of control values, days 6–15 of gestation), but was comparable with controls thereafter. Water intake was increased at the highest dose (139–205% of control values, days 6–15 of gestation). No treatment-related changes were observed at any dose at necropsy.

A total of 23, 23, 25 and 22 dams had live young at day 20 in the control group, and at the lowest, intermediate and highest dose, respectively. There was no significant influence of treatment on embryonic losses, litter size or sex ratio, but the litter weights and mean fetal weights were reduced at the highest dose, the latter being statistically significant (90% and 94% of control values, respectively). The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher at the highest dose and slightly higher at the intermediate dose; the incidences on the basis of fetuses (litters) were 1 (1), 0 (0), 3 (2), and 28 (11) for the control group, at the lowest, intermediate and highest dose, respectively. In addition, reduced ossification was seen slightly more frequently at the highest and intermediate doses. As result, the percentage of fetuses showing skeletal anomalies (variations) was significantly increased at the two higher doses, but the percentage of fetuses affected at the intermediate dose exceeded the historical background range (21.9–27.2%) only slightly (Table 30).

The NOAEL for maternal toxicity was 300 mg/kg per day on the basis of clinical signs and reduced body-weight gain at 1000 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 300 mg/kg per day on the basis of an increased incidence of delayed

Table 30. Incidence of fetal skeletal anomalies in a study of developmental toxicity in rats given glyphosate by gavage

	Dose (mg/kg bw per day)			
	0	300	1000	3500
No. of fetuses (litters) examined	155 (23)	143 (23)	166 (25)	142[a] (22)
No. of fetuses (litters) affected	19 (11)	36 (16)	46 (19)	55 (19)
Mean (% of fetuses)	11.7	22.6	28.4*	35.7**

From Brooker et al. (1991b)
* $p < 0.05$, ** $p < 0.01$; Kruskal-Wallis test, and distribution-free Williams' test
[a] Two malformed fetuses were excluded

ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day and greater (Brooker et al., 1991b).

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the OECD Guidelines for Testing of Chemicals No. 414, groups of 24 time-mated female Alpk:APfSD (Wistar-derived) rats were given glyphosate (purity, 95.6%; in deionized water) at a dose of 0, 250, 500 or 1000 mg/kg bw per day by gavage on days 7–16 of gestation (day 1 being the day of mating). The animals were observed routinely for physical appearance, behaviour, body-weight gain and food consumption. On day 22 of gestation, the dams were killed, and a macroscopic examination carried out post mortem. Pregnancy status was determined and numbers of corpora lutea, live fetuses and intrauterine deaths recorded. All fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and fixed, and sections of the head were examined for abnormalities of the brain. The carcasses were then prepared and examined for skeletal alterations.

One control animal was killed on day 7 as a result of mis-dosing; there were no other mortalities. There were no changes in the clinical condition of the dams given glyphosate that were considered to be treatment-related, and there was no effect on body weight, food consumption or macroscopic findings post mortem. There was no evidence of developmental toxicity attributable to glyphosate as assessed by the number, growth or survival of the fetuses. Observation of the external appearance of the fetuses, examination of the viscera and assessment of the skeletons revealed no treatment-related findings.

The NOAEL for both maternal and developmental effects was 1000 mg/kg bw per day, the highest dose tested (Moxon, 1996a).

Rabbits

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the OECD Guidelines for Testing of Chemicals No. 414, groups of 16–20 time-mated female New Zealand White rabbits were given glyphosate (purity, 98.6%; in aqueous solution/suspension with 1% methylcellulose) at a dose of 0, 50, 150 or 450 mg/kg bw per day by gavage on days 7–19 of gestation (day 0 being the day of mating). Dosage volumes were calculated for individual animals on day 7 of gestation and adjusted according to body weight on days 9, 11 and 15. All animals were observed daily for clinical signs and mortality, and body weight and food consumption were measured on days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation. On day 29 of gestation, the dams were killed, and a macroscopic examination post mortem was carried out. Pregnancy status was determined and numbers of corpora lutea, live fetuses and intrauterine deaths were recorded. All live fetuses were examined for external abnormalities, weighed, and prepared and examined for soft tissue abnormalities and for skeletal abnormalities (modified Dawson technique). Where appropriate, abnormalities were examined by additional procedures (e.g. microdissection, histopathology) to clarify initial observations.

One animal at the highest dose was found dead on day 20 of gestation after signs of abortion on day 19 of gestation, gastrointestinal disturbances, and a severe reduction in food intake and body-weight loss from the start of treatment. There was a dose-related increase in the incidence of females with soft/liquid faeces and inappetence (lack of appetite) at the intermediate and highest doses. Also, food consumption was slightly reduced at the

intermediate dose (by 88–89% of the value for controls, days 11–19 of gestation) and at the highest dose (by 83–90% of the value for controls, days 9–19 of gestation), while body-weight gain at these doses was 80% and 67% of control value (days 7–20 of gestation), respectively. No treatment-related changes were observed at any dose at necropsy.

There were 18, 12, 15 and 13 viable litters in the control group and at the lowest, intermediate, and highest doses, respectively. Pre-treatment events (corpora lutea, pre-implantation loss) showed no significant differences between groups. In the treated groups, there was a significant increase in the number of embryonic deaths per litter and, hence, in postimplantation loss when compared with these values in the concurrent control group, although no clear dose–response relationship was evident (Table 31). Consequently, litter size and litter weight showed a dose-related reduction in all treated groups (not statistically significant). No adverse effect of treatment was noted for mean fetal weight.

A total of three (three), three (three), five (three) and six (five) fetuses (litters) out of 163, 104, 112 and 95 fetuses examined showed malformations in the control group and at the lowest, intermediate and highest dose, respectively. The slightly higher number of fetuses with malformations at the intermediate and highest dose was caused by an apparent increase in the incidence of fetuses with interventricular septal defect and other abnormalities affecting the heart—the number of fetuses affected in the control group and at the lowest, intermediate and highest doses being one, one, four and five, respectively. The mean percentage of malformed fetuses per litter, however, was within the concurrent background range in all groups (13 studies performed in 1989; mean incidence of 3.8 with a range of 0.7 to 5.9).

The NOAEL for maternal toxicity was 50 mg/kg per day on the basis of clinical signs and reduced food consumption and body-weight gain at 150 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 150 mg/kg per day on the basis of a slightly increased incidence of late embryonic deaths and postimplantation loss at 450 mg/kg bw per day (Brooker et al., 1991a).

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the OECD Guidelines for Testing of Chemicals No. 414, groups of 20 time-mated female New Zealand White rabbits were given glyphosate (purity, 95.6%; in deionized water) at a dose of 0, 100, 175 or 300 mg/kg bw per day by gavage on days 8–20 of gestation (day 1 being the day of mating). Dosage volumes were calculated for

Table 31. Incidence of embryonic deaths in a study of developmental toxicity in rabbits given glyphosate by gavage

Parameter (mean No.)	Dose (mg/kg bw per day)				Range for historical controls (mean)[a]
	0	50	150	450	
Implantations	9.7	10.5	9.0	9.2	7.0–11.1 (9.5)
Early embryonic deaths	0.4	0.9	0.9	0.5	0.3–1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1–1.3 (0.7)
Abortions	0	0	0.1	0	0–0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8*	0.6–2.0 (1.2)
Postimplantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5–17.5 (12.9)
Live young	9.1	8.7	7.7	7.3	6.1–9.5 (8.2)

From Brooker et al. (1991a)

*$p < 0.05$, **$p < 0.01$; Kruskal-Wallis test, and distribution-free Williams' test

[a] From 21 studies performed between January 1989 and June 1990

individual animals according to their daily body weights. All animals were observed daily for clinical signs and mortality, while body weight and food consumption were measured on days 1, 4, 8–20, 23, 26 and 30 of gestation and on days 8, 11, 14, 17, 20, 23, 26 and 30 of gestation, respectively. On day 30 of gestation, the dams were killed, and a macroscopic examination was carried out post mortem. Pregnancy status was determined and numbers of corpora lutea, implantations, live fetuses and intrauterine deaths were recorded. All fetuses were examined for external abnormalities, weighed, and prepared and examined for soft tissue abnormalities and for skeletal abnormalities (modified Dawson technique). Additionally, assessment of ossification including scoring of *manus* and *pes* was performed.

The incidence of intercurrent maternal deaths was 1, 2, 2 and 2 in the control group, and at the lowest, intermediate and highest dose, respectively. There was a dose-related increase in the incidence of dams with signs of diarrhoea and reduced faecal output at the intermediate and highest doses. Food consumption was significantly reduced at the intermediate dose (by 72–86% of the value for controls, days 8–20 of gestation) and the highest dose (by 57–81% of the value for controls, days 8–20 of gestation), while body-weight gain at these doses was 70% and 38% of the value for controls (days 8–20 of gestation), respectively. No treatment-related changes were observed at any dose at necropsy.

There were 17, 18, 17 and 17 viable litters in the control group and at the lowest, intermediate and highest dose, respectively. The mean fetal weight (44.4, 43.3, 43.2 and 40.7 g for the control group and at the lowest, intermediate and highest doses, respectively) was statistically significantly reduced at the highest dose, which was attributed to the occurrence of two litters for which the mean fetal weight was particularly low (20.3 g and 29.6 g). There was no effect of treatment on the number or survival of the fetuses in utero. The number of fetuses with major defects was 3 out of 143, 1 out of 147, 0 out of 135 and 2 out of 144 in the control group and at the lowest, intermediate, and highest dose, respectively. Neither the type nor incidence of major defects indicated a treatment-related effect. The proportion of fetuses with minor skeletal defects was statistically significantly increased at the lowest and highest doses, when compared with that in the control group, but not at the intermediate dose. Consideration of the specific defects revealed a statistically significantly increased incidence of fetuses with partially ossified transverse processes of the seventh vertebra in the group receiving the highest dose (5.6%, compared with 0.7% in controls), unossified transverse processes of the seventh lumbar vertebra (9.7%, compared with 2.8% in controls) or partially ossified sixth sternebra (11.1%, compared with 2.8% in controls). Owing to the reduction in ossification, at the highest dose the mean *manus* score per litter (3.05, compared with 2.88 in controls) and the mean *pes* score per litter (1.18, compared with 1.07 in controls) were slightly increased.

The NOAEL for maternal toxicity was 100 mg/kg per day on the basis of clinical signs and reduced food consumption and body-weight gain at 175 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 175 mg/kg per day on the basis of reduced fetal weight and reduced ossification at 300 mg/kg bw per day (Moxon, 1996b).

2.6 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity conducted in compliance with the principles of GLP and according to OECD guideline 424, groups of 10 male and 10 female Alpk:AP_fSD rats were given glyphosate (purity, 95.6%; in deionized water) as a single dose at 0, 500, 1000

or 2000 mg/kg bw by gavage and sacrificed 2 weeks later. All animals were observed before the start of the study and daily throughout the study for changes in clinical condition. Detailed clinical observations including qualitative assessments of landing foot splay, sensory perception and muscle weakness were performed at weekly intervals. Locomotor activity was also monitored at weekly intervals. Body weights and food consumption were measured throughout the study. At the end of the study, five rats of each sex per group were sacrificed and subjected to whole-body perfusion fixation. Selected nervous system tissues including brain (seven levels including the cerebral cortex, hippocampus, cerebellum, pons and medulla), spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic, sural and tibial nerves removed and processed for microscopic examination. Brains were weighed and measured (length and width). Histopathological examination was performed on animals in the control group and at the highest dose only.

Administration of glyphosate produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 h after dosing in 3 out of 10 females at 2000 mg/kg bw. One of these females was found dead on day 2 of the study. The clinical signs seen were considered to reflect general toxicity attributable to treatment with glyphosate. One female dosed at 500 mg/kg bw was found dead approximately 6 h after dosing on day 1, but in the absence of any treatment-related clinical signs, this death was considered not to be treatment-related. There were no treatment-related clinical observations at 500 or 1000 mg/kg bw in either sex or in males at 2000 mg/kg bw. Mean food consumption at 2000 mg/kg bw was slightly reduced for females (92% of value for controls; $p < 0.05$) and males (95% of value for controls; not significant) during week 1, while body weights were not affected at any dose. There were no treatment-related changes in the FOB, landing foot splay, sensory perception, grip strength or motor activity. At necropsy, no treatment-related macroscopic changes and no effects on brain weight, length or width were observed. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving glyphosate at a dose of 2000 mg/kg bw.

The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested. The NOAEL for general toxicity was 1000 mg/kg bw on the basis of lethality and general clinical signs of toxicity at 2000 mg/kg bw (Horner, 1996a).

In a short-term study of neurotoxicity that was conducted in compliance with the principles of GLP and according to OECD guideline 424, groups of 12 male and 12 female Alpk:AP$_f$SD rats were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000 or 20 000 ppm for 13 weeks. Diets were appropriately controlled for concentration, homogeneity and stability at regularly intervals throughout the study. The overall calculated mean daily intake of glyphosate was 156, 617 and 1547 mg/kg bw per day for males and 166, 672 and 1631 mg/kg bw per day for females. All animals were observed before the start of the study and daily throughout the study for changes in clinical condition and behaviour. Detailed clinical observations, including qualitative assessments of landing foot splay, sensory perception and muscle weakness, were performed at intervals during the study. Locomotor activity was also monitored at intervals. Body weights and food consumption were measured throughout the study. At the end of the study, six rats of each sex (which had been pre-designated for neuropathology) per group were sacrificed and subjected to whole-body perfusion fixation. Selected nervous system tissues including brain (seven levels including the cerebral cortex, hippocampus, cerebellum, pons and medulla),

spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic, sural and tibial nerves were removed and processed for microscopic examination. Brains were weighed and measured (length and width). Histopathological examination was performed on animals in the control group and at the highest dose only. At termination, all animals not required for neuropathology were killed and discarded.

Administration of glyphosate resulted in treatment-related reductions in growth and food utilization for males fed diets containing glyphosate at 20 000 ppm, with no associated effects on food consumption (Table 32). There were no treatment-related effects on body weight, food consumption or food utilization for males fed 2000 or 8000 ppm glyphosate, or for females at any dose.

There were no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. At necropsy, no treatment-related macroscopic changes and no effects on brain weight, length or width were observed. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals dosed with glyphosate at a dietary concentration of 20 000 ppm.

The NOAEL for neurotoxicity was 20 000 ppm, equal to 1547 mg/kg bw per day, the highest dose tested. The NOAEL for general toxicity was 8000 ppm, equal to 617 mg/kg bw per day, on the basis of reduced growth and reductions in food utilization in male rats at 20 000 ppm (Horner, 1996b).

In a study of acute delayed neurotoxicity conducted in compliance with the principles of GLP and according to OECD guideline 418, 20 hens (hybrid brown laying strain—Lohmann Brown) were given a single oral dose of glyphosate (purity, 95.6%) at 2000 mg/kg bw. In addition, two groups of 12 hens were dosed with distilled water or tri-ortho-cresyl phosphate (TOCP) at a dose of 1000 mg/kg bw and served as negative and positive controls, respectively. Observations in the following 21/22 days included mortality, clinical signs, assessment of delayed locomotor ataxia and body weight. Measurements of brain acetylcholinesterase, and neuropathy target esterase in the brain and lumbar spine were made for three hens from each treatment group, 48 h after dosing. At the end of the obser-

Table 32. Body weights and food utilization in rats fed diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20 000	0	2000	8000	20 000
Body weights (g)								
Week 1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
Week 4	338.2	340.7	339.6	323.7*	214.3	228.3**	224.9**	219.2
Week 8	440.7	440.1	429.1	405.8**	253.6	262.1	260.4	255.4
Week 12	510.3	506.8	497.8	471.1**	278.9	288.2	279.8	276.0
Week 14	534.7	532.8	526.5	496.1**	285.1	291.5	287.9	281.0
Food utilization (g of growth/100 g of food)								
Weeks 1–4	18.13	17.16	16.94	16.28*	9.42	9.73	9.36	9.61
Weeks 5–8	11.52	10.69	10.35	9.93*	5.99	5.55	5.39	5.70
Weeks 1–13	12.00	11.45	11.38	10.87**	6.08	6.03	6.06	5.96

From Horner (1996b)
*$p < 0.05$, **$p < 0.01$; Student's t-test, two-sided

vation period, six hens from each treatment group were selected for histopathological examination of the forebrain, mid- and hindbrain, upper cervical, lower cervical, mid-thoracic and lumbo-sacral spinal cord, proximal sciatic nerve, distal sciatic nerve and tibial nerve.

There was no evidence of clinical ataxia in any of the negative controls or in any of the hens dosed with glyphosate. Five of the hens dosed with TOCP developed clinical ataxia, starting between days 11 and 21. There was no effect on body weights for hens dosed with glyphosate, but hens dosed with TOCP showed an overall weight loss. Acetylcholinesterase activity in brain samples was reduced by 19% in hens treated with TOCP. It was reduced by 6% in hens treated with glyphosate, but was not statistically significant and was considered of no toxicological significance. There was no effect on neuropathy target esterase activity in brain or spinal cord for the hens treated with glyphosate, but in the positive controls there was an 84% and 78% reduction in brain and spinal cord neuropathy target esterase activities, respectively, compared with the negative controls. At necropsy, no macroscopic abnormalities were seen in any of the hens examined. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment-related changes in hens treated with glyphosate. Hens treated with TOCP showed significant axonal degeneration in spinal cord, peripheral nerve and cerebellum, demonstrating the validity of the test system.

The NOAEL for acute delayed neurotoxicity of glyphosate in hens was 2000 mg/kg bw (Johnson, 1996).

In a non-guideline experiment, a cell culture model was used to determine if chronic exposure to organophosphate pesticides can alter the sensitivity of nerve cells to subsequent acute exposure to organophosphates or other compounds. NB2a neuroblastoma cells were grown in the presence of diazinon at a concentration of 25 µmol/l for 8 weeks. The organophosphate was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides, including glyphosate (purity, >99%). The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the IC_{50} for each organophosphate or formulation was calculated. The IC_{50} values in diazinon-pre-exposed cells were compared with the equivalent values in cells not pre-exposed to diazinon. The IC_{50} for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20% and 90% after pre-treatment with diazinon. According to the study authors, the data support the view that long-term exposure to an organophosphate may reduce the threshold for toxicity of some environmental agents (Axelrad et al., 2003).

(b) Mechanism of induction of salivary gland changes

In a study of the mechanism of induction of salivary gland lesions performed by the United States National Toxicology Program (NTP), two groups of four male F344/N rats were fed diets containing glyphosate (purity, 99%) at a concentration of 50 000 ppm (which was the highest dose used in a short-term study on toxicity), together with continuous subcutaneous infusion of propanolol (a β-blocker; 1.2 mg/kg bw per day) or a vehicle (water). Three additional groups of four male rats received control diet, together with continuous subcutaneous infusion of isoproterenol (a β-adrenergic agonist; 1.0 mg/kg bw per day), isoproterenol plus propanolol, or a vehicle (water). After 14 days of treatment, the animals were sacrificed and the parotid and submandibular/sublingual glands were removed, weighed and processed for electron and light microscopy.

All rats survived to the end of the study. Rats receiving isoproterenol were hypoactive and had increased respiratory rates on day 1, but were normal by the following day. While there was no effect on food consumption in any group, there was a significant decrease in body-weight gains in the groups that received glyphosate (6.3 g and 6.0 g compared with 16.0 g in controls). Both glyphosate and isoproterenol produced increased salivary gland weights, with the parotid gland being more affected (280% or 154% of weights in the control group for glyphosate or isoproterenol, respectively). When both compounds were given along with propanolol, parotid weights were 194% of those of the controls for glyphosate but only 109% of those of the controls for isoproterenol. In the parotid and in the submandibular gland, increased weights were associated with cytoplasmic changes of acinar cells (basophilic change, fine vacuolation, swelling, loss of the normal periodic acid–Schiff (PAS)-positive reactivity of the secretory granules). The study authors concluded that the salivary gland effects induced by glyphosate were mediated through an adrenergic mechanism (Chan & Mahler, 1992).

In a study conducted in compliance with the principles of GLP and designed for comparison of salivary gland effects in three strains of rats, groups of 24 male Alpk:ApfSD (Wistar-derived) (AP), Sprague-Dawley (Charles River CD) (CD), and Fischer 344 rats (F344) were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0 (control) or 20 000 ppm (equivalent to approximately 2000 mg/kg bw per day) for 28 days. Eight animals from each group were killed on day 29 and the remaining animals were retained on control diet for a further 4 weeks (eight rats per group) or 13 weeks (eight rats per group). Clinical observations, body weights and food consumption were measured, and at the end of the scheduled periods, the animals were killed and subjected to a gross examination of the salivary glands. The salivary glands were weighed, and the left salivary glands were taken for microscopic examination.

Treatment with glyphosate at 20 000 ppm produced significant reductions in body weight and minor reductions in food consumption in AP and CD rats, but no effects were seen in F344 rats. In contrast, weight of salivary glands was unaffected in CD rats, but was increased in AP and F344 rats at the end of the 4 weeks. Microscopic examination of the salivary gland showed that the most pronounced effect occurred in F344 rats, where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells only occurred in the AP and CD rats.

Recovery from effects was apparent in all strains during the recovery periods. Body weight and food consumption returned to control values in both AP and CD rats. After 4 weeks on control diet, significant recovery of the salivary gland changes, in terms of both weight and histopathology, was evident in the F344 rats, and the AP and CD rats were indistinguishable from their corresponding controls. After 13 weeks on control diet, more F344 rats treated with glyphosate showed minor focal changes in the salivary gland compared with the respective controls, and group mean weights of the salivary gland were increased slightly (Table 33).

In conclusion, administration of diets containing glyphosate at a concentration of 20 000 ppm (equivalent to approximately 2000 mg/kg bw per day) to male rats for 4 weeks produced minor strain differences in systemic toxicity (changes in body weight and food consumption) and marked strain differences in the severity of effects in the parotid salivary gland. The most pronounced effects in the salivary glands were seen in F344 rats and the

Table 33. Selected findings in three strains of male rats given diets containing glyphosate for 28 days

Finding	Dietary concentration (ppm)					
	AP rats		CD rats		F344 rats	
	0	20 000	0	20 000	0	20 000
Body weight (g) at termination						
After 4 weeks of treatment	353.4	344.9	379.9	346.4	213.9	209.5
After 4 weeks of recovery	471.1	428.4	462.4	424.0	254.0	265.6
After 13 weeks of recovery	523.1	518.9	514.1	534.4	336.0	325.5
Salivary gland weight (mg)[a]						
After 4 weeks of treatment:						
Left	655	736	694	716	460	667**
Right	518	664*	609	640	420	579*
After 4 weeks of recovery:						
Left	722	729	803	783	484	550
Right	608	654	650	677	438	495**
After 13 weeks of recovery:						
Left	749	762	803	806	610	625
Right	668	680	679	694	477	536**
Basophilia of parotid acinar cells						
After 4 weeks of treatment:						
Minimal	0	1	1	4	6	0
Slight	0	6	0	1	1	0
Moderate	0	1	0	2	0	0
Marked	0	0	0	0	0	8
After 4 weeks of recovery:						
Minimal	1	1	0	0	0	5
Slight	0	0	0	0	0	1
After 13 weeks of recovery:						
Minimal	1	1	1	1	1	2
Slight	0	0	0	0	0	2
Moderate	0	0	0	0	0	1

From Allen (1996)
AP, Alpk:ApfSD (Wistar-derived) rats; CD, Sprague-Dawley (Charles River CD); F344, Fischer 344 rats (F344)
$p > 0.05$, $^{**}p < 0.01$, Student's t-test, two-sided
[a]Organ weight adjusted for body weight

changes were not completely reversible after 13 weeks of recovery, while in AP and CD rats complete improvement was apparent after a 4-week recovery period (Allen, 1996).

The hypothesis that glyphosate produced the salivary gland changes via β-adrenergic activity was questioned in a recent review paper (Williams et al., 2000). The authors emphasized that, first, if glyphosate was a β-agonist, its effect would be to stimulate β-receptors in other effector organs and produce a characteristic set of cardiocirculatory effects, such as increased heart rate and cardiac output as well as decreased blood pressure and peripheral resistance. None of these effects were noted in other studies. Similarly, it is known that isoproterenol and other β-agonists cause myocardial necrosis and enlargement of heart ventricles after prolonged treatment. Glyphosate did not produce any effects in heart tissue, even after long-term exposure at very high doses, providing additional support for the argument that glyphosate does not act as a β-agonist. The authors concluded that glyphosate has no significant β-adrenergic activity and therefore could not produce salivary gland changes via β-agonist activity. They proposed a number of other potential mechanisms for salivary gland alteration, including non-chemical modes of action. For example, salivary gland secretion has been shown to be affected by the texture and moistness of feed, and salivary gland enlargement has been caused by malnutrition. Glyphosate could be acting by such a non-chemical mechanism. Because glyphosate is a strong organic acid, dietary administra-

tion at relatively high concentrations may cause mild oral irritation leading to increased salivary gland size and flow. In the long-term exposure studies with glyphosate there were several salivary gland changes. These changes were: most pronounced in the parotid gland, responsible for secretion of serous fluid in response to such stimuli as acidic materials; absent in the sublingual gland that releases mucous fluid in response to other stimuli; and observed to an intermediate degree in the submandibular gland that contains a mixture of mucous and serous secreting cells. This pattern of observations was considered to be consistent with the hypothesis that the salivary gland changes observed are a biological response to the acidic nature of glyphosate. These salivary gland alterations are not known to represent any pathological condition and were not considered to be either toxicologically significant or adverse by Williams et al. (2000).

(c) Potential for endocrine modulation

In short-term studies of toxicity performed by the NTP, glyphosate (purity, 99%) was administered to groups of 10 male and 10 female $B6C3F_1$ mice and to groups of 10 male and 10 female F344/N rats at dietary concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm for 13 weeks. Evaluations of reproductive tissue revealed a significant reduction (80% of values for controls) of caudal epididymal sperm concentrations in male rats at the two highest doses; however, all values were within the normal range for the historical controls for this strain. All other parameters examined (left caudal, epididymal and testicular weights, epididymal sperm motility, total spermatid heads/testes, and total spermatid heads/gram caudal tissue) were not different from controls in rats or mice. The length of the estrus cycle was slightly longer (5.4 days compared with 4.9 days) in female rats at the highest dose than in the controls, but the biological significance of these findings, if any, is not known (Chan & Mahler, 1992).

In a non-guideline in-vitro experiment, glyphosate and 48 other chemicals were tested in two complementary assays, one measuring activation of the estrogen receptor of the rainbow trout in a yeast system and the other evaluating vitellogenin production in a trout liver cell-culture system. Glyphosate had no estrogenic activity in either assay (Petit et al., 1997).

In a non-guideline in-vitro experiment, glyphosate and eight pesticide formulations were tested for their ability to inhibit steroidogenesis in mouse MA-10 Leydig tumour cells. While glyphosate did not alter steroidogenesis (progesterone production) or total protein synthesis at any dose tested (0–100 µg/ml), the glyphosate formulation Roundup decreased progesterone production in a dosage-dependant manner without a parallel decrease in total protein synthesis (Walsh et al., 2000).

(d) Studies on the metabolite aminomethylphosphonic acid (AMPA)

(i) Acute toxicity

In a study of acute oral toxicity that was performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 401), five male and five female Alpk:AP_fSD (Wistar-derived) rats received AMPA (purity, 100%; in 0.5% aqueous polysorbate 80) as a single dose at 5000 mg/kg bw by gavage. A standard volume of 10 ml/kg bw was given to each animal. Test substance application was followed by a 15-day post-observation period before all the animals were killed and subjected to a macroscopic examination post mortem. None of the rats died before scheduled termination.

Signs of slight toxicity were seen in all animals, but these did not persist and all animals had recovered by day 4. Initially, all animals lost weight, but had exceeded their initial weight by day 6. However, body-weight reduction was noted in two males and three females between days 8 and 15. No treatment related findings were seen at examination post mortem. Accordingly, the acute oral LD_{50} for AMPA was >5000 mg/kg bw in male and female rats (Leah, 1988).

In a study of acute oral toxicity that was performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, five male and five female Sprague-Dawley rats received AMPA (purity, 99.2%; dissolved in 0.5% carboxymethylcellulose) as a single dose at 5000 mg/kg bw by gavage (dose volume, 10 ml/kg bw). The animals were observed frequently on the day of dosing and then once daily over the 14-day observation period. They were weighed before dosing, 7 days after dosing and at sacrifice on day 14. All rats were subjected to a gross examination post mortem. There were no treatment-related mortalities. Clinical signs were observed 4 h after dosing and included piloerection, diarrhoea, subdued behaviour, hunched appearance, and soiled anal and perigenital areas. All animals recorded normal body-weight gain throughout the experiment. No abnormalities were detected at necropsy after 14 days observation. Thus, the acute oral LD_{50} of AMPA in rats is >5000 mg/kg bw (Cuthbert & Jackson, 1993a).

In a study of acute dermal toxicity performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, five male and five female Sprague-Dawley rats received AMPA (purity, 99.2%) as a single dose at 2000 mg/kg bw. The test substance was administered evenly onto a square dressing (5 cm × 5 cm) that was moistened with distilled water and then applied to the shaved back of each rat. The patch was covered with an occlusive dressing and kept in contact with the skin for 24 h. At the end of the exposure period the patch was removed and the exposed skin wiped with distilled water to remove any excess test material. The rats were observed frequently on the day of dosing and then once a day over the 14-day observation period. At study termination, animals were sacrificed and subjected to necropsy. There were no mortalities after a single dermal application of AMPA at 2000 mg/kg bw, no clinical signs were noted and no abnormalities detected at necropsy. Thus, the acute dermal LD_{50} of AMPA to rats must be above this limit dose (Cuthbert & Jackson, 1993b).

The dermal sensitization potential of AMPA (purity, 99.2%) was evaluated in a Magnusson & Kligman maximization test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 406). On the basis of the results of a preliminary test, a group of 20 female Dunkin-Hartley guinea-pigs received AMPA by an intradermal injection (10% w/v in carboxymethylcellulose) and 6 days later by topical application (25% w/v in carboxymethylcellulose). Slight to moderate skin irritation was observed at the treated sites. Two weeks after the topical induction, the animals were challenged with AMPA (25% w/v in carboxymethylcellulose). The skin reactions were scored 24 h and 48 h after removal of the patches. None of the animals showed a positive response at challenge (Cuthbert & Jackson, 1993c).

(ii) Short-term studies of toxicity

In a range-finding study performed in compliance with the principles of GLP, groups of five male and five female Sprague-Dawley rats were given AMPA (purity, 99.2%; in carboxymethylcellulose) at doses of 0, 10, 100, 350 or 1000 mg/kg bw per day by oral gavage for 28 days. Control animals were given carboxymethylcellulose alone (at a volume of

10 ml/kg bw). Animals were observed daily for mortalities and signs of reaction to treatment. Once per week all animals received a detailed clinical examination. Body weights and food consumption were calculated weekly, water consumption was monitored by visual inspection throughout the study. At study termination all animals were sacrificed and necropsied. Thirteen different tissues were weighed and fixed for histological examination.

There were no mortalities or clinical signs observed throughout the duration of the study. There were no notable intergroup differences with regards to body weight in males. Females receiving a dose of 1000 mg/kg bw per day displayed a slight reduction by 13% in body weight when compared with values for the controls. However, this change was not statistically significant. In males, a similar effect was not observed. There were no notable intergroup differences in food and water consumption for males and females. Furthermore, there were slight but statistically significant increases in kidney weights in males at the two higher doses when compared with values for the control group (by 7% and 8%, respectively). Histological examinations revealed a very mild reduction of serous secretion in the mandibular salivary gland of males at the highest dose. With regard to the salivary gland findings in some of the studies with glyphosate, it is equivocal whether or not this minor finding was related to treatment.

On the basis of an increase in kidney weight in male rats at 350 and 1000 mg/kg bw per day and a reduction of body weight in females at the highest dose, the NOAEL for AMPA was 100 mg/kg bw per day (Heath et al., 1993).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 10 male and 10 female Sprague-Dawley rats were given AMPA (purity, 99.2%; in carboxymethylcellulose) at a dose of 0, 10, 100 or 1000 mg/kg bw per day by oral gavage for 13 weeks. Animals in the control group were given carboxymethylcellulose alone (at a volume of 10 ml/kg bw). Animals were observed daily for mortalities and signs of reaction to treatment. Once per week all animals received a detailed clinical examination. Body weights and food consumption were recorded weekly; water consumption was monitored by visual inspection throughout the study. Ophthalmoscopy examinations were performed on all animals during pretrial and on all animals in the control group and at the highest dose during week 12 of dosing. Blood samples were collected from the orbital sinus of all animals during week 13 of dosing. The blood collected was analysed for 14 haematology and 14 clinical chemistry parameters. At study termination all animals were sacrificed and necropsied. Fourteen organs including submaxillary, sublingual and parotid salivary glands were removed and weighed. Fourty tissues from premature decedents and from animals in the control group and the group receiving the highest dose were collected and fixed for full histopathological examination.

There were no unscheduled deaths that could be attributed to treatment and no specific clinical signs were noted over the course of the study. Ophthalmoscopy examinations resulted in no abnormal findings. There were no dose-related intergroup differences with regard to body weight, food or water consumption in any sex throughout the study. Haematology and clinical chemistry revealed a few minor effects, however, in the absence of a clear dose–response relationship, these were considered to have occurred by chance. There were no significant organ weight changes attributed to treatment with AMPA. The effect on kidney weight as elucidated in the previous 4-week study was not confirmed. At necropsy, none of the findings could be attributed to administration of AMPA. The NOAEL in this study was 1000 mg/kg bw per day, i.e. the highest dose tested (Strutt et al., 1993).

(iii) Genotoxicity

In an Ames test performed under GLP conditions and in compliance with OECD 471, AMPA (purity, >99% w/w) was tested in the presence and absence of metabolic activation (S9 mix) in two independent tests using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) and one strain of *Escherichia coli* (WP2*uvrA* pKM101). AMPA was dissolved in sterile deionized water and tested at doses of between 1.6 and 5000 µg/plate. The incubation period was 72 h at 37 °C. Appropriate controls were included. The number of revertant colonies was determined using an automated electronic colony counter.

In two separate assays, the test substance did not induce any significant, reproducible increases in the observed number of revertant colonies in *S. typhimurium* strains TA1535, TA1538, TA98 and TA100 or *E. coli* strain WP2*uvrA* pKM101 either in the presence or absence of an auxiliary metabolizing system (S9 mix). In contrast, the positive control substance caused an increase in the mean number of revertant colonies, thus demonstrating the sensitivity of the test system to a known mutagen. In the first test, small and non-dose related increases in revertant colony numbers were observed with strain TA1537 both in the presence and absence of metabolic activation. Increases were not seen in two further independent tests with this strain. This lack of reproducibility proved that the originally observed increases were not indicative of mutagenic activity and that AMPA uniformly gave a negative, i.e. non-mutagenic, response under the conditions of this assay (Callander, 1988).

In an Ames test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 471), cultures of four mutant strains of *S. typhimurium* (TA100, TA98, TA1537 and TA1535) were exposed to AMPA (purity, 99.2%) at a concentration of 5.0, 2.5, 1.3, 0.63 and 0.31 mg per plate with and without metabolic activation (S9 mix). Two independent test series were performed, the first as a plate incorporation assay, the second as a preincubation assay using replicates of three plates for each dose. Positive and negative controls were included in both tests. After incubation for 48–72 h at 37 °C, the number of colonies (revertants) were counted.

The counts for negative and positive controls were all within the expected ranges. No depression of background growth was observed, indicating that AMPA was not cytotoxic at concentrations of up to 5.0 mg/plate. The number of revertants groups treated with glyphosate were generally similar to those in the concurrent controls. A single statistically significant increase in revertants was found (TA1535, plate incorporation assay, at a concentration of 0.63 mg/plate without metabolic activation). However, the increase was marginal and no dose–response relationship was seen. Thus, AMPA was found to be non-mutagenic in this test system (Jensen, 1993a).

In a test for mammalian cell gene mutation in vitro, performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 476), AMPA (purity, 99.2%) at a dose of 0.31, 0.63, 1.3, 2.5 or 5.0 mg/ml was applied to cell cultures of mouse lymphoma cells (L5178Y), with and without metabolic activation. Duplicate tests were performed for each dose. Solvent controls and positive controls were included in both assays. After incubation for 10 days at 37 °C, the numbers of cell clones were counted and the cloning efficiencies determined.

The mutation frequencies of the test cultures were generally similar to those of the concurrent negative controls at all doses, with and without metabolic activation. Statistical

analysis revealed no statistically significant differences at any dose. The mutation frequency for positive controls was in the expected range in all test series. Thus, AMPA was found to be non-mutagenic in L5178Y mouse lymphoma cells in vitro (Jensen, 1993b).

In an assay for micronucleus formation, performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 474), groups of five male and five female Bom:NMRI mice received AMPA (purity, 99.2%) as a single dose at 5000 mg/kg bw by oral gavage. The test substance was dissolved in aqueous sodium chloride and carboxymethylcellulose to give a dose volume of 20 ml/kg bw. Positive and solvent control groups of similar size were also included. The mice from the different AMPA test groups were killed at 24, 48 and 72 h, respectively, after dosing. The negative (solvent) control group mice were all sacrificed after 48 h and the positive control group terminated 24 h after dosing. Immediately after a mouse was killed, cell smears were prepared. Slides were coded in order to perform a blind counting. The following counts were made: percentage of polychromatic erythrocytes (PCE) in 2000 erythrocytes—% PCE; number of micronuclei (MN) observed in 2000 polychromatic erythrocytes (PCE)—MN/PCE; and the number of micronuclei in normochromatic erythrocytes (NCE) observed during the counting of 2000 PCE—MN/NCE.

The % PCE in the AMPA test groups was significantly lower than that in the control group, indicating a clear depression of erythropoiesis. The frequency of micronucleus formation in the positive and negative control groups were in accordance with historical data. The frequency of micronucleus formation in PCE was similar in the negative control and test groups. The results for AMPA were negative in this assay for micronucleus formation in vivo (Jensen, 1993c).

(iv) Developmental toxicity

In a study of devlopmental toxicity performed in compliance with the principles of GLP and roughly according to the test guidelines of the US EPA and the OECD (TG 414), groups of 10 mated female Sprague-Dawley rats received AMPA (purity, 99.2%; in carboxymethylcellulose) at a dose of 100, 350 or 1000 mg/kg bw by oral gavage from day 6 to day 16 of gestation. Control animals were dosed with carboxymethylcellulose and distilled water alone. Animals were observed daily for mortalities and reaction to treatment. Individual body weights were recorded on days 0, 6, 9, 13, 17 and 20 of gestation. Food consumption was recorded daily, starting on day 4 of gestation. On day 20 of gestation, animals were sacrificed to examine congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine the number of corpora lutea and number and position of all implantation sites in the uterus. Each implant was classified as being: (a) live; (b) a fetal death, judged to have occurred after day 16 of gestation; (c) a late embryonic death, judged to have occurred in the period between day 12–16 of gestation; or an early embryonic death, judged to have occurred before day 12 of gestation. Live fetuses were individually identified, weighed and examined for any externally visible abnormalities. Half the fetuses were examined for visceral and skeletal abnormalities and the remaining fetuses were examined for soft tissue abnormalities.

There were no mortalities, and there were no clinical observations related to treatment with AMPA throughout the duration of the study. Body-weight gain and food consumption of the test animals were similar to those of the controls. There were no notable intergroup differences in the incidence of intrauterine deaths, or in mean fetal weights. Examination

of fetuses for developmental abnormalities and variations of the viscera and skeleton (including state of ossification) showed no intergroup differences. Thus, the maternal and developmental NOAEL was 1000 mg/kg bw per day, the highest dose. No evidence of teratogenicity was obtained (Hazelden, 1992).

3. Observations in humans

There are various reports in the literature describing the effects observed after accidental and/or intentional ingestion of concentrated formulations of glyphosate. Large amounts of glyphosate-based herbicides are occasionally deliberately ingested to attempt suicide, mainly in Asian countries, and may result in serious gastrointestinal, cardiovascular, pulmonary and renal effects and possibly death (Talbot et al., 1991; Tominack et al., 1991; Lee et al., 2000). The nature of the clinical symptoms suggests that hypovolemic shock was the cause of death (Sawada et al., 1988; Tominack et al., 1989). It has been pointed out by these authors that the surfactant contained in glyphosate formulations may be responsible for the clinical syndrome, but that the available evidence on this point was inconclusive. In such cases, aggressive supportive care is recommended (Tominack et al., 1989). Accidental exposure to small volumes of glyphosate results in, at most, only mild effects; no deaths have been reported (Goldstein et al., 2002).

In spite of this experience, it has been stated that glyphosate is a leading cause of pesticide poisoning. This contradiction may be elucidated using data from California. The claims are based upon a counting of telephone calls to the California Environmental Protection Agency's Pesticide Poisoning Information System (PISP). Since the inception of the PISP database, glyphosate has been among the most frequently reported individual agents (California EPA, 1996). Review of the California data indicates that the number of reported cases simply reflects greater use of the product relative to other herbicides and shows that glyphosate has relatively low toxicity among pesticides used in California (Goldstein et al., 2002). PISP was created in 1982 as a clearinghouse for telephone calls of pesticide-related illness. Concurrently, the reporting of pesticide-related illness to PISP was made mandatory for health-care providers in California. The data collected there include cases of eye and skin irritation, systemic symptoms, as well as general inquiries and asymptomatic exposures. Thus, number of calls is a poor indicator of true "clinical poisoning", defined as a poisoning with the occurrence of systemic symptoms and excluding those cases involving only topical irritation of the skin and/or eye. An analysis of the database spanning 1982 to 1997 shows that there were 815 calls involving glyphosate herbicide products. Of those 815 calls, 399 were eye irritation-only cases, 250 were skin irritation-only cases, seven were respiratory-only cases and 32 were mixed cases (eye, skin and respiratory). Only 20 out of the 815 calls reported systemic symptoms after use of a glyphosate product only. The reported symptoms were not severe, expected to be limited in duration, and were frequently inconsistent with the route of exposure and/or previous experience with glyphosate.

The California Department of Pesticide Regulation noted in its 1994 report that most people (>80%) affected by glyphosate experienced only irritant effects and, of the 515 pesticide-related hospitalizations recorded over 13 years, none was attributed to glyphosate.

Acquavella et al. (1999) evaluated ocular effects in 1513 cases of exposure to glyphosate formulations reported to a certified regional centre of the American Association of Poison Control Centres from 1993 to 1997. The large majority of reported exposures were judged by specialists at the centre to result in either no injury (21%) or only transient

minor symptoms (70%). None of the reported exposures resulted in permanent change to the structure or function of the eye. This information is particularly important since glyphosate acid was irritant to the eyes in studies in animals.

Barbosa et al. (2001) published a single case report of a man aged 54 years who accidentally sprayed himself with a glyphosate-based formulation in his garden (manufacturer and formulation details unknown). According to the authors, within 6 h of the incident the man developed conjunctival hyperaemia and a generalized rash. One month later he presented with Parkinsonian symptoms in all four extremities and 1 year later developed a resting tremor of one hand and complained of memory deficits. However, this single case is not sufficient to prove the proposed relationship between exposure to glyphosate and the occurrence of Parkinson disease, since this finding is inconsistent with extensive testing in animals, and human experience. Furthermore, the hypothesis regarding a possible mechanism of action via production of glycine is not supported by existing metabolic data. There is no credible evidence so far that glyphosate is capable of inducing Parkinson disease or any other neurological illness in humans or animals.

Exposure related to the professional use of glyphosate-based formulations, through the monitoring of the single active ingredient glyphosate, has been the subject of a number of studies. The practices monitored in those studies represent a range of application techniques, use rates, workloads and reflect variety in use of personal protective equipment. Dermal contact is the most likely route of exposure for applicators; and activities such as mixing and loading of glyphosate and extended applications using hand sprayers have the highest potential for exposure. Inhalation is considered to be a minimal route of exposure under most circumstances because of glyphosate's extremely low vapour pressure.

Both passive dosimetry and biomonitoring have been used as techniques to assess exposure. Biomonitoring results represent systemic (internal) exposure, while the results obtained from passive dosimetry quantify external deposition. There is general agreement that biological measurements as obtained through biomonitoring provide the most relevant information for safety assessments (Chester & Hart, 1986; Franklin et al., 1986). Biomonitoring for glyphosate has been a particularly valuable technique because metabolism studies have shown that it is rapidly excreted by mammals unchanged, primarily via urine, facilitating interpretation of exposure with little need for adjustment of the results to account for pharmacokinetic factors.

Some biomonitoring studies were performed on silvicultural workers who sprayed a glyphosate formulation in a variety of forestry and tree farming activities. In one study, the United States Department of Agriculture's Forest Service, in collaboration with Monsanto Company and the University of Arkansas, sponsored a study to investigate exposure of workers to glyphosate at two forestry nurseries in Oregon and in Massachusetts where glyphosate was used for weed control (Lavy et al., 1992). At both nurseries, exposure of applicators, weeders, and scouts were measured while they performed their normal duties. They assessed the internal dose of glyphosate through analyses of the total daily urine excreted by each of the workers. Urine samples were collected from the weeders and scouts before working with glyphosate and for 8 months thereafter. Continuous sampling of total urine was conducted for the first 12 consecutive weeks of the study, after which a 24-h sample was collected each Wednesday for the next 5 months. Urine samples from applicators were collected for a 6-day period, including the day before, the day of, and the 4 days after the applications of herbicide. These samples were analysed as 24-h composites. Of the

355 daily samples of urine analysed, none were found to contain quantifiable concentrations of glyphosate. The limit of quantification was 10 ppb. Dermal exposure was likely for the workers; the lack of quantifiable glyphosate in the urine was attributed to the very limited ability of glyphosate to penetrate the skin of the exposed workers.

In a second collaborative study conducted by the US Department of Agriculture Forestry Service, Georgia Tech Research Institute, and Monsanto (Cowell & Steinmetz, 1990), the exposure of applicators to glyphosate during a hand-held directed spray foliar application at three sites maintained by the Forestry Service where glyphosate was used to control vegetative growth around pine seedlings planted in clear-cut forest areas was assessed. At each test site, in addition to applying the herbicide, one person (the mixer) measured and mixed a 3% (v/v) spray solution of the formulation and filled the backpack sprayers for all the other applicators. At all three sites, five workers per site applied glyphosate, and were monitored for exposure to glyphosate on the day the applications were made. In addition, at one site a supervisor also applied glyphosate and was monitored for exposure. Urine samples for biological monitoring from each participant were collected for a period of 5 days. Urine was collected the day before, the day of, and the 3 days after the application of glyphosate. Urine specimens for each worker were combined to form 12-h composite samples. Of the 96 urine samples analysed, five were found to contain quantifiable levels of glyphosate. The highest concentration of glyphosate measured was 14 ppb and the highest estimated internal dose was 0.0006 mg/kg bw, which is well below the proposed ADI.

Two other studies have been conducted to measure exposure of forestry workers to glyphosate during normal silvicultural applications: one in Finland (Jauhiainen et al., 1991) and the other in Canada (Center de Toxicologie du Quebec, 1988).

For the Finnish study, two groups of five forestry workers were used: an unexposed control group of workers that planted young trees, and a test group of workers that applied glyphosate using brush saws equipped with pressurized sprayers. The test group sprayed glyphosate each day for 5 consecutive days in August 1988. Each worker prepared fresh spray solutions each day. Urine samples were taken at the end of each working day that glyphosate was applied. Urine samples were also taken from each of the workers 3 weeks after the last day of herbicide application. In addition, each worker received a medical examination on the first and last days that glyphosate was applied and a follow-up examination 3 weeks after the last application day. These examinations included haematology, clinical chemistry, electrocardiogram (ECG), pulmonary function tests, an interview for a health questionnaire, and a general clinical examination (including blood pressure, pulse rate and pressure craft of hands). All urine samples had less than detectable concentrations of glyphosate. There were no statistically significant differences in the findings of the medical examinations conducted before and after exposure.

The Canadian study of exposure of forestry workers to glyphosate after normal silviculture uses of glyphosate was conducted over two growing seasons and involved 45 workers. During the summer of 1986 a crew of five forestry workers (foreman, mixer, operator, and two flagmen) in charge of spraying operations were monitored for exposure to glyphosate. Seven urine samples were collected from each worker on each day of herbicide application: one at the beginning of the work day, four during the course of the work day, one at the end of the work day, and one in the morning of the day after application. Glyphosate applications were made on 19 different days, with the total application times

ranging from 1 to 9 h per day. The active ingredient was not detected in most urine samples from the two flagmen and the operator, and concentrations of glyphosate in all urine samples were <0.03 ppm (the limit of quantitation). In contrast, 14 out of 33 urine samples from the mixer and two urine samples for the foreman contained glyphosate at concentrations of >0.03 ppm. Maximum glyphosate concentrations in the foreman's and mixer's urine were 0.043 and 0.055 ppm, respectively.

As a follow-up to the 1986 study, 40 forestry workers were monitored during the summer of 1987 for exposure to glyphosate during normal use of glyphosate in silviculture. Consistent with the results of previous studies, concentrations of glyphosate in the urine of exposed workers were very low. In most samples, glyphosate was not detectable. In those samples that did contain detectable levels of glyphosate, concentrations were <0.1 ppm in all cases, and typically <0.035 ppm.

Although the concentrations of glyphosate in some of the urine samples of workers in this study were greater than those found in other glyphosate worker exposure studies, the levels found were very low.

The most recent biomonitoring study, the Farm Family Exposure Study (FFES), was funded through a research contract with the University of Minnesota and sponsored by seven agricultural chemical companies in order to investigate real-world exposures to pesticides for farmers and their families using state-of-the-art field and analytic methods. FFES participants were randomly selected from licensed pesticide applicators in Minnesota and South Carolina. Families were eligible if there was a farmer, spouse, and at least one child aged 4–18 years living on the farm; if they owned or leased at least 10 acres (0.04 km^2) of cropland; if they planned to apply at least one of the target pesticides (glyphosate, 2,4-D, or chlorpyrifos) within 1 mile (1.6 km) of their residence; if they were willing to collect all their urine for 5 consecutive days (the day before, the day of, and the 3 days after a pesticide application); and if they were willing to fill out pre-application and post-application questionnaires. FFES field staff observed all pesticide applications and documented information relevant for exposure assessment. Forty-eight farm families, including 79 children, provided urine specimens relating to glyphosate application. Analysis of 24-h composite urine samples was performed for each family member the day before, the day of, and for 3 days after a glyphosate application. The limit of detection (LOD) was 1 ppb.

Twenty-nine out of 48 farmers (60%) were found to have detectable levels of glyphosate in their urine on the day of application. The geometric mean concentration was 3.2 ppb on the day of application and declined thereafter, and the maximum concentration was 233 ppb. Farmers who did use rubber gloves when mixing and loading glyphosate formulations had lower geometric mean urinary concentrations than those who did not (2.0 ppb compared with 9.7 ppb). The number of acres treated was not correlated with urinary concentration of glyphosate, but there was a trend between concentration and the number of times that farmers mixed and loaded the concentrated herbicide formulation. Other factors associated with urinary concentration of glyphosate were using an open cab tractor, observed skin contact with the glyphosate formulation, and repairing equipment during the application.

Detectable urinary concentrations of glyphosate were infrequent for farm spouses and farm children. Two out of 48 farm spouses (4%) had detectable values on the day of application, the highest individual concentration was 3 ppb. Of the 78 children who provided samples on the day of application, nine (12%) had a detectable glyphosate concen-

tration, and all except one of the children had been present for or assisted with mixing, loading, or application activities. The maximum urinary concentration of glyphosate, 29 ppb, was for a teenage boy who assisted his father with the mixing and application. The maximum systemic dose of glyphosate for farmers, spouses, and children was estimated to be 0.004, 0.00004 and 0.0008 mg/kg bw, respectively (Acquavella et al., 2004).

Widely used pesticides, like glyphosate, have recently become a focus of epidemiological research. In the past few years several epidemiological studies have been published that reported weak associations of glyphosate with lymphopoeitic cancers (Nordstrom et al., 1998; Hardell & Erikson, 1999; McDuffie et al., 2001), self-reported adverse reproductive outcomes (Savitz et al., 1997; Curtis et al., 1999; Arbuckle et al., 2002) and self-reported attention deficit hyperactivity disorder in children (Garry et al., 2002). However, the results of these studies do not meet generally accepted criteria from the epidemiology literature for determining causal relationships. Generally, the associations were rather weak and rarely statistically significant. Control for potential confounding factors, including other pesticides, was not possible owing to limited available information and small numbers of subjects. It was not measured whether there actually was any internal exposure or the extent of such exposure and, accordingly, a possible dose–response relationship could not be evaluated.

Comments

After oral administration to rats, [^{14}C]glyphosate was only partially absorbed (about 30–36%) from the gastrointestinal tract. Absorption was not significantly dose-dependent over the range of 10 to 1000 mg/kg bw. Peak plasma concentrations of radiolabel were observed at 0.5–1 h after dosing in rats and hens, respectively, and at 6–8 h after dosing in goats. The highest tissue concentrations were found in bone, with lower concentrations being found in bone marrow, kidney and liver. After oral administration, about 60–70% of the administered dose was eliminated in the faeces. Of the glyphosate that was absorbed, most was excreted in the urine and <0.2% in expired air. After intravenous application, faecal excretion via bile was only about 2–8% of the administered dose. Whole-body clearance (about 99% of an oral dose) occurred within approximately 168 h. The estimated half-life for whole-body elimination of the radiolabel was 2.1–7.5 h for the alpha phase and 69–337 h for the beta phase. Repeated dosing did not alter absorption, distribution, and excretion. There was very little biotransformation of glyphosate; the only metabolite, AMPA, accounted for ≤0.7% of the administered dose in excreta; the rest was unchanged glyphosate.

Glyphosate has low acute oral toxicity in mice (LD_{50}, >2000 mg/kg bw; no deaths at this dose) and rats (LD_{50}, >5000,mg/kg bw), low acute dermal toxicity in rats (LD_{50}, >2000 mg/kg bw) and rabbits (LD_{50}, >5000 mg/kg bw), and low acute inhalation toxicity in rats (LC_{50}, >4.43 mg/l). Clinical signs after acute oral exposure included reduced activity, ataxia and convulsions.

Glyphosate was not irritating to the skin, but produced moderate to severe eye irritation with irreversible corneal opacity in one study. Glyphosate salts were slightly irritating to the eye, with minimal to moderate conjunctival irritation and slight iritis that usually disappeared within 48 h after exposure. Glyphosate was not a skin sensitizer in guinea-pigs.

In short-term studies of toxicity in different species, the most important effects were clinical signs related to gastrointestinal irritation, salivary gland changes (hypertrophy and increase in basophilia of cytoplasm of acinar cells) and hepatotoxicity. In mice, reduced body-weight gain was seen at a dietary concentration of 25 000 ppm. Alterations of the salivary glands were present in mice in one of two short-term studies at dietary concentrations of ≥6250 ppm; the NOAEL for this finding was 3125 ppm (equal to 507 mg/kg bw per day). In rats, findings included soft faeces, diarrhoea, reduced body-weight gain, decreased food utilization and slightly increased plasma enzyme activities (ALP, ALT) at dietary concentrations of ≥20 000 ppm. Additionally, in two out of four 90-day studies in rats, increased incidences of alterations of the salivary glands were observed. At the lower doses, these changes were only minimal with respect to severity and incidence. The overall NOAEL was 300 mg/kg bw per day.

In dogs, the NOAEL in a 90-day feeding study was 10 000 ppm (equal to 323 mg/kg bw per day) on the basis of reduced body-weight gain, marginal reductions in albumin and calcium concentrations, and increased plasma ALP activities at 50 000 ppm. In a 1-year study in dogs given capsules containing glyphosate, the NOAEL was 30 mg/kg bw per day, on the basis of clinical signs (soft faeces, diarrhoea) and reduced body-weight gain at ≥300 mg/kg bw per day. In a 1-year feeding study, the NOAEL was 15 000 ppm (equal to 440 mg/kg bw per day) on the basis of reduced body-weight gain at 30 000 ppm.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In the study of carcinogenicity in mice, no toxic effects were observed at up to the highest dose tested (1000 mg/kg bw per day), and there was no evidence of carcinogenicity.

In a 1-year study of toxicity in rats, the NOAEL was 2000 ppm (equal to 141 mg/kg bw per day) on the basis of a reduction in body weight and clinical chemistry findings at 8000 ppm. Three new long-term studies in rats were evaluated. In the first study, the NOAEL was 8000 ppm (equal to 362 mg/kg bw per day) on the basis of a reduction in body weight in females and an increased incidence of cataracts and lens abnormalities in males at 20 000 ppm. In the second study, the NOAEL was 100 mg/kg bw per day on the basis of more pronounced alterations of the parotid and submaxillary salivary glands at ≥300 mg/kg bw per day. In the most recent 2-year study in rats, the NOAEL was 6000 ppm (equal to 361 mg/kg bw per day) on the basis of a reduction in body weight and food consumption, and indications of kidney, prostate, and liver toxicity at 20 000 ppm. There was no evidence of a carcinogenic response to treatment in rats.

The genotoxic potential of glyphosate has been extensively tested in a wide range of assays both in vitro and in vivo, including end-points for gene mutation, chromosomal damage and DNA repair. Negative results were obtained in studies performed in compliance with current test guidelines. The Meeting concluded that glyphosate is unlikely to be genotoxic.

In view of the absence of a carcinogenic potential in animals and the lack of genotoxicity in standard tests, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans.

Glyphosate had no effects on fertility in both two-generation studies of reproductive toxicity in rats. The overall NOAEL for parental and offspring toxicity was 3000 ppm (equal to 197 mg/kg bw per day) on the basis of increased food and water consumption and reduced

body-weight gain in F_1 animals, and an increased incidence of alterations of the parotid and submaxillary salivary glands in F_0 and F_1 animals at 10 000 ppm.

In studies of developmental toxicity in rats, the NOAEL for maternal and developmental toxicity was 300 mg/kg bw per day, on the basis of clinical signs and reduced body-weight gain in the dams and increased incidences of fetuses with delayed ossification and skeletal anomalies.

In studies of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of clinical signs and reduced food consumption and body-weight gain. The NOAEL for developmental toxicity was 175 mg/kg bw per day on the basis of reduced fetal weight and delayed ossification, and an increased incidence of postimplantation loss. The Meeting concluded that glyphosate is not teratogenic.

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

Hypertrophy and cytoplasmic alterations of the salivary glands (parotid and/or mandibular) was a common and sensitive end-point in six studies: in three 90-day studies (one in mice, two in rats), a 1-year study in rats, a 2-year study in rats and a two-generation study of reproductive toxicity in rats. Mechanistic studies available to the Meeting hypothesized that the mechanism was adrenergic. However, the inability of a β-blocker to significantly inhibit these effects indicates that glyphosate does not act as a β-agonist. Other proposed mechanisms for the salivary gland alterations include oral irritation caused by dietary administration of glyphosate, a strong organic acid. Although the mechanism of the cytoplasmic alterations in the salivary glands was unclear, the Meeting concluded that this treatment-related effect is of unknown toxicological significance.

In a study of acute neurotoxicity in rats, the NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested. In a short-term study of neurotoxicity in rats, the NOAEL for neurotoxicity was 20 000 ppm, equal to 1547 mg/kg bw per day, the highest dose tested. In a study of acute delayed peripheral neuropathy in hens, clinical and histopathological examination found no evidence for acute delayed peripheral neuropathy at a dose of 2000 mg/kg bw.

New toxicological data on AMPA (the primary degradation product of glyphosate in plants, soil and water, and the only metabolite of glyphosate found in animals) was submitted to the present Joint Meeting for evaluation. AMPA was of low acute oral and dermal toxicity in rats (LD_{50}, >5000 and >2000 mg/kg bw, respectively), and was not a skin sensitizer in guinea pigs. In a 90-day study of toxicity in rats, the NOAEL was 1000 mg/kg bw per day, the highest dose tested. AMPA had no genotoxic potential in vitro or in vivo. In a study of developmental toxicity in rats, no evidence for embryo- or fetotoxicity was found and the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

On the basis of the new toxicological data, the present Joint Meeting concluded that AMPA is of no greater toxicological concern than its parent compound, thus confirming the conclusion of the 1997 JMPR.

Routine medical surveillance of workers in production and formulation plants revealed no adverse health effects attributable to glyphosate. In operators applying

glyphosate products, cases of eye, skin and/or respiratory tract irritation have been reported. Acute intoxication was reported in humans after accidental or intentional ingestion of concentrated glyphosate formulations, resulting in gastrointestinal, cardiovascular, pulmonary, and renal effects and occasionally death. The acute toxicity of glyphosate formulations was likely to be caused by the surfactant in these products.

The Joint Meeting established a group ADI for glyphosate and AMPA of 0–1.0 mg/kg bw on the basis of the NOAEL of 100 mg/kg bw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. The ADI is supported by NOAELs of 141 and 197 mg/kg bw per day from the 1-year study and the two-generation study of reproductive toxicity in rats, respectively.

The Joint Meeting concluded that it was not necessary to establish an ARfD for glyphosate in view of its low acute toxicity, the absence of relevant developmental toxicity in rats and rabbits that could have occurred as a consequence of acute exposure, and the absence of any other toxicological effect that would be elicited by a single dose.

The NOAEL of 30 mg/kg bw per day in a 1-year study in dogs was not considered to be relevant for establishing either the ADI or ARfD, since the gastrointestinal effects seen in this study at 300 and 1000 mg/kg bw per day were related to high local concentrations of test substance resulting from the administration of glyphosate in capsules.

Toxicological evaluation

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	3-month study of toxicity[a,e]	Toxicity	3 125 ppm, equal to 507 mg/kg bw per day	6 250 ppm, equal to 1 065 mg/kg bw per day
	2-year study of carcinogenicity[a]	Toxicity	1 000 mg/kg bw per day[d]	—
		Carcinogenicity	1 000 mg/kg bw per day[d]	—
Rat	3-month study of toxicity[a,e]	Toxicity	300 mg/kg bw per day	12 500 ppm, equal to 811 mg/kg bw per day
	1-year study of toxicity[a]	Toxicity	2 000 ppm, equal to 141 mg/kg bw per day	8 000 ppm, equal to 560 mg/kg bw per day
	2-year study of toxicity and carcinogenicity[a,e]	Toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Carcinogenicity[d]	20 000 ppm, equal to 1 214 mg/kg bw per day[d]	—
	Multigeneration reproductive toxicity[a,e]	Parental toxicity	3 000 ppm, equal to 197 mg/kg bw per day	10 000 ppm, equal to 668 mg/kg bw per day
		Offspring toxicity	3 000 ppm, equal to 197 mg/kg bw per day	10 000 ppm, equal to 668 mg/kg bw per day
	Developmental toxicity[b,e]	Maternal toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo- and fetotoxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
Rabbit	Developmental toxicity[b,e]	Maternal toxicity	100 mg/kg bw per day	150 mg/kg bw per day
		Embryo- and fetotoxicity	175 mg/kg bw per day	300 mg/kg bw per day
Dog	3-month study of toxicity[a]	Toxicity	10 000 ppm, equal to 323 mg/kg bw per day	50 000 ppm, equal to 1 680 mg/kg bw per day
	1-year study of toxicity[a,c,e]	Toxicity	30 mg/kg bw per day[c,f]	300 mg/kg bw per day[c]

[a] Dietary administration
[b] Gavage administration
[c] Capsules
[d] Highest dose tested
[e] Two or more studies combined
[f] Not used for establishing the ADI (or ARfD) since the NOAEL was based on an effect induced by high local concentrations

Estimate of acceptable daily intake for humans

0–1.0 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

- Additional information on the mechanism of the changes in the salivary glands
- Further observations in humans

Summary of critical end-points for glyphosate

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, approximately 30–36%
Dermal absorption	No information
Distribution	Widely distributed
Rate and extent of excretion	Largely complete within 48 h; approximately 30% in urine and 70% in faeces
Potential for accumulation	No evidence of accumulation (<1% after 7 days)
Metabolism in mammals	Very limited (<0.7%), hydrolysis leading to AMPA
Toxicologically significant compounds (animals, plants and the environment)	Parent compound, AMPA
Acute toxicity	
Rat, LD_{50}, oral	>5000 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	>4.43 mg/l (4-h, nose-only exposure)
Rabbit, dermal irritation	Non-irritant
Rabbit, eye irritation	Moderately to severely irritant
Skin sensitization	Not sensitizing (Magnusson & Kligman test, Buehler test)
Short-term toxicity	
Target/critical effect	Clinical signs (soft faeces, diarrhoea), reduced body-weight gain; liver (toxicity), salivary glands (hypertrophy)
Lowest relevant oral NOAEL	300 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	—
Lowest relevant inhalation NOAEC	—
Genotoxicity	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body-weight gain; liver (toxicity), salivary glands (hypertrophy), eye (cataract, lens fibre degeneration)
Lowest relevant NOAEL	100 mg/kg bw per day (2-year study in rats)
Carcinogenicity	No evidence of carcinogenicity in rats or mice
Reproductive toxicity	
Reproductive target/critical effect	Reduced pup weight at parentally toxic doses
Lowest relevant reproductive NOAEL	197 mg/kg bw per day (two-generation study in rats)
Developmental target/critical effect	Embryo- and fetotoxicity at maternally toxic doses (rat, rabbit)
Lowest relevant developmental NOAEL	175 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	No evidence of neurotoxicty in any study conducted
Medical data	Medical surveillance of workers in plants producing and formulating glyphosate did not reveal any adverse health effects. In operators applying glyphosate products, cases of eye, skin and/or respiratory irritation have been reported. Cases of acute intoxication have been observed after accidental or intentional ingestion of glyphosate formulations.

Summary

	Value	*Study*	*Safety factor*
ADI[a]	0–1.0 mg/kg bw	2-year study in rats (salivary gland effects)	100
ARfD	Unnecessary	—	—

[a] For the sum of glyphosate and AMPA

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