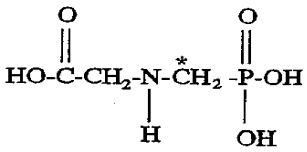
1. Biochemical aspects

The absorption, distribution, metabolism and excretion of glyphosate was studied in rats following a single oral low dose, a single oral high dose and a single oral low daily dose repeated for 14 days followed by a radioactive dose. In addition, absorption and excretion of glyphosate was studied via intravenous and intraperitoneal administration in rats and intramuscular administration in Rhesus monkeys.

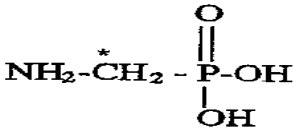
Fig. 1 shows the structure of radiolabelled glyphosate

Fig. 1. Structure of glyphosate – ^{14}C-labelled at the methylene carbon at C1 or C2-glycine carbon



*** Denotes position of ^{14}C label.**

Fig. 2. Structure of aminomethylphosphonic acid (AMPA)



*** Denotes position of ^{14}C label.**

1.1 Absorption, distribution and excretion

(a) Oral route

The excretion and residue levels found by various studies following a single oral dose or repeated oral administration of glyphosate in rats and rabbits are shown in the Table 1.

Table 1. Total elimination and residues of administered radioactivity after single or repeated oral administration of ^{14}C-labelled glyphosate

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
6.7 mg/kg bw Single dose 120 hours	Rat	14–16	35–43	81–85	49–55	0.14–0.65	0.83–1.02	Colvin & Miller[a] (1973a)

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
10 mg/kg bw Single dose 24/48 hours	Rat	17.9/34.0	12.8/12.5	59.3/60.5	80.3/91.2	ND	ND	Davies, (1996a)
10 mg/kg bw Single dose 72 hours	Rat	13	10.6	88.5	88.7	0.59	0.49	Davies, (1996d)
10 mg/kg bw Single dose 7 days	Rat	28.6	22.5	62.4	69.4	0.44	0.31	Ridley & Mirly, (1988)
10 mg/kg bw Repeated dosing 72 hours	Rat	10.6	10.7	86.6	90.7	0.46	0.41	Davies[b] (1996c)
10 mg/kg bw Repeated dosing 7 days	Rat	30.9	23.1	61.0	70.9	0.54	0.35	Ridley & Mirly[b] (1988)
30 mg/kg bw Single dose 168 hours	Rat	29.04	30.71	58.84	56.53	0.62	0.64	Powles, (1992a)
30 mg/kg bw Repeated dosing 168 hours	Rat	34.28	34.63	49.64	46.73	0.96	0.83	Powles, (1992b)
1000 mg/kg bw Single dose 72 hours	Rat	16.7	17.5	89.6	84.5	0.52	0.58	Davies (1996b)
1 000 mg/kg bw Single dose 168 hours	Rat	30.55	22.41	53.27	60.37	0.47	0.40	Powles (1992b)
1 000 mg/kg bw Single dose 7 days	Rat	17.8	14.3	68.9	69.4	0.28	0.24	Ridley & Mirly (1988)
10 mg/kg bw Single dose 168 hours	Rat	22.5	19.4	74.6	84.3	0.33	0.27	McEwen[c] (1995)
10 mg/kg bw Single dose 168 hours	Rat	30.3	29.5	74.7	74.2	0.31	0.39	McEwen[c] (1995)
1 mg/kg bw Single dose 168 hours	Rat	18.4	27.2	72.6	62.4	0.8	1.0	Knowles & Mookherje e (1996[c])
100 mg/kg bw Single dose 168 hours	Rat	39.4	43.1	41.2	42.4	0.8	1.0	Knowles & Mookherje e (1996[c])

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
5.7–8.8 mg/kg bw Single dose 120 hours	Rabbit	7–11	ND	80–97	ND	0.1–1.2	ND	Colvin & Miller[a] (1973c)

bw: body weight; ND: not determined; no. number

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

[b] Groups of male and female rats were given 14 consecutive daily oral doses of 10 mg/kg bw of unlabelled glyphosate followed by a single oral dose 10 mg/kg bw of [^{14}C]glyphosate.

[c] Residual activity in carcass only.

The excretion and residue levels found by various studies following single intraperitoneal, intravenous or intramuscular administration in rats and Rhesus monkeys are shown in Table 2.

Table 2. Residues of administered ^{14}C-labelled glyphosate[a] after single dose administration

Dose / Means of administration / Length of observation	Species	Percentage of administered dose (%)						Reference
		Total excretion via urine		Total faecal excretion		Total tissue and residual carcass residues		
		Males	Females	Males	Females	Males	Females	
6.7 mg/kg bw Intraperitoneal 120 hours	Rat	82–90	ND	6–14	ND	< 1	ND	Colvin & Miller[a] (1973a)
10 mg/kg bw Intravenous 7 days	Rat	79.0	74.5	4.65	8.3	1.27	1.09	Ridley & Mirly (1988)
30 mg/kg bw Intravenous 168 hours	Rat	85.98	84.18	3.42	1.48	1.35	1.09	Powles (1992b)
4 mg Intramuscular 7 days	Monkey	89.9	ND	ND	ND	ND	ND	Maibach (1983)

bw: body weight; ND: not determined

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

Rats

In a pre-GLP study, aqueous solutions of glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon and at the C2-glycine carbon were administered to Wistar rats by gavage. The radiochemical purity of the labelled materials used were 95% and higher for ^{14}C-methylene glyphosate, ^{14}C-C1-glycine glyphosate and ^{14}C-C2-glycine glyphosate. For the first series of experiments, eight male and four female rats were fasted for four hours and then administered, by gavage, aqueous solutions of [^{14}C]glyphosate at a dose level of 6.7 mg/kg body weight (bw). Two male rats and one female rat were administered ^{14}C-methylene glyphosate, three male rats and one female rat were administered ^{14}C-C1-glycine glyphosate, and three male rats and two female rats were administered ^{14}C-C2-glycine glyphosate. In a second series of experiments, three treatment groups of

Body and organ weights indicated that the continuous administration of feed containing 1, 10 and 100 ppm of glyphosate for 14 days had no detrimental effect on the growth or relative organ size of rats. Of the [^{14}C]glyphosate ingested, 8.3–10.5% of the daily intake was excreted in the urine. The combined urinary and faecal excretion of radioactivity was approximately equal to the total intake of [^{14}C]glyphosate after 6 days, indicating that a plateau had been reached. By day 4 of dosing, radioactivity in the urine plus faeces exceeded 90% of the cumulative intake, and by the end of the 14-day dosing period the combined excretion of radioactivity was 96, 115 and 93% of the cumulative intake of the 1, 10 and 100 ppm dosing levels, respectively. Since the amount of radioactivity excreted was directly proportional to the intake, the elimination kinetic of [^{14}C]glyphosate could be described as a first-order process, precluding the potential of unlimited accumulation. Most tissues reached maximum [^{14}C]glyphosate residue levels during the dosing period in 10 days or less. There was a modest cumulative effect in the body as a result of chronic [^{14}C]glyphosate administration, but the effect was not localized in a single tissue type or organ system. The order of decreasing tissue propensity for [^{14}C]glyphosate, on a fresh-weight basis, was kidney, spleen, fat, liver, ovaries, heart, muscle, brain and testes. On a dry-weight base the order was spleen, kidney, ovaries, heart, liver, testes, fat, brain and muscle. Accumulation of [^{14}C]glyphosate in muscle tissue was very low on either a fresh- or dry-weight basis, indicating a very low propensity for accumulation. The residues in the tissues were reversibly bound and began to deplete as soon as the dosed feed was withdrawn (Colvin & Miller, 1973b).

Seven different test groups of Sprague Dawley (Crl:CD[SD]BR) rats, each with an equal number of males and females, were dosed with [^{14}C]glyphosate labelled in the methylene position between the nitrogen and phosphorous atoms (radiochemical purity ≥ 98%). Single oral doses (10 and 1000 mg/kg bw) were administered by gastric intubation, and intravenous doses (10 mg/kg bw) were injected into the lateral tail vein. Another group of five male and five female rats was treated with 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. Blood, urine and faeces were sampled at various time points. At the end the study, the animals were terminated and different tissues as well as the carcass analysed for radioactivity.

The distribution of radioactivity in the excreta and the tissue samples are summarized in Table 3.

Table 3. Recovery of radioactivity as a percentage of the administered ^{14}C-labelled glyphosate dose

	Per cent of administered radioactive dose (%)							
	Single IV dose 10 mg/kg bw		**Single oral dose 10 mg/kg bw**		**Repeated oral dose 10 mg/kg bw**		**Single oral dose 1 000 mg/kg bw**	
Excreta/tissue	**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	0.09	0.05	0.05	0.02	0.05	0.03	0.04	0.03
Residual carcass	1.18	1.04	0.40	0.29	0.50	0.32	0.25	0.21
Gastrointestinal contents	0.04	0.04	0.02	0.01	0.01	0.01	0.03	0.04
Cage wash	0.89	1.30	1.30	1.96	0.82	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

bw: body weight; IV: intravenous

[a] Total recovery is the mean of individual animal data.

Source: Ridley & Mirly (1988)

In a study of absorption, distribution and excretion, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material = 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw. A group of five male and five female rats was administered unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by [^{14}C]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. The rats were terminated after 90% of the dose had been eliminated or 7 days after dosing, whichever was sooner. At necropsy, a blood sample was drawn and selected tissues removed.

Following administration of the single intravenous dose of 30 mg/kg, more than 84% of the radioactivity was eliminated in urine, mostly within 8 hours. Faecal elimination accounted for less than 3.5% of the administered radioactivity and only a very small proportion was eliminated in exhaled air; less than 1.4% remained in tissues and the residual carcass after termination. In contrast, faeces were the major route of elimination when [^{14}C]glyphosate was administered orally. Approximately 56–59% of the oral dose of 30 mg/kg was excreted in faeces, mostly within 12–36 hours. Urinary elimination of the oral dose was slower than for the intravenous dose, with 29–31% eliminated, mostly within 36 hours of dosing. Excretion was unaffected by administering unlabelled glyphosate for 14 days prior to dosing with [^{14}C]glyphosate, and the routes and rates of excretion of a high dose of [^{14}C]glyphosate (1000 mg/kg) were essentially identical to that of the low dose. There was no significant sex difference in the elimination of glyphosate for any dose regimen. Irrespective of the dose, route or frequency of duration, less than 1.4% of a dose was retained in tissues. The highest concentration of radioactivity was in bone and lower concentrations were in bone marrow, kidneys, liver, lungs and the residual carcass (Powles, 1992b).

In a study of absorption, distribution, excretion and metabolism, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material 98.9%; radiochemical purity > 98%) as a single dose at 10 or 600 mg/kg bw by gavage in water. For the excretion study, urine and faeces (5/sex) were collected at selected intervals for 168 hours. Animals were terminated at 168 hours post dosing and the radioactivity in blood and selected tissues analysed. For the plasma concentration study, blood samples (total nine per sex per dose) were drawn at selected intervals up to 168 hours. For the tissue distribution study, 12 rats (six male, six female) were administered single oral doses of either 10 or 600 mg/kg bw per day by gavage. The animals were divided into two groups of six (three per sex) and terminated by cervical dislocation 6 and 18 hours (the low-dose study) or 3 and 9 hours (for the high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC).

During the 7-day observation period, up to about 23% and 30% of the radioactivity of the low dose was excreted in the urine of low- and high-dose animals, respectively. At both doses, about three quarters of the radioactivity was detected in the faeces within 7 days (75% for males and 84% for females, 10 mg/kg bw; 75% and 74%, 600 mg/kg bw; Table 4) (McEwen, 1995).

Table 4. Radioactivity in rat excreta and tissue over 168 hours after a single dose of ^{14}C-labelled glyphosate

	Percentage of administered radioactive dose (%)			
	10 mg/kg bw		**600 mg/kg bw**	
Excretion intervals (h)	**Males**	**Females**	**Males**	**Females**
Urine				
0–6	2.63	3.25	11.55	9.08

	Percentage of administered radioactive dose (%)			
	10 mg/kg bw		600 mg/kg bw	
Excretion intervals (h)	**Males**	**Females**	**Males**	**Females**
6–24	15.85	12.69	13.85	13.36
24–48	2.82	2.41	2.33	4.40
48–72	0.54	0.44	0.59	1.07
72–96	0.24	0.19	0.30	0.40
96–120	0.15	0.13	0.21	0.24
120–144	0.09	0.07	0.17	0.17
144–168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal (urine plus cage wash)	22.51	19.37	30.26	29.50
Faeces				
0–24	60.28	74.59	58.94	46.28
24–48	11.72	7.56	13.41	22.87
48–72	1.18	1.34	1.36	3.83
72–96	0.29	0.36	0.35	0.47
96–120	0.17	0.27	0.36	0.23
120–144	0.35	0.08	0.08	0.12
144–168	0.64	0.10	0.15	0.35
Subtotal faeces	74.63	84.30	74.65	74.15
Residual carcass	0.33	0.27	0.31	0.39
Total	97.47	103.94	105.22	104.04

bw: body weight

Source: McEwen (1995)

After a single dose of 10 mg/kg bw, peak mean concentrations of radioactivity in plasma occurred at 6 and 2 hours in males (0.22 µg eq/mL) and females (0.28 µg eq/mL) (Table 5). After a single oral dose of 600 mg/kg bw, peak mean concentrations of radioactivity in plasma occurred at 3 hours in both males (26 µg eq/mL) and females (29 µg eq/mL). The area under the concentration versus time–curve (AUC_t) was calculated at 400 and 355 µg eq/mL*hour in males and females, respectively. These values were around 120 times higher than the AUCt obtained in the low-dose group.

Table 5. Pharmacokinetic parameters of total rat plasma radioactivity following single oral doses of ^{14}C-labelled glyphosate

	Measures per administered dose			
	10 mg/kg bw		600 mg/kg bw	
Parameter	**Males**	**Females**	**Males**	**Females**
C_{max} (µg eq/mL)	0.2219	0.2789	25.97	28.84
T_{max} (hour)	6.00	2.00	3.00	3.00
AUC_t (µg eq/mL*hour)	3.20	3.70	399.90	355.30
AUC (µg eq/mL*hour)	3.80	4.20	419.00	–[a]

Terminal rate constant (per hour)	0.0840	0.0887	0.1174	–[a]
Terminal half-life (hour)	8.30	7.80	5.90	–[a]
Absorption rate constant (per hour)	0.2963	0.4239	0.2845	0.4477

AUC: area under the plasma concentration–time curve; AUC_t: area under the curve calculated up to the last detectable sample (calculations done up to 24 hours); bw: body weight; C_{max}: maximum concentration; eq: equivalent; T_{max}: time to reach the maximum concentration

[a] Could not be calculated accurately as the values were at or close to the limit of reliable measurement.

Source: McEwen (1995)

There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract, the stomach, muscles and the kidneys, the organs of excretion contained concentrations of radioactivity higher than the plasma (Table 6). High levels of radioactivity were detected in the content of stomach and the gastrointestinal tract. The radioactivity in most tissues had decreased to around the limit of detection 7 days after dosing.

Table 6. Radioactivity in male and female rat tissue over 168 hours after a single oral dose of 10 mg/kg bw ^{14}C-labelled glyphosate

	Proportion of administered dose over time (%)					
	Male[a]			**Females[a]**		
Tissue	**6 hours**	**18 hours**	**168 hours**	**6 hours**	**18 hours**	**168 hours**
Bone[b]	0.12	0.10	0.02	0.10	0.09	0.03
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
Gastrointestinal tract contents	31.56	4.89	0.01	34.54	14.30	0.01
Kidneys	0.79	0.36	< 0.01	0.67	0.26	< 0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	< 0.03
Stomach	3.47	0.60	0.60	2.56	0.62	< 0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Plasma	0.12	0.03	< 0.01	0.13	0.03	< 0.01
Whole blood	0.20	0.04	< 0.03	0.15	0.05	< 0.03

bw: body weight

Results expressed as mean percentage (%) of applied dose, except bone, which is expressed as percentage (%) of applied dose/g.

[a] $N = 5$

[b] $n = 3$

Source: McEwen (1995)

A major component of urine or the [14C]phosphonomethyl-labelled glyphosate-treated animals was unchanged glyphosate, accounting for 18–27% of both the administered doses. A minor component, accounting for 0.1–0.3% of the administered dose, was shown to co-chromatograph (using normal phase TLC and reverse phase HPLC) with aminomethylphosphonic acid.

Unchanged glyphosate was the major component of the faecal extract of the [^{14}C]phosphonomethyl-labelled glyphosate-treated animals, accounting for 65–78% of both the administered doses. Two minor metabolites accounted for 0.3–1.6% of the administered dose; one of these was shown to co-chromatograph with aminomethylphosphonic acid (McEwen, 1995).

In a study of absorption, distribution, excretion and metabolism, groups of five male and five female Sprague Dawley (Crl:CD BR) rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (two batches of unlabelled test material, purity 95.3% and 96.0%; radiochemical purity > 99%) as a single gavage dose of 1 or 100 mg/kg bw in water. For the excretion study, urine and faeces (5/sex per dose) were collected at selected times for 168 hours and samples pooled and analysed directly by TLC or HPLC. At 168 hours, the animals were terminated and radioactivity in blood and selected tissues analysed. For the pharmacokinetic study, blood was drawn (5/sex per dose) at selected intervals up to 72 hours after dosing. For the tissue distribution study, 12 male and 12 female rats were administered a single daily gavage dose of either 10 or 100 mg/kg bw. The treated animals were divided into four groups (three per sex) and terminated at 4, 12, 24 and 72 hours after dosing. For the biliary excretion study, seven male and seven female cannulated rats were administered a single gavage dose of 1 mg/kg bw. Urine, faeces and bile were collected periodically up to 48 hours after dosing.

Following a single gavage dose of 1 mg/kg bw, the major route of elimination was the faeces with 72.62% recovered in males and 62.40% in females, mostly within 24 hours of dosing (63.93% in males and 49.69% in females), suggesting this proportion of the dose was not systemically absorbed. During the 7-day observation period, 18.44% (male) and 27.15% (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose. The remainder of the radioactivity was recovered in the cage wash (6.48% in males and 7.71% in females), cage debris (0.03% in males and 0.58% in females) and carcass (0.75% in males and 0.98% in females).

Following the single gavage dose of 100 mg/kg bw, elimination of radioactivity in the urine (39.42% in males and 43.07% in females) was quantitatively more significant than in to the low-dose group. Faecal elimination accounted for 41.23% in males and 42.37% in females. The remainder of the radioactivity was recovered in the cage wash (13.85% in males and 11.96% in females), cage debris (0.98% in male and 0.10% in female) and carcass (0.84% in male and 0.98% in female). Renal elimination was essentially complete in 48 hours.

In the cannulated rats dosed with 1 mg/kg bw by gavage, the majority of the administered dose was recovered in faeces (55.33% in male and 60.97% in female) in 48 hours. Renal elimination accounted for 27.45% in males and 24.21% in females. The remainder of the radioactivity was recovered in the cage wash (6.57% in male and 6.77% in female), cage debris (0.26% in male and 0.15% in female) and carcass (4.99% in male and 3.82% in female).

The mean terminal elimination half-lives were 10.86 hours and 8.07 hours with corresponding area under the plasma concentration–time curve (AUC) of 0.319 and 0.340 µg eq/mL*hour in males and females, respectively (Table 7). As the elimination half-lives could not be calculated for several high-dose animals, mean $AUC_{0–24}$ (0.257 and 0.338 µg eq/mL*hour in males and females) were calculated to compare the results of both groups. Following a single oral dose of 100 mg/kg bw, mean $AUC_{0–24}$ were 58.2 and 50.7 µg eq/mL*hour in males and females, respectively.

Table 7. Kinetic parameters in male and female rat plasma after a single oral dose of ^{14}C-labelled glyphosate

	Measures per administered dose			
	1 mg/kg bw		100 mg/kg bw	
Kinetic parameters	**Males**	**Females**	**Males**	**Females**
C_{max} (µg eq/mL)	0.016	0.037	8.909	7.634
T_{max} (hour)	3.900	8.000	3.600	4.000
$AUC_{0–24}$ (µg eq/mL*hour)	0.257	0.338	58.200	50.700
AUC (µg eq/mL*hour)	0.319	0.340	–	–

Terminal half-life (hour)	10.860	8.065	–	–

AUC: area under the plasma concentration–time curve; AUC_{0-24}: area under the plasma concentration–time curve from time 0 to 24 hours; bw: body weight; C_{max}: maximum concentration; eq: equivalent; T_{max}: time to reach the maximum concentration

Source: Knowles & Mookherjee (1996)

At 1 mg/kg bw, radioactivity was detected in all tissues 4 hours post dose, indicating rapid absorption and distribution in the body. Apart from the gastrointestinal tract (and contents) and the carcass, the kidneys was the only tissue with any notable amounts throughout the observation period. At 72 hours, post-dose concentrations had decreased or plateaued to less than 2% of the administered dose in all tissues in both males and females, with the carcass containing most of the remaining radioactivity. At 100 mg/kg bw, all the tissues were exposed to radiolabelled material 4 hours post dose. Again, only the gastrointestinal tract, carcass and kidneys contained significant amounts of radioactivity. After 72 hours, concentrations had decreased or plateaued to less than 2% of the dose in all tissues in both sex, with the carcass containing most of the remaining radioactivity.

In conclusion, following oral administration of glyphosate at 1 mg/kg bw and 100 mg/kg bw, the absorption, distribution, metabolism and excretion was independent of dose level and sex. Metabolism of glyphosate was very low with more than 90% of the administered dose eliminated unchanged in the urine and faeces. Elimination was essentially completed by 48 hours, and the majority of the radioactivity was recovered in faeces (Knowles & Mookherjee, 1996).

Rabbits

In a pre-GLP study, glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon and at the C2-glycine carbon was dissolved in isotonic saline and administered by gavage to male New Zealand White rabbits fasted for 3 hours. In two replicate experiments, three rabbits were administered ^{14}C-methylene glyphosate, two were administered ^{14}C-C1-glycine glyphosate and two were administered ^{14}C-C2-glycine glyphosate. All the doses were within a range of 5.7–8.8 mg/kg bw.

Approximately, 80–97% of the oral dose of [^{14}C]glyphosate was excreted in the faeces and 7–11% in the urine over 120 hours. Less than 1% of the dose was exhaled. Approximately 1.2%, 0.7% and 0.1% of the dose was retained in the tissues (excluding gastrointestinal tract contents) for ^{14}C-C2-glycine, ^{14}C-C1-glycine and ^{14}C-methylene glyphosate, respectively. The radioactivity in the tissues differed between ^{14}C-C2-glycine and ^{14}C-C1-glycine by 4 or 5 times, but the ranking was similar: the liver had the highest concentrations followed by the kidney, the spleen, the heart, skeletal muscle and gonads, in that order. Only ^{14}C-C2-glycine radioactivity was incorporated in the fat (Colvin & Miller, 1973c).

(b) Intraperitoneal route

In the previously described Colvin & Miller (1973a) study, three treatment groups each with three male Wistar rats were dosed via intraperitoneal injection with ^{14}C-methylene glyphosate (2.33 mg/kg bw), ^{14}C-C1-glycine glyphosate (2.91 mg/kg bw) and ^{14}C-C2-glycine glyphosate (3.63 mg/kg bw). Within 12 hours, 74–78% of the ^{14}C-glyphosate was excreted in the urine. At 96 hours post-administration, total urinary excretion was 81–90% of the administered radioactivity and faecal excretion was 6–14% of the administered radioactivity, indicating that [^{14}C]glyphosate is also eliminated via the bile. The percentage of radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that observed following oral administration (Section 1.1 (a)), but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater than after oral administration, but was in all cases less than or equal or 1% of the administered dose (Colvin & Miller, 1973a).

Table 8. Quantification of glyphosate metabolites as percentages of single doses of ^{14}C-labelled glyphosate administered orally to rats

		Percentage of administered dose (%)					
		Low-dose study 10 mg/kg bw		Repeat dose study[a] 10 mg/kg bw		High-dose study 1000 mg/kg bw	
Sample	**Analyte**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Urine	Glyphosate	12.7	10.5	10.5	10.5	16.0	16.7
	AMPA	0.2	0.1	< 0.1	< 0.1	0.6	0.7
Faeces	Glyphosate	74.8	55.2	52.9	72.1	79.3	63.9
Total	Glyphosate	87.5	65.7	63.3	82.6	95.3	80.6
	AMPA	0.2	0.1	< 0.1	< 0.1	0.6	0.7

AMPA: aminomethylphosphonic acid; bw: body weight

[a] Following 14 repeated oral doses of 10 mg/kg bw unlabelled glyphosate.

Source: Macpherson, 1996

Urine and faeces samples from the previously described Knowles & Mookherjee (1996) study (Section 1.1 (a)) were analysed for identification of glyphosate metabolites. Briefly, five female Sprague Dawley (Crl:CD BR) rats were administered [^{14}C]phosphonomethyl-labelled glyphosate as a single dose at 1 or 100 mg/kg bw by gavage in water. For the excretion study, urine and faeces (5/sex per dose) were collected at selected times for 168 hours.

Metabolite profiles of pooled urine and faecal samples were investigated by HPLC. Only one major peak was detected in urine and faeces (> 90% of the total activity); this was subsequently identified as glyphosate. A minor component observed in the radiochromatograms had a similar retention time to AMPA; however, it could not be positively identified due to very low levels (Knowles & Mookherjee, 1996).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies of glyphosate (including skin and eye irritation and dermal sensitization studies) are summarized in Table 9.

Table 9. Summary of acute toxicity studies with glyphosate

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Oral					
Mouse	ICR	M + F	96.7	> 10 000	Shirasu & Takahashi (1975)
Mouse	NMRI	M + F	98.6	> 2 000	Dideriksen (1991)
Mouse	ICR(Crj:CD-1)	M + F	95.68	> 5 000 (M) > 5 000 (F) > 5 000 (combined)	Komura (1995a)
Mouse	ICR(Crj:CD-1)	M + F	62.34% glyphosate isopropylamine salt	> 5 000	Enami & Nakamura (1995)
Rat	Sprague Dawley	F	96.40 & 96.71	> 5 000	Komura (1995b)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rat	HanRcc: WIST	F	96.66	> 2 000	Simon (2009a)
Rat	CD/Crl:CD(SD)	F	97.52	> 2 000	Haferkorn (2009a)
Rat	Sprague Dawley	F	96.40 & 96.71	> 5 000	You (2009a)
Rat	CD/Crl:CD(SD)	F	95.23	> 2 000	Haferkorn (2010a)
Rat	CD/Crl:CD(SD)	F	97.3	> 2 000	Haferkorn (2010b)
Rat	Sprague Dawley derived	F	97.23	> 5 000	Merkel (2005a)
Rat	Wistar Hannover	F	98.05	> 2 000	Do Amaral Guimaraes (2008a), with addendum dated 2010
Rat	HanRcc: WIST(SPF)	F	95.1	> 2 000	Talvioja (2007a)
Rat	Sprague Dawley	M + F	97.76	> 5 000 (M) > 5 000 (F) > 5 000 (combined)	Reagan & Laveglia (1988a)
Rat	Wistar	M + F	99	5 600 (combined)	Heenehan, Rinehart & Braun (1979)
Rat	Sprague Dawley	M + F	85.5	> 5 000	Blaszcak (1988a)
Rat	Sprague Dawley	M + F	98.6	> 5 000	Cuthbert & Jackson (1989a)
Rat	Alpk:AP_fSD (Wistar derived)	M + F	95.6	> 5 000 (male) > 5 000 (female) > 5 000 (combined)	Doyle (1996a)
Rat	HanRcc:WIST(SPF)	F	96.1	> 5 000	Arcelin (2007a)
Rat	RjHan:WI	F	96.3	> 5 000	Tavaszi (2011a)
Rat	Wistar	M + F	99	5 600	Heenehan (1979a)
Rat	Sprague Dawley derived	M + F	62% glyphosate isopropylamine salt	> 5 000	Moore (1999)
Acute dermal					
Rat	Sprague Dawley	M + F	Not reported	> 2 000	Cuthbert & Jackson (1989b)
Rat	Sprague Dawley	M + F	96.40 & 96.71	> 5 050	You (2009b)
Rat	SD(Crj:CD)	M + F	95.68	> 2 000	Komura (1995c)
Rat	HanRcc: WIST(SPF)	M + F	96.66	> 2 000	Simon (2009b)
Rat	CD/Crl:CD(SD)	M + F	97.52	> 2 000	Haferkorn (2009b)
Rat	CD/Crl:CD(SD)	M + F	95.23	> 2 000	Haferkorn (2010c)
Rat	CD/Crl:CD(SD)	M + F	96.6	> 2 000	Haferkorn (2010d)
Rat	Sprague Dawley	M + F	97.23	> 5 000	Merkel (2005b)
Rat	Wistar Hannover	M + F	98.05	> 2 000	Do Amaral Guimaraes (2008b)
Rat	HanRcc: WIST(SPF)	M + F	95.1	> 2 000	Talvioja (2007b)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rat	Alpk:AP_fSD (Wistar derived)	M + F	95.6	> 2 000	Doyle (1996b)
Rat	HanRcc: WIST(SPF)	M + F	96.1	> 5 000	Arcelin (2007b)
Rat	RjHan (WI) Wistar	M + F	96.3	> 5 000	Zelenak (2011a)
Rabbit	New Zealand White	M + F	85.5	> 5 000	Blaszcak (1988b)
Rabbit	New Zealand White	M + F	97.76	> 5 000	Reagan (1988a)
Rabbit	New Zealand White	M + F	99	> 5 000	Heenehan (1979b)
Inhalation (nose only)					
Rat	CD/Crl:CD(SD)	M + F	96.6	> 5.18	Haferkorn (2010e)
Rat	F344/DuCrj(SPF)	M + F	97.56	> 5.48	Koichi (1995)
Rat	HsdRcc Han	M + F	96.66	> 5.04	Griffiths (2009)
Rat	CD/Crl:CD(SD)	M + F	97.52	> 5.12	Haferkorn (2009c)
Rat	CD/Crl:CD(SD)	M + F	95.23	> 5.02	Haferkorn (2010f)
Rat	Sprague Dawley	M + F	96.40 & 96.71	> 2.24	Carter (2009)
Rat	Sprague Dawley	M + F	97.23	> 2.04	Merkel (2005c)
Rat	Not reported	M + F	98.05	> 5.21	Dallago (2008)
Rat	HanRcc: WIST(SPF)	M + F	95.1	> 3.252	Decker (2007)
Rat	Alpk:AP_fSD (Wistar derived)	M + F	95.6	> 4.43	Rattray (1996)
Rat	Wistar RjHan (WI)	M + F	96.9	> 5.04	Nagy (2011)
Rat	Sprague Dawley	M + F	62% glyphosate isopropylamine	> 2.08	Wnorowski (1999)
Rat	Hsd:Sprague Dawley	M + F	47.2% glyphosate acid equivalent	> 5.27	Bonnette (2004)
Primary dermal irritation					
Rabbit	New Zealand White	M + F	95.1	Non-irritating	Talvioja (2007c)
Rabbit	Himalayan	M	95.23	Non-irritating	Leuschner (2009a)
Rabbit	New Zealand White	F	97.56	Non-irritating	Hideo (1995a)
Rabbit	Himalayan	M	97.52	Non-irritating	Leuschner (2009c)
Rabbit	Himalayan	M	96.6	Non-irritating	Leuschner (2010a)
Rabbit	New Zealand White	M + F	96.71	Non-irritating	You (2009c)
Rabbit	New Zealand White	M	97.23	Non-irritating	Merkel (2005d)
Rabbit	New Zealand White	F	98.05	Non-irritating	Canabrava Frossard de Faria (2008a)
Rabbit	New Zealand White	M + F	97.76	Non-irritating	Reagan & Laveglia (1988b)
Rabbit	New Zealand White	M + F	99	Slightly irritating	Heenehan (1979c)
Rabbit	New Zealand White	F	95.6	Non-irritating	Doyle (1996c)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rabbit	New Zealand White	M + F	96.1	Non-irritating	Arcelin (2007c)
Rabbit	New Zealand White	M	96.3	Mildly irritating	Zelenak (2011b)
Rabbit	New Zealand White	M + F	85.5	Slightly irritating	Blaszcak (1988c)
Eye irritation					
Rabbit	New Zealand White	M + F	95.1	Mildly irritating	Talvioja (2007d)
Rabbit	Himalayan	M	95.23	Moderately irritating	Leuschner (2009b)
Rabbit	New Zealand White	F	97.56	Severely irritating	Hideo (1995b)
None	n/a	–	Not stated	pH of a 1% solution in water was 1.93. Not tested because pH < 2 indicates corrosive properties	Simon (2009c) [a]
Rabbit	Himalayan	M	97.52	Mildly irritating	Leuschner (2009d)
Rabbit	Himalayan	M	96.6	Mildly irritating	Leuschner (2010b)
Rabbit	New Zealand White	M + F	96.40 & 96.71	Moderately irritating	You (2009d)
Rabbit	New Zealand White	M	97.23	Moderately irritating	Merkel (2005e)
Rabbit	New Zealand White	M + F	98.05	Severely irritating	Canabrava Frossard de Faria (2008b)
Rabbit	New Zealand White	Not reported	97.76	Severely irritating	Reagan & Laveglia (1988c)
Rabbit	New Zealand White	F	95.6	Mildly irritating	Johnson (1997)
Rabbit	New Zealand White	M + F	96.1	Mildly irritating	Arcelin (2007d)
Rabbit	New Zealand White	M	96.3	Severely irritating	Tavaszi (2011b)
Rabbit	New Zealand White	M + F	85.5	Moderately irritating	Blaszcak (1988d)
Rabbit	New Zealand White	M + F	46.6	Non-irritating	Blaszcak (1998e)
Rabbit	New Zealand White	M + F	57.8% glyphosate potassium (47.13% glyphosate acid equivalent)	Mildly irritating	Bonnette (2001)
Rabbit	New Zealand White	M + F	Not reported (MON 0139)	Non-irritating	Branch (1981)
Rabbit	New Zealand White	Not specified	90.8% (MON 8722)	Mildly irritating.	Busch (1987a)
Rabbit	New Zealand White	Not specified	70.7% (MON 8750)	Mildly irritating	Busch (1987b)
Rabbit	New Zealand White	Not specified	99	Moderately irritating	Heenehan (1979d)
Rabbit	New Zealand White	Not specified	97.76	Severely irritating	Reagan (1988b)

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

[a] According to Simon (2009c): "A 1% w/w solution of glyphosate technical in purified water was found to have a pH of 1.93. According to Council Regulation (EC) No. 440/2008, B.5. and OECD Guidelines 405, a test item is not required to be tested if the pH value is less than 2, because it is assumed that the test item has corrosive properties… Therefore, no eye irritation with glyphosate technical will be performed"

(f) Dermal sensitization

Results of studies of skin sensitization with glyphosate are shown in Table 10.

Table 10. Results of skin sensitization studies with glyphosate

Species	Strain	Sex	Route	Purity (%)	Results	Reference
Mouse	CBA/Ca	F	LLNA	96.1	Negative	Betts (2007)
Mouse	CBA/J Rj	F	LLNA	96.3	Negative	Török-Bathó (2011)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	95.1	Negative	Talvioja (2007e)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	97.52	Negative	Haferkorn (2009d)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	Two analyses: 95.23 & 96.4	Negative	Haferkorn (2010g)
Guinea pig	Hartley	F	Magnusson–Kligman Maximization	97.56	Negative	Hideo (1995c)
Guinea Pig	Hartley	M	Magnusson–Kligman Maximization	96.66	Negative	Simon (2009d)
Guinea pig	Dunkin Hartley	M	Magnusson–Kligman Maximization	Two analyses: 97.52 & 98.8	Negative	Haferkorn (2010h)
Guinea pig	Short-haired Hartley albino	M + F	Buehler	Two analyses: 96.4 & 95.71	Negative	You (2009e)
Guinea pig	Hartley albino	M + F	Buehler	97.23	Negative	Merkel (2005f)
Guinea pig	Hartley	M	Buehler	98.05	Negative	Lima Dallago (2008)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	95.7	Negative	Richeux (2006)
Guinea pig	Albino Crl (HA) BR	F	Magnusson–Kligman Maximization	95.6	Negative	Doyle (1996d)
Mouse	CBA/Ca	F	LLNA	96.1	Negative	Betts (2007)
Mouse	CBA/J Rj	F	LLNA	96.3	Negative	Török-Bathó (2011)

F: female; LLNA: local lymph node assay; M: male

Mouse

In a local lymph node assay, about 25 µL of a 10, 25 or 45% w/v preparation of glyphosate technical (96.1% glyphosate acid) in dimethyl sulfoxide (DMSO) was applied to the dorsal surface of each ear of groups of four female CBA/Ca mice. A vehicle control group was similarly treated with DMSO alone. The procedure was repeated daily for 3 consecutive days.

Three days after the third application, all the animals were injected in the tail vein with about 250 µL of phosphate buffered saline containing 20 µCurie (µCi; 74×10^{10} Bq) [methyl-^{3}H]thymidine. The mice were terminated after about 5 hours. The drained auricular lymph nodes were removed from

kidneys, liver and lungs of animals at the lowest (200 mg/kg) and intermediate (1000 mg/kg) doses underwent a full histopathological examination.

No treatment-related mortalities, clinical signs, haematological or biochemical findings and no organ-weight changes were observed. Gross or histopathological examination did not show any effects of glyphosate administration.

Taking into account the limited range of clinical chemistry parameters evaluated, the NOAEL in the 13-week toxicity study in mice was 4500 mg/kg bw per day, the highest dose tested in this study (Perry et al., 1991a).

In a 13-week oral toxicity study, groups of 10 male and 10 female B6C3F1 mice were fed diets containing glyphosate (purity 99%) at concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm (equal to 0, 507, 1065, 2273, 4776 and 10 780 mg/kg bw per day for males and 0, 753, 1411, 2707, 5846 and 11 977 mg/kg bw per day for females). All tissues from the highest-dose and control animals were examined microscopically. The 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 both males and females. There were no differences in feed 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 11). 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 and had fewer ducts. No histological changes were observed in the submandibular and sublingual glands.

Table 11. Incidence and severity of cytoplasmic alteration of the parotid and submandibular salivary glands (combined) in mice administered glyphosate for 13 weeks

	No. of cases per dietary concentration of glyphosate					
	0 ppm	**3 125 ppm**	**6 250 ppm**	**12 500 ppm**	**25 000 ppm**	**50 000 ppm**
Males	0/10	010	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)

no.: number; ppm: parts per million

Results presented as number of mice showing cytoplasmic alterations / total number of mice in the group, with average severity score in parentheses. Severity score is based on a scale of 1 = minimal, 2 = mild, 3 = moderate or 4 = marked.

Source: Chan & Mahler (1992)

The NOAEL in the 13-week toxicity study in mice was 3125 ppm (equal to 507 mg/kg bw per day) based on parotid salivary gland lesions at 6250 ppm (equal to 1065 mg/kg bw per day) (Chan & Mahler, 1992).

In a 13-week oral toxicity study, groups of 12 male and 12 female ICR(Crj:CD-1)SPF mice were administered glyphosate (purity 97.56%) at dietary concentrations of 0, 5000, 10 000 or 50 000 ppm (equal to a mean daily glyphosate intake of 0, 600, 1221 and 6295 mg/kg bw per day for males and 0, 765, 1486 and 7435 mg/kg bw per day for females).

There were no treatment-related clinical signs, mortality or ophthalmological and haematological findings. At 50 000 ppm, mean body weights of the males were 91% that of the controls from week 2 to the end of the treatment; body weights of females were comparable to that of the controls. Similarly, feed consumption was slightly decreased in males at the highest dose. At

50 000 ppm, feed efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment.

At 50 000 ppm, females showed a significant treatment-related increase in creatine phosphokinase ($P < 0.01$). Other statistically significant ($P < 0.01$) changes in clinical chemistry were observed in high-dose male and female mice; however, these changes were minor and not associated with any histological findings and not considered adverse. In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

At 50 000 ppm, males and females showed significant ($P < 0.01$) increases in both absolute and relative caecum weights (238% and 263%, respectively, for males, and 187% and 195%, respectively, for females) (Table 12).

Table 12. Caecum weights of mice administered glyphosate for mice 13 weeks

	Absolute and relative weight per dietary concentration of glyphosate			
	0 ppm	**5 000 ppm**	**10 000 ppm**	**50 000 ppm**
Males				
Absolute weight ± SD (mg) [a]	624 ± 86	609 ± 116	718 ± 177	1 484 ± 359
Relative weight ± SD (%)	1.45 ± 0.19	1.38 ± 0.26	1.61 ± 0.33	3.82 ± 1.15**
Females				
Absolute weight ± SD (mg) [a]	497 ± 96	474 ± 115	604 ± 123	958 ± 163**
Relative weight ± SD (%)	1.43 ± 0.26	1.37 ± 0.30	1.67 ± 0.42	2.79 ± 0.53**

ppm: parts per million; SD: standard deviation; **: $P < 0.01$

Relative weight expressed as (organ weight / body weight) × 100.

[a] At 50 000 ppm, both males and females showed significant increases in absolute weights (238% for males and 187% females).

Source: Kuwahara (1995)

At 50 000 ppm, males and females showed a significant increase in incidence of distension of the caecum (12/12 males and 10/12 females, in contrast to none in the control group). In addition, at this dose males showed significant increases in incidence of cystitis (4/12 compared to none in the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distension of the caecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the caecum.

The NOAEL in the 13-week toxicity study in mice was 10 000 ppm (equal to 1221 mg/kg bw per day) based on the decrease in body weights in males, increase in absolute and relative caecum weights in both sexes and increased incidence of distension of the caecum in both sexes at 50 000 ppm (equal to 6295 mg/kg bw per day) (Kuwahara, 1995).

Rats

In a 4-week range-finding study of oral toxicity, groups of five male and five female Sprague Dawley rats were fed diets containing glyphosate (purity 97.7%) at concentrations of 0, 30 000, 40 000 or 50 000 ppm (equivalent to approximately 1500, 2000 and 2500 mg/kg bw per day).

No animals died during 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. Slightly reduced body-weight gains were noted in both sexes at all the 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 feed

consumption was reduced for males at the intermediate and highest dose during the first week of the study. Feed 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 the frequent occurrence of soft stools and/or diarrhoea at all doses, no NOAEL could be derived from this 4-week dietary toxicity study in rats (Reyna & Thake, 1989).

In a 4-week oral toxicity study, 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. All the animals were terminated and necropsied, and the livers, hearts, kidneys, spleens and adrenals of control and highest-dose animals processed and examined histopathologically. Examination was subsequently extended to include the kidneys from all females in all the groups.

Soft faeces were noted in three males in the highest-dose group during weeks 3 to 4, but not in any other group. No treatment-related effects were observed on mortality, clinical signs of toxicity, body weights, feed and water consumption or haematological parameters. In males, equivocal increases in plasma alanine transaminase [alanine aminotransferase] and alkaline phosphatase activities were observed at 250, 1000 or 2500 mg/kg bw. In females, plasma alanine transaminase activity was significantly increased at the highest dose, as was total bilirubin. In addition, increased plasma concentrations of phosphate were noted in males at 1000 or 2500 mg/kg bw. 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 in female rats at 250 mg/kg bw and higher doses (Table 13).

Table 13. Nephrocalcinosis in rats administered glyphosate for 4 weeks

	No. per dietary concentration of glyphosate									
	Males					**Females**				
	0 mg/kg bw per day	**50 mg/kg bw per day**	**250 mg/kg bw per day**	**1 000 mg/kg bw per day**	**2 500 mg/kg bw per day**	**0 mg/kg bw per day**	**50 mg/kg bw per day**	**250 mg/kg bw per day**	**1 000 mg/kg bw per day**	**2 500 mg/kg bw per day**
No. of cases	0	NI	NI	NI	NI	0	0	2	2	4
No. of very mild/minimal cases	0	NI	NI	NI	NI	0	0	1	1	2
No. of mild/slight cases	0	NI	NI	NI	NI	0	0	1	1	2

bw: body weight; NI: not investigated; no.: number

Source: Atkinson et al. (1989)

The NOAEL in the 4-week dietary toxicity study in rats was 50 mg/kg bw per day for slight nephrocalcinosis in female rats at 250 mg/kg bw per day (Atkinson et al., 1989). This finding was not confirmed in a separate study by Perry et al., 1991b.

In a 90-day oral toxicity study, groups of 12 male and 12 female Sprague Dawley rats were fed diets containing glyphosate (purity 95.2%) at concentrations of 0, 1000, 5000 or 20 000 ppm (calculated mean intakes equal to 0, 63, 317 and 1267 mg/kg bw per day for males and 0, 84, 404 and 1623 mg/kg bw per day for females). Clinical signs, body weight, feed consumption, haematology

and clinical chemistry parameters were monitored routinely. Gross examinations were performed for all groups, and the kidneys, liver and testes weighed after termination. A standard range of tissues from control and highest-dose animals was microscopically examined as well as the kidneys, livers and lungs from animals at all 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 in the 90-day dietary toxicity study in rats was 20 000 ppm (equal to 1267 mg/kg bw per day), the highest dose tested (Stout & Johnson, 1987).

In a 13-week oral toxicity study, groups of 10 male and 10 female Sprague Dawley rats were fed diets containing glyphosate (purity 98.6%) at concentrations that were adjusted weekly to doses of 0, 30, 300 or 1000 mg/kg bw per day. All tissues from control and highest-dose animals, in addition to the kidneys, liver, lungs and parotid salivary glands of all the test animals, underwent a full histopathological examination.

There were no mortalities, clinical signs or changes in body or organ weights, feed and water consumption, haematological parameters and ophthalmoscopic and macroscopic findings. Females at the highest dose showed slight but statistically significant increases in concentrations of glucose (11%; $P < 0.05$), total protein (9%; $P < 0.001$), albumin (9%; $P < 0.05$) and creatinine (8%; $P < 0.01$) compared with those in the control group. Urinalysis revealed a reduction in pH in males at the highest dose.

In contrast to results from a 4-week study in rats conducted at the same testing facility (Atkinson et al., 1989), the incidence of nephrocalcinosis in this 13-week study was evenly distributed in dose groups and sexes and was not dose dependant; it is therefore clearly not treatment related.

An increase in the incidence of cellular alterations (deep basophilic staining and enlargement of cytoplasm) was observed 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 only reached statistical significance in males at the highest dose (Table 14), suggesting these changes are of equivocal toxicological significance.

Table 14. Cytoplasmic alteration of the parotid salivary gland in rats administered glyphosate in the diet for 13 weeks

	No. per dietary concentration of glyphosate							
	Males				**Females**			
	0 mg/kg bw per day	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Severity [a]								
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**

bw: body weight; no.: number; *: $P < 0.05$; **: $P < 0.01$

[a] Severity graded as very mild, mild, moderate, severe and very severe.

Source: Perry et al. (1991b)

The NOAEL in this 90-day toxicity study in rats was 300 mg/kg bw per day based on the more pronounced severity of cellular alterations in the parotid salivary gland at 1000 mg/kg bw per day (Perry et al., 1991b).

In a 13-week oral toxicity study, groups of 10 male and 10 female F344/N rats were fed diets containing glyphosate (purity 99%) at concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm. Ten more animals of each sex were included at each dietary concentration for evaluation of haematological and clinical pathology parameters. The calculated mean intakes were equal to 0, 205, 410, 811, 1678 and 3393 mg/kg bw per day, respectively, for males and 0, 213, 421, 844, 1690 and 3393 mg/kg bw per day, respectively, for females. All tissues from the control and highest-dose animals were examined microscopically. Salivary glands were also examined for the animals at all lower doses.

Diarrhoea was seen in males at the highest dose and in all 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. Small increases in several erythrocyte parameters were noted in males at 12 500 ppm and higher doses. These changes were unremarkable and generally consistent with a mild dehydration. Plasma alkaline phosphatase and alanine transaminase 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 not toxicologically significant.

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 both 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, suggesting that any changes are of equivocal toxicological significance.

Table 15. Cytoplasmic alterations of the parotid and submandibular salivary glands (combined) in rats administered glyphosate for 13 weeks

	Incidence per dietary concentration of glyphosate					
	0 ppm	**3 125 ppm**	**6 250 ppm**	**12 500 ppm**	**25 000 ppm**	**50 000 ppm**
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 (1.0)

ppm: parts per million

Results presented as number of rats showing cytoplasmic alterations / total number of rats in the group, with average severity score in parentheses. The severity score is based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Source: Chan & Mahler (1992)

The NOAEL in the 13-week dietary toxicity study in rats was 6250 ppm (equal to 410 mg/kg bw per day) based on the more pronounced cellular alterations in the salivary glands at 12 500 ppm and above (Chan & Mahler, 1992).

In a 90-day range-finding study, groups of 10 Sprague Dawley rats per sex were administered daily doses of glyphosate technical (purity 97.5%) at concentrations of 0, 2000, 6000 and 20 000 ppm

Table 16. Caecum weights of rats administered glyphosate for 13 weeks

	Absolute and relative weight per dietary concentration of glyphosate			
	0 ppm	**3 000 ppm**	**10 000 ppm**	**30 000 ppm**
Males				
Absolute weight ± SD (mg)	2 823 ± 794	3 187 ± 609	3 383 ± 1 081 (11%)	5 854 ± 2 053**
Relative weight ± SD (%)	0.55 ± 0.16	0.62 ± 0.13	0.64 ± 0.20 (11%)	1.22 ± 0.41**
Females				
Absolute weight in mg ± SD	2 367 ± 582	2 586 ± 462	3 546 ± 959*	5 268 ± 1 189**
Relative weight ± SD (%)	0.79 ± 0.17	0.84 ± 0.17	1.22 ± 0.32*	1.92 ± 0.41**

ppm: parts per million; SD: standard deviation; *: $P < 0.05$; **: $P < 0.01$

Relative weight = (organ weight/body weight) × 100.

Results expressed as absolute weight or relative weight and, in parentheses, this weight as a percentage of that of controls for males only.

Source: Kinoshita (1995)

At 30 000 ppm, 9 of the 12 males and 7 of the 12 females had statistically significantly distended caeca ($P = 0.01$). At 10 000 ppm, 3 of the 12 males showed distension of the caecum, but there were no macroscopic abnormalities in females. At 3000 ppm, there were no macroscopic abnormalities attributable to the treatment in either sex.

Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male at 10 000 ppm and one female at 30 000 ppm had renal lesions (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). However, these were considered of a genetic nature and not treatment related.

The NOAEL in this 90-day toxicity study in rats was 3000 ppm (equal to 168.4 mg/kg bw per day) based on increased caecum weight at 10 000 ppm and above (Kinoshita, 1995).

In a 90-day oral toxicity study, groups of 12 male and 12 female Alpk:AP Wistar-derived rats were fed diets containing glyphosate (purity 97.4%) at concentrations of 0, 1000, 5000 or 20 000 ppm (equal to mean intakes of 0, 81, 414 and 693 mg/kg bw per day for males and 90, 447 and 1821 mg/kg bw per day for females).

There were no mortalities. A low incidence of diarrhoea and light-coloured faeces was 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 body-weight gain and food utilization efficiency compared with controls. There was some evidence for a reduction in platelet count in males and females at 5000 and 20 000 ppm. A marginal dose-related increase in prothrombin time was observed in males at all doses. The differences, however, were small and considered not of haematological significance. Plasma alkaline phosphatase and alanine transaminase activities were increased in both sexes at 20 000 ppm and, to a lesser extent, in males at 5000 ppm. In addition, plasma aspartate aminotransferase activity was increased in females at the highest dose at this early time point, but not at study termination. The changes in clinical chemistry parameters were small, often lacking a clear dose–response relationship, and therefore not considered biologically relevant. There were no treatment-related effects on urine biochemistry and organ weights.

The only notable histopathological finding was a uterine leiomyosarcoma in a female at 5000 ppm. Although these are rare, finding such a tumour in an animal at the intermediate dose was considered incidental to treatment.

The NOAEL in the 90-day toxicity study in rats was 5000 ppm (equal to 414 mg/kg bw per day) based on the reduced growth in males at 20 000 ppm (Botham, 1996).

In a 90-day feeding study, groups of 10 Sprague Dawley rats per sex were administered daily doses of glyphosate (purity 95.3%) at concentrations of 0, 1000, 10 000 or 50 000 ppm (equal to 0, 79, 730 and 3706 mg/kg bw per day for males and 0, 90, 844 and 4188 mg/kg bw per day for females) in the diet.

There were no deaths. Animals of both sexes treated with 50 000 ppm had soft faeces and diarrhoea throughout the study period from day 4. Both sexes at 50 000 ppm showed a reduction in body-weight gain over the first 4 weeks of treatment. Body-weight development was unaffected at the other doses. Both males and females at 50 000 ppm showed a reduction in dietary intake and feed efficiency over the first 4 weeks of treatment compared with controls. Water consumption, measured ocular parameters or haematological parameters for either sex were unaffected. Both males and females at 10 000 or 50 000 ppm showed a statistically significant ($P < 0.05$ at 10 000 ppm and $P < 0.01$ at 50 000 ppm) reduction in plasma calcium concentration and an increase in alkaline phosphatase compared with controls. A statistically significant ($P < 0.05$) increase in inorganic phosphorus and reduction in plasma creatinine were also evident in males and females at 50 000 ppm, while females at this dose level showed statistically significant ($P < 0.01$) reductions in total plasma protein and albumin compared with controls. There were no other treatment-related effects. Both males and females at 50 000 ppm showed statistically significant increases in relative liver and kidney weights compared with controls (Table 17).

Table 17. Group mean relative organ-weights of rats administered glyphosate for 90 days

Dietary concentration of glyphosate (ppm)	Mean relative organ weight (%)			
	Liver		Kidney	
	Male	Female	Male	Female
0	2.974 9 ± 0.2629	2.973 4 ± 0.1558	0.586 1 ± 0.0575	0.651 6 ± 0.0523
1 000	2.886 8 ± 0.2552	2.909 3 ± 0.2146	0.590 1 ± 0.0804	0.6257 ± 0.0375
10 000	2.885 3 ± 0.3758	2.980 1 ± 0.1556	0.607 0 ± 0.0552	0.645 4 ± 0.0532
50 000	3.243 3 ± 0.2452*	3.198 9 ± 0.2098*	0.6963 ± 0.0436**	0.718 0 ± 0.0707*

ppm: parts per million; *: $P < 0.05$; **: $P < 0.001$

Results expressed as mean organ-weight as a percentage of mean body-weight, ± standard deviation.

Source: Coles et al. (1996)

At 50 000 ppm all animals had enlarged and fluid-filled caeca while one female had gaseous distension of the stomach at the final termination. There were no treatment-related macroscopic abnormalities at 10 000 or 1000 ppm.

Treatment-related changes were observed in the caeca. Atrophy, characterized by flattening of the intestinal mucosa, was observed in five rats of both sexes at 50 000 ppm ($P < 0.05$ for male rats) and for one male and two female rats at 10 000 ppm. The etiology of this change is uncertain and may represent no more than atrophy of the mucosa resulting from caecal distension. There were no other treatment-related changes.

The NOAEL in this 90-day toxicity study in rats was 1000 ppm (equal to 79 mg/kg bw per day) based on the reduced plasma calcium concentration and increased alkaline phosphatase concentrations at 10 000 ppm (Coles et al., 1996).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an unpublished non-GLP carcinogenicity study, glyphosate (purity 99.7%) was administered in the diet to groups of 50 male and 50 female CD-1 mice per dose at concentrations of 0, 1000, 5000 or 30 000 ppm (equal to 0, 157, 814, 4841 mg/kg bw per day, respectively, for males and 0, 190, 955, and 5874 mg/kg bw per day, respectively, for females) for 24 months. Cage-side and detailed clinical observations were conducted and body weight and feed intake monitored throughout the study. Water consumption was measured during months 12 and 24. Erythrocyte, as well as total white blood cell counts and differentials, were conducted at months 12, 18 and 24. Tissues and organs were collected from all mice whether they died during the study or were terminated. Microscopic analyses were conducted on all collected tissues.

Analysis of treated diets demonstrated that glyphosate homogeneously mixed with rodent diet remained stable for the 1-week feeding period used in this study. Glyphosate test concentrations averaged approximately 95% of the target concentrations throughout the study. No treatment-related physical or behavioural signs of toxicity or mortality were observed. Yellow staining of the anogenital area, scabbing on the ears, alopecia, excessive lacrimation, displacement of the pupils and ocular opacities seen in all groups of male and female mice were not dose related; all occurred at low incidences. Body weights for both males and females at 30 000 ppm were consistently less than the controls throughout the study. Although the decreases were slight (1%–11%), several were statistically significant. Other statistically significant decreases were noted in the mid- and low-dose animals; however, these were sporadic and did not reflect a recognizable dose–response relationship. Although sporadic statistically significant effects were noted for feed consumption in treated male and female mice, none were dose or treatment related. Also, no treatment-related effects were observed for water consumption. No biologically or toxicologically relevant effects were noted on total erythrocyte or white blood cell counts, haemoglobin, haematocrit or platelet counts. No treatment-related changes were observed in absolute or relative organ weights. Several statistically significant changes in organ/body weight ratios were observed, but these were attributed to the statistically significant decreases in terminal (fasted) body weights rather than to specific organ effects. There were no dose–response relationships or any correlated gross or microscopic observations in any of the organs.

No remarkable treatment-related effects were noted at necropsy. Statistically significant positive trends were observed for central lobular hepatocyte hypertrophy, centrilobular hepatocyte necrosis (Table 18) and chronic interstitial nephritis in males, and for proximal tubule epithelial basophilia and hypertrophy in females. Statistically significant increases in the incidence of lesions were observed for centrilobular hepatocyte necrosis in high-dose males and proximal tubule epithelial basophilia and hypertrophy in high-dose females. While the incidences and/or dose–response trends of these individual microscopic kidney lesions were found to be statistically significant, they were considered part of a spectrum of lesions which, as a whole, constitute spontaneous renal disease.

Table 18. Hepatocellular lesions in mice administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Lesion		**0 ppm**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Centrilobular hypertrophy	M	9/49 [a]	5/50	3/50	17/50
	F	0/49	5/50	1/49	1/49
Centrilobular necrosis	M	0/49[b]	2/50	2/50	10/50 [a,b]

F: female; M: male; ppm: parts per million

Results presented as number of mice showing hypertrophy or necrosis / number of mice examined.

[a] Statistically significant linear trend ($P \leq 0.01$) using the Cochran–Armitage test.

[b] Statistically significant increase compared to control ($P \leq 0.01$) using the Chi squared test.

Source: Knezevich & Hogan (1983)

Neoplastic outcomes were of the type common in mice of this age and strain. Of the tumour types observed, bronchiolar-alveoli tumours of the lungs, hepatocellular neoplasms and tumours of the lymphoreticular system, none were dose related and all were seen in all treatment groups (Table 19). Lymphoreticular tumours were more frequently observed in female mice, but the incidences were low and did not approach statistical significance (nonsignificant trend and pair wise comparison). With the possible exception of kidney tumours (renal tubular adenomas) in males, all tumour types were considered spurious and unrelated to treatment (see Table 19).

Table 19. Neoplasia in male and female mice treated with glyphosate for 24 months

	Incidence per dietary concentration of glyphosate							
	Males				**Females**			
Site / Neoplasia	**0 ppm** [a]	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**	**0 ppm** [a]	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Lung								
Bronchiolar alveolar adenoma	5/48	9/50	9/50	9/50	10/49	9/50	10/49	1/50
Bronchiolar alveolar adenocarcinoma	4/48	3/50	2/50	1/50	1/49	3/50	4/49	4/50
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/48	4/50	3/50	1/50	–	–	–	–
Liver								
Hepatocellular adenocarcinoma	5/49	6/50	6/50	4/50	1/49	2/50	1/49	0/49
Hepatocellular carcinoma	0/49	0/50	0/50	2/50	2/49	1/50	0/49	4/49
Lymph node (mediastinal)								
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/45	2/49	1/41	2/49	–	–	–	–
Kidney								
Renal tubular adenoma	0/49	0/49	1/50	3/50	–	–	–	–
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49	3/49	2/50	2/50	–	–	–	–
Total lymphoreticular neoplasms (sum of lymphoblastic lymphosarcoma, composite lymphosarcoma and histiocytic sarcoma)	2/48	6/49	4/50	2/49	5/50	6/48	6/49	10/49

ppm: parts per million; PWG: Pathology Working Group

Results presented as number of neoplasm-bearing animals / number of animals examined.

[a] Incidence of effect in controls from the study report prior to PWG re-evaluation.

Source: (Knezevich and Hogan, 1983)

At the request of the USEPA, the Pathology Working Group (PWG) examined all sections of the kidneys from this study as well as additional renal sections. The PWG evaluation included a renal tubule adenoma in one control male mouse that was identified during a re-evaluation of the original renal section. The PWG noted that because differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and because both lesions are derived from the same cell type, it appropriate to combine the incidences for statistical analysis. Statistical analyses performed by the PWG are presented in Table 20. The PWG concluded that these lesions are not treatment-related based on the following considerations: 1) renal tubular-cell tumours are spontaneous lesions for which there is a paucity of historical control data for this mouse stock; 2) there was no statistical significance

in a pairwise comparison of treated groups with the controls and there was no evidence of a significant linear trend; 3) multiple renal tumours were not found in any animal; and 4) treatment-related nephrotoxic lesions, including pre-neoplastic changes, were not present in male mice in this study. In addition, there was no increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/regeneration, hyperplasia or hypertrophy). Although the incidence of tubular adenomas exceeded the testing laboratory's historical control range (0–3.3%), the increase at the high dose was not statistically significant compared to the concurrent controls. However, the re-analysis of the tumour indicated that kidney adenomas and kidney adenoma/carcinoma combined showed statistically significant positive trend.

Table 20. Results of re-examination of incidence of renal tumours in male mice treated with glyphosate for 24 months

	Incidence of renal tumours per dietary concentration of glyphosate			
Tumour type	**0 ppm**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Adenomas	1/49 (2%)	0/49 (0%)	0/50 (0%)	1/45 (2%)
	P = 0.442 2	*P* = 1.000 0	*P* = 1.000 00	*P* = 0.757 6
Carcinomas	0/49 (0%)	0/49 (0%)	1/50 (2%)	2/50 (4%)
	P = 0.063 5	*P* = 1.000 0	*P* = 0.505 1	*P* = 0.252 5
Combined	1/49 (2%)	0/49 (0%)	1/50 (2%)	3/50 (6%)
	P = 0.064 8	*P* = 1.000 0	*P* = 0.757 6	*P* = 0.316 3

ppm: parts per million

Results presented as the number of tumour-bearing animals / number of animals examined, with the resulting percentage in parentheses.

P values determined using the Cochran–Armitage test and Fisher Exact test.

Source: Knezevich & Hogan (1983)

The NOAEL for the systemic toxicity in the two-stage carcinogenicity study in mice was 5000 ppm (equal to 814 mg/kg bw per day) based on the slightly reduced body weights, increased centrilobular hepatocellular necrosis in high-dose males and proximal tubular epithelial basophilia in high-dose females seen at the systemic LOAEL of 30 000 ppm; equal to 4841 mg/kg bw per day for males and 5874 mg/kg bw per day for females (Knezevich & Hogan, 1983).

The present Meeting concluded that there is some indication, by trend test but not pairwise comparison, of induction of kidney adenomas in male mice.

In a 22-month carcinogenicity study, trimethylsulfonium carboxymethylamino-methylphosphonate (Company code SC-0224; glyphosate trimethylsulfonium; purity 56.17%) was administered in the diet to groups of 80 ICR(Crl:CD-1)BR mice per sex per dose at concentrations of 100, 1000 or 8000 ppm for 22 months (mean test material intake 11.7, 118 and 991 mg/kg bw per day for male mice and 16.0, 159 and 1341 mg/kg bw per day for female mice, respectively). One control group of 60 male and female mice were fed the basal diet only. An additional control group of 80 male and female mice were fed the basal diet plus 1% propylene glycol vehicle. Interim terminations of different numbers of mice occurred at 6, 12 and 18 months. The number of mice scheduled for the full 22-month study was 50/sex per dose. Blood samples were drawn from 10 fasted male and female mice per dose at 6, 12, 18 and 22 months for haematology and clinical chemistry measurements. At the same time points, brain cholinesterase concentrations from left and right sides of the brains of five mice/sex per dose were measured; urine analysis for 10 fasted mice/sex per dose was performed; and ophthalmoscopic examinations of all the mice were conducted. Macroscopic examinations of all the animals and histopathological examinations of selected tissues from all the animals were conducted. Selected organs were weighed.

There were no statistically significant increases in the incidence of any tumours, benign and malignant, in either sex; however, the number of animals with multiple tumour types was slightly increased in the high-dose group of both sexes (males: 16/50; females: 11/50) compared to the control (males: 11/50; females: 6/50). This led to a slight increase in the total number of tumours in the high-dose group of both sexes (males: 60; females: 43) compared to the control (males: 49; females: 36).

Haemangiosarcoma in the vascular system was evident in 4/50 high-dose males, 2/50 low-dose females and 1/50 high-dose females compared to 0/50 controls. Of the high-dose mice, one had tumours in the liver and spleen; one had a tumour in the liver only; one had tumours in the liver, spleen and prostate; and one had a tumour in the spleen only. The incidence of haemangiosarcoma in males was positive in Exact trend test and nonsignificant in pairwise comparison (Table 21). In female mice, incidence of haemangiosarcoma did not achieve statistical significance.

Table 21. Haemangiosarcomas in male mice administered glyphosate for 104 weeks

	Measure per dietary dose of glyphosate			
	0 mg/kg bw per day	**100 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Haemangiosarcomas	0/47 (0%)	0/46 (0%)	0/50 (0%)	4/45 (9%)
	$P = 0.002\ 96$**	$P = 1.000\ 00$	$P = 1.000\ 00$	$P = 0.053\ 32$

bw: body weight; **: significance of trend ($P < 0.01$) denoted at control, using Fisher Exact test and Exact Trend test.

Results presented as number of tumour-bearing animals / number of animals examined less those that died before week 52, with the resulting percentage in parentheses.

Source: Atkinson et al., 1993a

Histiocytic sarcoma in the lymphoreticular/haematopoietic tissue was evident in 2/50 low- and high-dose males and 3/50 low- and intermediate-dose females and 1/50 high-dose female (none were evident in the respective controls). Due to a lack of dose relationship and statistical significance, these changes are not considered treatment related. Other tumours seen were considered typical for mice of this age and strain.

The NOAEL for systemic toxicity in the 104-week carcinogenicity study in mice was 1000 mg/kg bw per day, the highest dose tested (Atkinson et al., 1993a).

In an 18-month carcinogenicity study, glyphosate (two lots of HR-001, purity 97.56% and 94.61%) was fed in the diet to groups of 50 male and 50 female ICR(Crj:CD-1)(SPF) mice at 0, 1600, 8000 or 40 000 ppm (equal to 0, 165, 838.1 or 4348 mg/kg bw per day for males and 0, 153.2, 786.8 or 4116 mg/kg bw per day for females) for 18 months. During treatment, all animals were observed for clinical signs and changes in body weight, and feed consumption was measured. At week 21, urine analysis was carried out on 20 males from all groups. Differential leukocyte counts were determined in blood smears from 10 males and 10 females from all groups at week 52 and after 78 weeks of treatment and also in animals terminated in extremis during the treatment, as possible. At final necropsy after 78 weeks of treatment, organ weights of 10 males and 10 females were analysed to determine differential leukocyte counts. All animals of both sexes were necropsied and their histopathology examined.

At 1600 ppm, there were no treatment-related changes in either sex in any parameters. At 8000 ppm, retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9 to 24. No treatment-related changes were seen in males. At 40 000 ppm, the incidence of pale skin increased in males. In addition, loose stools were found in all the cages from week 21 in males and week 20 in females. Retarded growth was persistently observed during treatment, with statistically significant differences in weight from week 16 to 36 in males and from week 6 to the end of the treatment in females. These changes were associated with depressed feed

consumption and feed efficiency. At necropsy, the increased incidences of distension of the caecum were noted in males and females in all the animals examined, which were consistent to increases in absolute and relative weights of the caecum. However, no histopathological abnormalities were recorded in the caecum. In males, a significant increase was noted for the overall incidence of anal prolapse that corresponded with erosion/ulcer of the anus.

The incidence of lymphoma was increased in the high-dose males but lacked a clear dose–response (see Table 22). It was significant by trend test and not by pairwise comparison. In female mice, the increased incidences of lymphoma were not statistically significant (trend test and pairwise comparison). The overall incidences of lymphomas observed were well below the historical control range of 0–18% (Baldrick & Reeve, 2007). Kidney adenomas and carcinomas in male mice were slightly increased at the high dose of 40 000 ppm. The statistical significance was achieved by the trend test and not by pairwise comparison. The incidences of kidney tumours in males exceeded the historical control range. Incidence of haemangiosarcomas was statistically significantly increased in the mid and high dose according to the trend test but not in a pairwise comparison.

Table 22. Selected neoplastic findings in male and female mice administered glyphosate for 18 months

	Incidence per dietary concentration of glyphosate			
Neoplastic findings	**0 ppm**	**1 600 ppm**	**8 000 ppm**	**40 000 ppm**
Males				
Lymphoma	2/50	2/50	0/50	6/50
Kidney (adenoma/carcinoma)	0/50	0/50	0/50	2/50
Haemangiosarcoma (various organs)	1/50	0/50	0/50	0/50
Females				
Lymphoma	6/50	4/50	8/50	7/50
Kidney (adenoma/carcinoma)	0/50	0/50	0/50	0/50
Haemangiosarcoma (various organs)	0/50	0/50	3/50	5/50

No.: number; ppm: parts per million

Results presented as number of tumour-bearing animals / number of animals examined.

Source: Sugimoto (1997)

Based on these results, the NOAEL was 1600 ppm (153.2 mg/kg bw per day) and the LOAEL was 8000 ppm (838.1 mg/kg bw per day) for females based upon retarded growth with statistically significant decreases in weight at week 6 and weeks 9 to 24 (Sugimoto, 1997).

In a 78-week carcinogenicity study, glyphosate (purity 97.5%) was fed to groups of 50 male and 50 female Crj:CD-1 mice per dose at dietary concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 67.6, 685 and 7470 mg/kg bw per day for males and 0, 93.2, 909 and 8690 mg/kg bw per day for females) for 78 weeks. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were conducted and body weight and feed intake monitored throughout the study. Differential white blood cell counts were performed at week 52, and haematological parameters evaluated at the end of the treatment. Gross pathological examinations were conducted at termination and on euthanized moribund and pre-terminally dead mice. Selected organs (brain, liver, both kidneys, both adrenal glands and both testes) were weighed. The tissue samples from control and high-dose animals and animals that died or were terminated in extremis were histopathologically examined.

Prepared diets were stable at room temperature for 4 months and the test material was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations ranged from 80–110% of the nominal concentrations. At 50 000 ppm, all the mice had loose stools throughout the treatment period, although some showed improvement as treatment continued. In the same group, nine males and eight females had treatment-related anus prolapse at week 10 or later. Other clinical signs and incidences were similar in both control and treated groups. A statistically significant difference in mortality rate in males was noted between the 50 000 ppm group and the control group at week 26 or later. Mortality in mid- and low-dose males and females at all doses was unaffected. At 50 000 ppm, body-weight gain significantly decreased or appeared to decrease throughout the treatment in males and at week 24 or later in females. No effects of treatment were observed in treated males and females in the mid and low dose at any time compared to controls. In both males and females at 50 000 ppm, feed consumption decreased compared with controls; the change was considered treatment related. No treatment-related changes were observed in haematology parameters. In the females at 50 000 ppm, the relative weights of kidneys (total) significantly increased. These changes were considered treatment related, though no corresponding histopathological findings were observed. In addition, decreases in the absolute weights of liver and right and left kidneys and significant increases in the relative weights of brain, left kidney, left adrenal gland, and right and left testes in males, and a decrease in the absolute weight of brain in females were noted at 50 000 ppm. The changes in the adrenal and brain were not considered adverse since they were not accompanied with histopathological findings. Macroscopic examination revealed luminal dilation of the large intestine, which may be associated with loose stool, in most of the terminated males and females at 50 000 ppm. Treatment-related non-neoplastic lesions were found in the kidneys in males and the rectums in males and females at 50 000 ppm. The renal findings included significant increases in tubular epithelial cell hypertrophy, tubular dilation, degeneration/necrosis and an increasing tendency in basophilic tubules proliferation (based on data from all animals). The rectal findings included significant increases in anus prolapse-associated erosion and luminal dilation (Table 23).

Table 23. Non-neoplastic lesions in mice administered glyphosate for 78 weeks

	Incidence per dietary concentration of glyphosate							
	Male				**Female**			
Non-neoplastic lesion	**0 ppm**	**500 ppm**	**5 000 ppm**	**50 000 ppm**	**0 ppm**	**500 ppm**	**5 000 ppm**	**50 000 ppm**
Kidney								
Tubular dilation	4/50	7/50	4/50	20**/50	8/50	12/50	5/50	8/50
Tubular epithelial cell hypertrophy	13/50	10/50	13/50	25*/50	13/50	17/50	14/50	13/50
Basophilic tubules	21/50	16/50	17/50	28/50	14/50	14/50	10/50	13/50
Tubular degeneration/necrosis	9/50	6/50	5/50	15/50	5/50	8/50	8/50	7/50
Rectum								
Luminal dilation	0/48	0/12	0/7	6*/46	0/44	0/11	0/10	6*/44
Erosion	0/48	0/12	0/7	3/46	0/44	0/11	0/10	6*/44

ppm: parts per million; *: $P < 0.05$, **: $P < 0.01$ (Fisher Exact test).

Results presented as number of tumour-bearing animals / number of animals examined.

Source: Takahashi (1999a)

Incidences of lymphomas in female mice were 3/50, 1/50, 4/50 and 6/50 in the control, 500, 5000 and 50 000 ppm dose group, respectively. The increased incidences of lymphoma at high doses were statistically significant in the trend test but not in a pairwise comparison. Renal cell adenoma was observed in three males and renal cell carcinoma in one male at 50 000 ppm; renal cell adenoma

was also observed in one male at 5000 ppm and none in any of the females (based on data from all animals). The incidence of other tumour types in glyphosate-treated groups and controls were similar.

These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel, Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. The haematoxylin-and-eosin–stained kidney sections prepared in the original study had faded and could not be evaluated; the paraffin-embedded blocks of 50 males from each group which had been stored for each observation period were sectioned and stained by haematoxylin and eosin for microscopic re-examination. The data from the re-examination and the original data are shown in Table 24.

Table 24. Renal tumours in male mice administered glyphosate for 78 weeks

Dietary concentration of glyphosate (ppm)	Findings	No. of cases		Incidence[a]
		Original study	Re-examination	
50 000	Renal cell adenoma	3	1	1/50 (2%)
	Renal cell carcinoma	1	1	1/50 (2%)
5 000	Renal cell adenoma	1	1	1/50 (2%)
500	Renal cell adenoma	0	1	1/50 (2%)

no.: number; ppm: parts per million

[a] Results presented as number of tumour-bearing animals / number of animals examined, with the resulting percentage in parentheses.

Source: Nippon Experimental Medical Research Institute (2012)

Upon re-examination (using Fisher Exact probability test, $P > 0.05$), the incidence of renal tumours in each treatment group no longer significantly differed from that in the control group. The historical control data for the Takahashi (1999a) study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13%) in males and 0/725 (0%) in females and for renal cell adenoma were 3/564 (0.53%) in males and 0/564 (0%) in females (Chandra & Frith, 1994; Baldrick & Reeve, 2007). The re-examination report also provides reference data: 0/55, 0/55, 1/55, 0/55 and 0/55 (0–1.8%) in males and 0/55 for all doses (0%) in females for renal cell carcinoma; and 0/55, 1/55, 1/55, 1/55, 0/55 (0–1.8%) in males and 0/55, 0/55, 0/55, 0/55, 1/55 (0–1.8%) in females for renal cell adenoma. The results of the re-examination revealed that the incidence of tubular epithelial cell hypertrophy in each treatment group did not significantly differ from that in the control group. In addition, the tubular epithelial cell hypertrophy was localized. These findings indicate no association between the tubular epithelial cell hypertrophy and the development of renal tumours.

In conclusion, the renal cell tumours observed in this study are not relevant for human risk assessment because (1) the incidence of renal tumours in males at 50 000 ppm did not significantly differ from that in the control group up on re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the highest dose (50 000 ppm) used in this study far exceeded the limit dose for mice (7000 ppm) specified by the Organisation for Economic Co-operation and Development (OECD) and USEPA.

The NOAEL in the 78-week carcinogenicity study in mice was 5000 ppm (equal to 685 mg/kg bw per day) for loose stools, decreased body-weight gain, decreased feed consumption and increased incidences of rectal and renal non-neoplastic lesions observed in male and female mice at the LOAEL of 50 000 ppm (equal to 7470 mg/kg bw per day), the highest dose tested (Takahashi, 1999a).

In an 18-month carcinogenicity study, glyphosate (purity > 95%) was fed to groups of HsdOla:MF1 Swiss Albino mice (50/sex per dose) in the diet at concentrations of 0, 100, 1000 or

Table 25. Malignant lymphoma in glyphosate-treated mice

			Measure per dietary concentration of glyphosate							
			Males				Females			
	M	F	0 ppm	100 ppm	1 000 ppm	10 000 ppm	0 ppm	100 ppm	1 000 ppm	10 000 ppm
Dead and moribund mice										
No. examined	75	77	22	20	22	27	16	16	20	20
No. affected	20	49	9	12	13	13	9	10	13	12
Incidence (%) [a]	26.7	63.6	41.0	60.0*	59.0*	48.0	56.0	63.0	65.0	60.0
Terminated mice										
No. examined	175	173	28	30	28	23	34	34	30	30
No. affected	26	50	1	3	3	6*	9	10	6	13
Incidence (%) [a]	14.9	28.9	3.6	10.0	10.7	26.1*	26.5	29.4	20.0	43.3*
Mean percentage	14.9	28.8	–	–	–	–	–	–	–	–
Range of percentage	8–24	2–43	–	–	–	–	–	–	–	–
All fates										
No. examined	250	250	50	50	50	50	50	50	50	50
No. affected	46	99	10	15	16	19*	18	20	19	25
Incidence (%) [a]	18.4	39.6	20.0	30.0	32.0	38.0*	36.0	40.0	38.0	50.0*
Mean percentage	18.4	41.6	–	–	–	–	–	–	–	–
Range percentage	6–30	14–58	–	–	–	–	–	–	–	–

F: females; M: males; –: not examined/not determined; *: significant increase compared with historical controls (no *P* value provided)

[a] Incidence expressed as number of animals affected as a percentage of the number examined.

Source: Kumar (2001)

The increased incidences of kidney tumours at high doses (0/50, 0/50, 1/50 and 2/50 at 0, 100, 1000 and 10 000 ppm, respectively) were statistically significant in the trend test but not in a pairwise comparison. No historical control data were available.

The NOAEL for systemic toxicity in the 18-month carcinogenicity study in mice was 1000 ppm (equal to 149.7 mg/kg bw per day) for increased mortality at 10 000 ppm. Glyphosate was not carcinogenic in mice at doses up to 10 000 ppm, the highest dose tested (Kumar, 2001).

In a carcinogenicity study, glyphosate (purity 95.7%) was fed in the diet to groups of 51 male and 51 female CD-1 mice per dose at concentrations of 0, 500, 1500 and 5000 ppm (equal to 0, 71.4, 234.2 and 810 mg/kg bw per day for males and 0, 97.9, 299.5 and 1081.2 mg/kg bw per day for females) for 79 weeks. An additional 12 mice per sex, designated as veterinary controls, were housed and maintained alongside the treated animals. Ten animals per sex from each group were set aside for an interim termination (toxicity assessment) at week 39. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were conducted, and body weight and feed intake monitored throughout the study. Water consumption was observed daily. Blood smear samples were collected after 12 months and at termination from all animals and from mice terminated in extremis. Differential white blood cell counts were performed on all control and high-dose animals and on the animals terminated in extremis. Gross pathological examinations were conducted at termination and on moribund and pre-terminally dead mice. Selected

The ophthalmic examination prior to study termination revealed a statistically significant difference ($P < 0.05$) in the incidence of cataractous lens changes between control and high-dose males (0/15 vs 5/20). This incidence (25%) was within the range (0–33%) observed in previous studies of untreated male CD rats at this laboratory (Monsanto Agricultural Company, St. Louis, MO, USA). The incidences of cataractous lens changes in low- and mid-dose males, as well as all treated female groups, were comparable to their respective controls. An examination by an independent pathologist from Monsanto (Dr Rubin) also showed a statistically significant increase ($P < 0.05$) in cataractous lens changes in high-dose male animals (8/19 vs 1/14 for controls) and concluded that a treatment-related occurrence of lens changes affected high-dose males. Further histopathological re-evaluation of eyes by Experimental Pathology Laboratories Incorporation revealed cataract and/or lens fibre degeneration (Table 26). Because the number of rats ophthalmologically examined and affected at termination was small, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes appears to be exacerbated by treatment in high-dose males.

Table 26. Cataract and lens fibre degeneration in male rats administered dietary glyphosate for 24 months

	Incidence per dietary concentrations of glyphosate			
	0 ppm	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Terminal kill	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

ppm: parts per million

Results presented as number of rats affected / number of rats examined.

Source: Strout & Ruecker (1990)

While there were various changes in haematology and serum chemistry parameters, these were not consistently noted at more than one time point; were small and within historical control ranges; and/or did not occur in a dose-related manner and so were considered either unrelated to treatment or toxicologically insignificant. There was a statistically significant increase in urine specific gravity in high-dose males at 6 months and statistically significant reductions in urine pH in high-dose males at months 6, 18 and 24 months; this may have been due to the excretion of glyphosate, which is an acid. Statistically significant increases in liver-to-body weight ratio at 12 months and absolute liver weight and liver-to-brain weight ratio at 24 months occurred in males at 20 000 ppm. There were no other statistically significant changes in organ weights. Gross abnormalities seen at necropsy were not glyphosate related.

Histopathological examination revealed an increase in the number of mid-dose females with inflammation of the stomach squamous mucosa, the only statistically significant occurrence of non-neoplastic lesions. Although the incidence (15%) of this lesion in mid-dose females was slightly outside the laboratory historical control range (0–13.3%), there was no dose-related trend across all groups of treated females and no significant difference in any male group, leading to the conclusion that the finding was not treatment related (Table 27).

Table 27. Inflammation of the stomach squamous mucosa in rats administered glyphosate for 24 months

	Incidence per dietary concentrations of glyphosate			
	0 ppm	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Males	2/58	3/58	5/59	7/59
Females	0/59	3/60	9/60**	6/59

ppm: parts per million; **: $P \leq 0.01$ (Fisher Exact test with Bonferroni inequality)

Results presented as number of rats with the inflammation / number of rats examined.

Source: Strout & Ruecker (1990)

The only statistically significant difference in neoplastic lesions between control and treated animals was an increase in the number of low-dose males (14%) with pancreatic islet cell adenomas (Table 28). The historical (1983–1989) control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of reported studies revealed a prevalence of 0–17% in control males with several values greater than or equal to 8%. The incidences of islet cell adenomas did not follow a clear dose-related trend in the treated male groups as indicated by the lack of statistical significance in the Peto trend test, meaning that the distribution of incidences in the four groups was most likely random. There was also considerable intergroup variability in the numbers of females with this tumour (5/60, 1/60, 4/60 and 0/59 in the control, low-, mid- and high-dose groups, respectively) and no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a control male, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support a conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to glyphosate administration.

Table 28. Incidence of pancreatic islet cell findings in rats administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Finding	**Sex**	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Hyperplasia	M	2/58 (3%)	0/57 (0%)	4/60 (7%)	2/59 (3%)
	F	4/60 (7%)	1/60 (2%)	1/60 (2%)	0/59 (0%)
Adenoma	M	1/58 (2%)	8/57** (14%)	5/60 (8%)	7/59*** (12%)
	F	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)
Carcinoma	M	1/58 (2%)	0/57 (0%)	0/60 (0%)	0/59 (0%)
	F	0/60 (0%)[a]	0/60 (0%)	0/60 (0%)	0/59 (0%)
Adenoma + carcinoma (combined)	M	2/58 (3%)	8/57*** (14%)	5/60 (8%)	7/59 (12%)
	F	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)

ppm: parts per million; **: $P < 0.01$ (Fisher Exact test with Bonferroni inequality); ***: noted to be statistically significant but not analysed in the original report

Results presented as number of rats affected / number of rats examined with the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

There was a statistically significant trend for hepatocellular adenomas in males only, but a significant trend was not seen for adenomas and carcinomas combined ($P > 0.05$) (Table 29). These tumours were not considered to treatment related since 1) their incidences were within the testing facility's historical control range (1–18%); 2) pre-neoplastic lesions (i.e. cell hyperplasia or pre-neoplastic foci) were absent; and 3) there was no evidence of progression to malignancy (adenoma to carcinoma).

An increased incidence of thyroid C-cell adenomas was observed at 8000 and 20 000 ppm in both sexes but this did not reach statistical significance compared to the control animals (Table 29). There was a statistically significant dose trend for C-cell adenomas and adenomas/carcinomas combined in females. The testing laboratory historical control range for C-cell adenomas was 1.8–10.6% for males and 3.3–10% for females; the range for C-cell carcinomas was 0–5.2% for males and 0–2.9% for females. These tumours are not considered relevant to human risk assessment because 1) the increased incidences in males were not statistically significant; 2) there was no evidence of progression from adenoma to carcinoma; 3) and there were no dose-related increases in the incidence or severity of pre-neoplastic lesions (hyperplasia); and 4) they occurred in only one study.

Table 29. Thyroid C-cell tumours in male and female rats administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Finding	**Sex**	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Adenoma	M	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
	F	2/57 (4%)*	2/60 (3%)	6/59 (10%)	6/55 (11%)
Carcinoma	M	0/54 (0%)	2/55 (4%)	0/58 (0%)	1/58 (2%)
	F	0/57 (0%)	0/60 (0%)	1/59 (2%)	0/55 (0%)
Adenoma + carcinoma (combined)	M	2/54 (4%)	6/55 (11%)	8/58 (14%)	8/58 (14%)
	F	2/57 (4%)*	2/60 (3%)	7/59 (12%)	6/55 (11%)

F: females; M: males; ppm: parts per million; *: $P < 0.05$ (Cochran–Armitage Trend Test)

Results presented as number of rats affected / number of animals examined, excluding those that died or were terminated prior to study week 55, and the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

The incidence of benign keratoacanthoma was increased in male rats, but as there was no dose–response relationship, it was not considered treatment related (Table 30).

Table 30. Skin keratoacanthoma in male rats administered glyphosate for 24 months

	Incidence per dietary concentration of glyphosate			
Finding	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Benign keratoacanthoma (dead and moribund animals)	0/36 (0%)	1/31 (3%)	2/33 (6%)	1/32 (3%)
Benign keratoacanthoma (terminal kill)	0/13 (0%)	2/19 (11%)	2/17 (12%)	2/17 (12%)

ppm: parts per million

Results presented as number of rats with skin keratoacanthoma / number of rats assessed, with the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

Lymphoma/lymphosarcoma was observed in multiple tissues in male and female rats; however, the incidences in treatment groups were lower than in the controls and no dose relationship was observed.

The NOAEL for toxicity in rats was 8000 ppm (equal to 362 mg/kg bw per day) for decreased body-weight gains in females and cataractous lens changes in males seen at the LOAEL of 20 000 ppm (Strout & Ruecker, 1990).

was significantly decreased in males and significantly increased in females compared to their respective controls.

At 30 000 ppm, neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period, with statistical significance most weeks. In all other groups, mortality was comparable to the control. Males had significant increases in incidence of bradypnea, palpable masses and soiled fur (at the external genital or perianal region) compared to controls. Palpable masses in the tail were present in 27 males, a high incidence compared to 11 for the controls; the incidences of masses in other locations were comparable to the controls. Males at 30 000 ppm also showed significant decreases in incidence of tactile hair loss, incidence of wounds and hair loss. In females, a significant increase in incidence of wet fur, mainly in the external genital area, was observed. In addition, loose stools were observed in all cages from week 24 in males and week 23 in females until the end of the treatment.

There was an increase in benign keratoacanthoma in males at 24 months that was statistically significant in trend wise comparison but not in pair wise comparison (Table 31). However, skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats (Chandra, Riley & Johnson, 1992). Adenomas of the kidney were observed in four males in the 30 000 ppm grouped compared to zero in the controls. The background incidence of this tumour in this strain of rat is reported to be 0.7% (0–2.9%), and the incidence of the tumour in the 30 000 ppm group was only slightly higher than this background incidence. Because there was no statistically significant difference in incidence between the control and the 30 000 ppm group, the slightly higher incidence was not considered due to the treatment with glyphosate.

Table 31. Skin keratoacanthoma in male rats administered HR-001 for 24 months

	Incidence per dietary concentration of HR-001			
Finding	**0 ppm**	**3 000 ppm**	**10 000 ppm**	**30 000 ppm**
Benign keratoacanthoma (dead and moribund animals)	2/32 (6%)	1/30 (3%)	0/32 (0%)	1/21 (5%)
Benign keratoacanthoma (terminal kill)	1/18 (6%)	2/20 (10%)	0/18 (0%)	6/29 (21%)

ppm: parts per million

Results presented as number of male rats with skin keratoacanthoma / number assessed, with resulting percentage in parentheses.

Source: Enomoto (1997)

The NOAEL for chronic toxicity was 3000 ppm (104 mg/kg bw per day) and the LOAEL 10 000 ppm (354 mg/kg bw per day) based on an increase in ptosis and of tactile hair loss in female rats in 24-month study. There was an increased incidence of multiple clinical signs at 30 000 ppm (Enomoto, 1997).

In a combined chronic toxicity and carcinogenicity study, groups of Fischer F344/DuCrlCrlj rats (50/sex per dose) were fed diets containing glyphosate (purity 97.5%) at concentrations of 0, 500, 4000 or 32 000 ppm (equal to 0, 25, 201 and 1750 mg/kg bw per day for males and 0, 29.7, 239 and 2000 mg/kg bw per day for females) for 104 weeks. An interim termination was conducted on 14 rats per sex per dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet determined. Clinical observations (including ophthalmoscopy), body weights, feed consumption, haematology and clinical biochemistry (blood and urine) were measured throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment of the study. At the end of the scheduled period the animals were terminated and necropsied. Blood samples were taken for

Macroscopic findings consisting of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes were seen in males at 6000 and 20 000 ppm. A minor increase in the incidence but not the severity of proliferative cholangitis in the liver was observed at interim and terminal kills in high-dose males. Moreover, an increased incidence of hepatitis and periodontal inflammation was observed in high-dose males. There were a number of changes in the kidneys of high-dose males and females, notably renal papillary necrosis, with or without papillary mineralization, and transitional cell hyperplasia; the incidence was greater in males than females. These findings are considered treatment related but are consistent with ingesting high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation. The decrease in the incidence of tubular degeneration of the testis in high-dose males is considered of no consequence (Table 32). The incidence of prostatitis was higher than the control groups in all treated males but it was within historical background levels in all treated groups; however, as the control value in this study was low, the relationship to treatment at the high-dose level cannot be entirely dismissed.

Table 32. Selected microscopic findings in rats administered glyphosate for 2 years

	No. per dietary concentration of glyphosate							
	Males				**Females**			
Organ / Finding	**0 ppm**	**2 000 ppm**	**6 000 ppm**	**20 000 ppm**	**0 ppm**	**2 000 ppm**	**6 000 ppm**	**20 000 ppm**
Liver: Proliferative cholangitis	56	57	55	64	55	58	59	61
Liver: Hepatitis	8	6	9	13	6	7	4	6
Kidney: Papillary necrosis	0	1	0	14	0	1	2	5
Kidney: 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

no. number; ppm: parts per million

Results presented as number of rats with the finding. $N = 64$ for male and for female rats.

Source: Brammer (2001)

In contrast to a previously described 1-year feeding study in rats (Milburn, 1996), microscopic changes were seen in the liver and kidneys of high-dose rats but not the salivary glands, even though the study was conducted on the same strain of the rats and in the same laboratory.

The incidence of hepatocellular adenomas in male rats at the high dose increased compared to the controls (0/52 at 0 ppm, 2/52 [4%] at 2000 ppm, 0/52 [0%] at 6000 ppm and 5/52 [10%] at 20 000). However, this increase was considered incidental rather than treatment related, for the following reasons: 1) the absence of a dose–response relationship; 2) the lack of progression to malignancy; 3) no evidence of pre-neoplastic lesions; 4) the incidences were within the range (0–11.5%) of historical controls for this strain (Wistar) of rats in 26 studies conducted between 1984 and 2003 at the testing laboratory; and 5) the 0% incidence in the concurrent controls is lower than the average background incidence for liver adenomas in male Wistar rats, which distorts the comparison.

In conclusion, the NOAEL for chronic toxicity of glyphosate in rats was 6000 ppm (equal to 361 mg/kg bw per day) based on kidney, prostate and liver toxicity seen at 20 000 ppm (equal to 1214 mg/kg bw per day) in this 2-year study. There was no evidence of carcinogenicity in rats at glyphosate doses up to 20 000 ppm (Brammer, 2001).

synthesis in isolated rat hepatocytes. Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

(a) In vitro studies

Bacteria

Glyphosate or Roundup was used in approximately 40 studies of mutagenicity in bacteria. Most were conducted with and without metabolic activation (using S9, 9000 × *g* supernatant fraction from induced male rat liver homogenate). The actual number of tests performed was well over 150 as multiple tester strains with and without S9 were used in most studies. Glyphosate or Roundup was found to be negative for genotoxic effects in almost all of these; weak positive results were reported in only one or two studies. Glyphosate was also reported to be negative in three assays measuring DNA repair (rec) in *Bacillus subtilis* and positive in one SOS-chromotest assay in *Escherichia coli*. Several studies reported that glyphosate could enhance DNA strand breaks or interfere with DNA strand break repair in cyanobacteria following exposure to ultraviolet-B radiation.

In the case of AMPA or the acetylated metabolites, no increases in mutation in bacteria were seen in the in vitro studies (Table 33).

Table 33. Summary of in vitro genotoxicity studies with glyphosate, glyphosate formulations, AMPA or their metabolites in bacteria

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Point mutations	*Salmonella typhimurium* TA98, 100, 1535, 1537	0.1–1 000 µg/plate	Glyphosate (98.4%)	No	Negative	Negative	Kier (1978)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538	0.005–50 µL/plate	Glyphosate trimesium SC-0224 (19.2%)	Yes	Negative	Negative	Majeska (1982)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	10– 5 000 µg/plate	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	1.6–5 000 µg/plate	Glyphosate trimesium ICIA 0224	Yes	Negative	Negative	Callander (1988a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	313–5 000 µg/plate	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Negative	Yanagimoto (1991)
Point mutations	*S. typhimurium* TA98, 100, 1535 and 1537	160–5000 µg/plate	Glyphosate (98.6%)	Yes	Negative	Negative	Jensen (1991a)
Point mutations	*S. typhimurium* TA97, 98, 100, 1535	33–10 000 µg/plate	Glyphosate (98.6%)	No	Negative	Negative	Chan & Mahler (1992)
Point mutations	*S. typhimurium* strains TA98, 100, 1535, 1537	50–5 000 µg/plate	Rodeo (40% glyphosate)	Yes	Negative	Negative	Kier et al. (1992)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					–S9	+S9	
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2, WP2 uvrA	100–5 000 µg/plate	Glyphosate trimesium TMSC (95%)	Yes	Negative	Negative	Callander (1993)
Point mutations	*S. typhimurium* TA98, TA100	180–1 440 µg/plate	Roundup	No	Weak positive / equivocal	Weak positive / equivocal	Rank et al. (1993)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537	156–5 000 µg/plate	HR-001 (95.7%)	Yes	Negative	Negative	Akanuma (1995a)
Point mutations	*S. typhimurium* strains TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	50–5 000 µg/plate	Glyphosate (95.3%)	Yes	Negative	Negative	Thompson (1996)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2,WP2 uvrA	100–5 000 µg/plate	Glyphosate (95.6%)	Yes	Negative	Negative	Callander (1996)
Point mutations	*S. typhimurium* TA97a, 98, 100, 1535	1–5 000 µg/plate	Glifos (360 g/L glyphosate)	No	Negative	Negative	Vargas (1996)
Point mutations	*S. typhimurium* TA97a, 98, 100, 102	0.025–0.3 µg/plate	Glyphosate formulation Perzocyd 10, soluble liquid concentrate	No	Negative	Negative	Chruscielska et al. (2000b)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	10–5000 µg/plate	Glyphosate technical (97%)	Yes	Negative	Negative	Schreib (2012)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	648–5000 µg/plate	Glyphosate technical Helm (98%)	Yes	Negative	Negative	Riberri do Val (2007)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (95.1%)	Yes	Negative	Negative	Sokolowski (2007a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (97.7%)	Yes	Negative	Negative	Sokolowski (2007b)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (95%)	Yes	Negative	Negative	Sokolowski (2007c)
Point mutations	*S. typhimurium* TA97a, 98, 100, 102, 1535	1–1000 µg/plate	Glyphosate TC (98%)	Yes	Negative	Negative	Miyaji (2008)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–3160 µg/plate	Glyphosate TC (97.5%)	Yes	Negative	Negative	Flügge (2009a)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2, WP2 uvrA	3–5000 µg/plate	Glyphosate (96.3%)	Yes	Negative	Negative	Sokolowski (2009)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–5000 µg/plate	Glyphosate (> 96%)	Yes	Negative	Negative	Donath (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–3160 µg/plate	Glyphosate TC (95.2%)	Yes	Negative	Negative	Flügge (2010)
Point mutations	*S. typhimurium* A98, 100, 1535, 1537; *E. coli* WP2 uvrA	31.6–5000 µg/plate	Glyphosate (96%)	Yes	Negative	Negative	Schreib (2010)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (> 95%) spiked with glyphosine (0.63%)	Yes	Negative	Negative	Sokolowski (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–5000 µg/plate	Glyphosate (> 95.8%)	Yes	Negative	Negative	Wallner (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	10–2000 µg/plate	Glyphosate (> 95.4%)	Yes	Negative	Negative	Donath (2011a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate (98.8%)	Yes	Negative	Negative	Donath (2011b)
Point mutations	*S. typhimurium* TA98, 100, 1535 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate (97.8%)	Yes	Negative	Negative	Donath (2011c)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	1.5–5000 µg/plate	Glyphosate (85.8%)	Yes	Negative	Negative	Thompson (2014)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate technical (94.1%)	Yes	Negative	Negative	Schreib (2015)
DNA damage	*B. subtilis* Rec assay H17 and M45	20–2 000 µg/disk	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
DNA damage	*B. subtilis* Rec assay H17 and M45	15–240 µg/disc	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Negative	Yanagimoto (1992b)
DNA damage	*B. subtilis* Rec assay H17 and M45	7.5–240 µg/disk	Glyphosate (95.7%)	Yes	Negative	Negative	Akanuma (1995b)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
DNA damage	*E. coli* SOS chromotest	0.1–0.25 µg	Roundup	No	Positive	N/A	Raipulis et al. (2009)
Enhanced UV-induced DNA strand breaks	Cyanobacteria (*Scytonema javanicum*)	10 µmol/L	Glyphosate	No	Positive	Negative	Wang et al. (2012)
Delayed UV–B-induced DNA strand break repair	Cyanobacteria (*Anabaena* sp.)	10 µmol/L	Glyphosate	No	Positive	N/A	Chen et al. (2012)
Delayed UV-B-induced DNA strand break repair	Cyanobacteria (*Microcystis viridis*)	10 µmol/L	Glyphosate	No	Positive	N/A	Chen et al. (2012)
DNA damage	Acellular prophage superhelical PM2 DNA	75 mmol/L	Glyphosate (98.4%)	No	Negative	N/A	Lueken et al. (2004)
AMPA							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	200–5 000 µg/plate	AMPA (99.3%)	Yes	Negative	Negative	Akanuma (1996)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	1.6–5 000 µg/plate	AMPA (> 99%)	Yes	Negative	Negative	Callander (1988b)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537	310–5 000 µg/plate	AMPA (99.2%)	Yes	Negative	Negative	Jensen (1993a)
***N*-Acetyl-AMPA**							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	50–5 000 µg/plate	*N*-acetyl-AMPA (76%; IN-EY252)	Yes	Negative	Negative	Wagner & Klug (2007)
***N*-Acetyl-glyphosate**							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	100–5 000 µg/plate	*N*-acetyl-glyphosate sodium salt (84.3%)	Yes	Negative	Negative	Mecchi (2004)

AMPA, aminomethylphosphonic acid; GLP: good laboratory practice; N/A: not applicable; S9: 9000 × *g* supernatant fraction from induced male rat liver homogenate; −S9: without metabolic activation; +S9: with metabolic activation; UV: ultraviolet

Mammalian cells

Glyphosate and its formulation products were tested for various types of genetic damage in mammalian cells in vitro (Table 34). The results are summarized as follows. Of the four in vitro studies of gene mutation in mammalian cells induced by glyphosate or its formulation products, no increases were reported. In contrast, nine of 10 studies investigating DNA strand breaks induced by glyphosate or Roundup in mammalian cells reported positive results, 4 of 11 studies of chromosome aberrations reported positive results. For two of these (Lioi et al., 1998a,b), the effects were seen at much lower concentrations than the other studies reporting negative results. Two studies reported

negative results for polyploidy. One study of the glyphosate formulation product Herbazed (Amer et al., 2006) reported an induction of chromosome aberrations in mouse splenocytes in vitro (see further discussion of Herbazed below). Five of eight studies of micronuclei were positive, two were negative and one was equivocal; three of the positive studies required S9 whereas two did not. Of the eight studies of sister chromatid exchanges induced in peripheral blood lymphocytes, seven were positive; four were in human peripheral blood lymphocytes, two were in bovine peripheral blood lymphocytes, and one was in mouse splenocytes. Both in vitro studies of unscheduled DNA synthesis in rat hepatocytes were negative.

AMPA was negative in two studies of unscheduled DNA synthesis in isolated rat hepatocytes (Bakke, 1991; Nesslany, 2002). Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

Table 34. Summary of in vitro genotoxicity studies with glyphosate, AMPA, metabolites of AMPA and formulants in mammalian cells

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					–S9	+S9	
Glyphosate							
Gene mutation (*HPRT*)	CHO cells	2–25 mg/mL	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	0.094–5 mg/mL	Glyphosate trimesium ICIA 0224 (57.6%)	Yes	Negative	Negative	Cross (1988)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	0.52–5 mg/mL	Glyphosate (98.6%)	Yes	Negative	Negative	Jensen (1991b)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	44–1 500 µg/mL	Glyphosate (95.6%)	Yes	Negative	Negative	Clay (1996)
Chromosomal aberrations	Mouse splenocytes	0.1–50 mmol/L	Herbazed (glyphosate, 84%)	No	Positive	N/A	Amer et al. (2006)
Chromosomal aberrations	CHO cells	4–10 µL/mL	Glyphosate trimesium SC-0224 (55.6%)	Yes	Negative	Negative	Majeska (1985)
Chromosomal aberrations	Chinese hamster cells (CHL/IU)	37.5–1 200 µg/mL	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Positive	Yanagimoto (1992a)
Chromosomal aberrations	Chinese hamster lung cells	62.5–1 000 µg/mL	HR-001 (95.7%)	Yes	Negative	Negative	Matsumoto (1995)
Chromosomal aberrations	Human peripheral blood lymphocytes	33–562 µg/mL	Glyfosaat	Yes	Negative	Negative	Van de Waart (1995)
Chromosomal aberrations	Chinese hamster lung cells	39–1250 µg/mL	Glyphosate (technical grade; 95.3%)	Yes	Negative	Negative	Wright (1996)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Chromosomal aberrations	Bovine lymphocytes	17–170 µmol/L	Glyphosate	No	Positive	N/A	Lioi et al. (1998a)
Chromosomal aberrations	Human peripheral blood lymphocytes	100–1250 µg/mL	Glyphosate (95.6%)	Yes	Negative	Negative	Fox (1998)
Chromosomal aberrations	Human peripheral blood lymphocytes	5–51 µmol/L	Glyphosate (≤ 98%)	No	Positive	N/A	Lioi et al. (1998b)
Chromosomal aberrations	Human peripheral blood lymphocytes	100–4 000 µg/mL	TMS Chloride (95%) [Glyphosate trimesium]	Yes	Equivocal	Equivocal	Griffiths & Mackay (1993)
Chromosomal aberrations	Human peripheral blood lymphocytes	0.2–6 mmol/L	Glyphosate (analytical grade; 96%)	No	Negative	N/A	Manas et al. (2009a)
Micronucleus	CHO K1 cells	5–100 µg/mL	Glyphosate	No	Negative	Positive	Roustan et al. (2014)
Micronucleus	Bovine lymphocytes	28–560 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Equivocal	N/A	Piesova (2004)
Micronucleus	Bovine lymphocytes	28–560 µg/mL	Glyphosate isopropylamine salt mixture (62%)	No	Equivocal	Negative	Piesova (2005)
Micronucleus	Bovine lymphocytes	28–1 120 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Negative	N/A	Sivikova et al. (2006)
Micronucleus	Human peripheral blood lymphocytes	0.5–580 µg/mL	Glyphosate (technical grade; 98%)	No	Negative	Positive	Mladinic et al. (2009)
Micronucleus	Human epithelial cancer cell line TR146	10–20 mg/L	Glyphosate (95%)	No	Positive	N/A	Koller et al. (2012)
Micronucleus	Human epithelial cancer cell line TR146	10–20 mg/L	Roundup	No	Positive	N/A	Koller et al. (2012)
Micronucleus	CHO K1 cells	5–100 µg/mL	Glyphosate	No	Negative	Positive	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM5757	75 mmol/L	Glyphosate (98.4%)	No	Negative alone; positive in presence of H_2O_2	N/A	Lueken et al. (2004)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
DNA strand breaks (Comet assay)	Human fibrosarcoma cell line HT1080	4.5–6.5 nmol/L	Glyphosate (technical grade)	No	Positive	N/A	Lopez et al. (2005)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM38	4.5–6.5 nmol/L	Glyphosate (technical grade)	No	Positive	N/A	Lopez et al. (2005)
DNA strand breaks (Comet assay)	Human liver HepG2 cell line	1–10 ppm	Roundup (R400)	No	Positive	N/A	Gasnier et al. (2009)
DNA strand breaks (Comet assay)	Human Hep2 cell line	3–7.5 mmol/L	Glyphosate (analytical grade; 96%)	No	Positive	N/A	Manas et al. (2009a)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	0.5–580 µg/mL	Glyphosate (technical grade; 98%)	No	Positive	Positive	Mladinic et al. (2009)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	10–2 000 mg/L	Glyphosate (95%)	No	Positive	N/A	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	10–2 000 mg/L	Roundup	No	Positive	N/A	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	N/A	Alvarez-Moya et al. (2014)
DNA strand breaks	Mouse spermatogonia	60–180 mg/L	Glyphosate		Positive	N/A	Ming et al. (2014)
Sister chromatid exchange	Mouse splenocytes	0.1–50 mmol/L	Herbazed (glyphosate, 84%)	No	Positive	N/A	Amer et al. (2006)
Sister chromatid exchange	CHO cells	4–10 µL/mL	Glyphosate trimesium SC-0224 (55.6%)	Yes	Negative	Negative	Majeska (1985)
Sister chromatid exchange	Bovine lymphocytes	28–1 120 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Positive	N/A	Sivikova et al. (2006)
Sister chromatid exchange	Bovine lymphocytes	17–170 µmol/L	Glyphosate	No	Positive	N/A	Lioi et al. (1998a)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.25–25 mg/mL	Roundup	No	Positive	N/A	Vigfusson & Vyse (1980)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.33–6 µg/mL	Glyphosate (analytical grade; 99.9%)	No	Positive	N/A	Bolognesi et al. (1997a)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Sister chromatid exchange	Human peripheral blood lymphocytes	0.1–0.33 µg/mL	Roundup (30.4% glyphosate)	No	Positive	N/A	Bolognesi et al. (1997a)
Sister chromatid exchange	Human peripheral blood lymphocytes	5–51 µmol/L	Glyphosate (≥ 98%)	No	Positive	N/A	Lioi et al. (1998b)
Unscheduled DNA synthesis	Rat hepatocytes	0.000 012 5–0.125 mg/mL	Glyphosate (98%)	No	Negative	N/A	Li & Long (1988)
Unscheduled DNA synthesis	Rat hepatocytes	0.2–111.7 mmol/L	Glyphosate (≥ 98%)	Yes	Negative	N/A	Rossberger (1994)
AMPA							
Gene mutation	Mouse lymphoma cells (L5871Y)	0.31–5.0 mg/mL	99.2%	Yes	Negative	Negative	Jensen (1993b)
Chromosomal aberrations	Human peripheral lymphocytes	0.9–1.8 mmol/L	99%	No	Weak positive	N/A	Manas et al. (2009b)
Micronucleus	CHO K1 cells	0.005–0.1 µg/L	AMPA (purity unspecified)	N/S	Positive	Positive	Roustan et al. (2014)
Micronucleus	CHO K1 cells	5–100	Glyphosate + AMPA	N/S	Negative	Negative	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human Hep2 cell line	2.5–7.5 mmol/L	99%	No	Positive	N/A	Manas et al. (2009b)
Unscheduled DNA synthesis	Rat hepatocytes	5–2 500 µg/mL	94.4%	N/S	Negative	N/A	Bakke (1991)
Unscheduled DNA synthesis	Rat hepatocytes	0.078–10 mmol/L	99.9%	N/S	Negative	N/A	Neslany (2002)
***N*-Acetyl-AMPA**							
Chromosomal aberrations	Human peripheral blood lymphocytes	191–1 530 µg/mL	76%; IN-EY252	Yes	Negative	Negative	Gudi & Rao (2007)
Gene mutation (*HPRT*)	CHO cells	100–1 531 µg/mL (active ingredient, adjusted for purity)	72%; IN-EY252	Yes	Negative	Negative	Glatt (2007)
***N*-Acetyl-glyphosate**							
Gene mutation (*HPRT*)	CHO cells	250–2 091 µg/mL (active ingredient, adjusted for purity)	*N*-acetyl-glyphosate sodium salt (63%)	Yes	Negative	Negative	Glatt (2006)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Chromosomal aberrations	CHO cells	960–2 800 µg/mL	*N*-acetyl-glyphosate sodium salt (84.3%)	Yes	Negative	N/A	Murli (2004)

AMPA: aminomethylphosphonic acid; CHO: Chinese hamster ovary; GLP: good laboratory practice; HepG2: hepatocellular carcinoma; Hep2: epidermoid cancer; HPRT: hypoxanthine-guanine phosphoribosyltransferase; N/A: not applicable; N/S: not stated; ppm: parts per million; S9: 9000 × *g* supernatant fraction from male rat liver homogenate; −S9: without metabolic activation; +S9: with metabolic activation; TK: thymidine kinase

(b) In vivo studies

Mammalian studies

Oral route

Thirty-three in vivo genotoxicity studies assessed the effect of orally administered glyphosate or its formulation products on rodents (29 in mice and four in rats). The end-points investigated included chromosomal alterations, micronuclei, sister chromatid exchanges, unscheduled DNA synthesis and dominant lethal mutations (Table 35). Fourteen of the studies were conducted using glyphosate (≥ 90% pure) with the remainder involving formulation products or less pure forms of glyphosate. The results were negative for 29 of the 33 studies. The majority of the studies were of good or acceptable quality, and included sponsored GLP studies conducted in compliance with OECD Guideline 474.

The four positive studies are briefly described here. A twofold statistically significant increase in micronucleus frequency was reported by Suresh (1993a) in female (but not male) mice treated with two 5000 mg/kg doses of glyphosate. (The JMPR committee noted that this dose exceeds the limit dose of 2000 mg/kg recommended by the OECD [2014] and the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [2011]. The micronucleus frequencies in the concurrent control were also higher than normal, and historical control frequencies for the lab were not provided. In addition, a study published the following year by the same group using the same doses of glyphosate did not see an increase in glyphosate-induced chromosome aberrations.] The three other positive studies were described in one article, a study published by Amer et al. (2006). In this article, positive results in both bone marrow cells and spermatocytes were reported after the administration of seven or more doses of a glyphosate formulation product called Herbazed (other positive results from that study are presented below). In contrast, in a repeated-dose study conducted by the United States National Toxicology Program (Chan & Mahler, 1992), increases in micronuclei were not seen in bone marrow erythrocytes of male and female mice administered glyphosate in the diet for 13 weeks. In another repeated-dose study, increases in chromosome aberrations were not seen in rat bone marrow cells harvested after 5 days of treatment with glyphosate trimesium (Matheson, 1982). Amer et al. (2006) also reported an increase in sister chromatid exchanges in mouse bone marrow cells after a single Herbazed dose.

Intraperitoneal injection

The JMPR committee concluded that genotoxic effects in animals treated with glyphosate or its formulation products by intraperitoneal injection were of limited value in assessing risks due to low-level dietary exposure. The following description of results is presented for completeness.

Twenty-one studies of micronuclei and chromosomal alterations were performed in the bone marrow cells of rodents administered glyphosate or its formulation products by intraperitoneal injection. Positive results were reported in approximately one third of the studies and negative/equivocal results for the remaining two thirds. The positive studies were reported in articles by four groups (Bolognesi et al., 1997; Prasad et al., 2009, Manas et al., 2009a; Rodrigues et al.,

2011) and involved the administration of both glyphosate and its formulation products. The Rodrigues et al. (2011) and Prasad et al. (2009) studies reported increases in micronuclei at doses (≥ 0.75 mg/kg bw and ≥ 25 mg/kg bw of Roundup, respectively) that were considerably lower than those reported as negative by other investigators (e.g. Jensen, 1991c [5000 mg/kg bw] and Kier, Flowers & Huffman, 1992 [850–3400 mg/kg bw]). When positive results were seen and when a direct comparison could be made, the formulation product was more potent than glyphosate itself (Bolognesi et al., 1997). Positive results in mouse spermatocytes were also reported with administration of 50 mg/kg bw of the glyphosate formulation product Herbazed for 5 days or more (but not 1 or 3 days) (Amer et al., 2006).

Increases in DNA strand breaks in the liver and kidney of mice were reported for both glyphosate and Roundup by Bolognesi et al. (1997). Heydens et al. (2008) conducted a follow-up study using the same Roundup formulation and reported that significant toxicity occurred in the liver and kidney when dosing was by intraperitoneal injection. They postulated that the DNA damage reported by Bolognesi et al. (1997) was likely a secondary effect of toxicity.

Bolognesi and colleagues (Peluso et al., 1998) also reported an increase in DNA adducts in mouse liver and kidney by the sensitive but nonspecific ^{32}P-postlabelling method following intraperitoneal administration of Roundup, but not glyphosate. They attributed the adducts to an unknown component of the herbicide mixture. This same group of investigators reported that intraperitoneal administration of glyphosate and Roundup resulted in an increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG) DNA adducts in the liver (glyphosate) and kidney (Roundup). A follow-up study on Roundup by Heydens et al. (2008) was unable to replicate the 8-OHdG adduct results.

Table 35. Summary of in vivo genotoxicity studies with glyphosate, glyphosate formulation products and AMPA and their metabolites in mammalian species

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Glyphosate						
Oral administration						
Dominant lethal test	Mouse fetuses and resorptions	200–2 000 mg/kg	Glyphosate (98.7%)	Yes	Negative	Rodwell (1980)
Chromosomal aberrations	Mouse bone marrow cells	50–5 000 mg/kg on 2 days	Glyphosate (96.8%)	Yes	Negative in males and females	Suresh (1994)
Chromosomal aberrations	Mouse bone marrow cells	1 080 mg/kg bw	Roundup (> 90% purity)	No	Negative in males	Dimitrov et al. (2006)
Chromosomal aberrations	Mouse bone marrow cells	50 and 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse spermatocytes	50 and 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Rat bone marrow cells	21–188 mg/kg	Glyphosate trimesium SC-0224 (58.5%)	No	Negative in males at all time points up to 5 days of exposure	Majeska (1982b)
Micronucleus	Mouse bone marrow erythrocytes	400–1 100 mg/kg	Glyphosate trimesium SC-0224 (55.3%)	Yes	Negative in males and females	Majeska (1986)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	3–50 mg/kg in the diet	Glyphosate (98.6%)	No	Negative in males and females	Chan & Mahler (1992)
Micronucleus	Mouse bone marrow erythrocytes	50–5 000 mg/kg bw; administered twice	Glyphosate (96.8%)	Yes	Negative for males; weak positive / equivocal for females at highest dose	Suresh (1993a)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw	Glyphosate (95.6%)	Yes	Negative in males and females	Fox & Mackay (1996)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate potassium salt (49% glyphosate acid by analysis) [indicated 59.3% in text]	Yes	Negative in males	Jones (1999)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78634 (65.2% glyphosate)	Yes	Negative in males	Erexson (2003)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	AK-01 Technical (99.1%)	Yes	Negative in males	Inoue (2004)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate technical (97.73%)	Yes	Negative in males and females	Honarvar (2005)
Micronucleus	Mouse bone marrow erythrocytes	1 080 mg/kg bw	Roundup (> 90% purity)	No	Negative in males	Dimitrov et al. (2006)
Micronucleus	Mouse bone marrow erythrocytes	8–30 mg/kg bw	Glyphosate technical Helm (≥ 95%)	Yes	Negative / equivocal in males	Zoriki Hosomi (2007)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate (99.1%)	Yes	Negative in males	Honarvar (2008)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 79864 (38.7% glyphosate)	Yes	Negative in males	Xu (2008a)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76171 (31.1% glyphosate)	Yes	Negative in males	Xu (2008b)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76313 (30.9% glyphosate)	Yes	Negative in males	Xu (2008c)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate (A17035A) (280 g/L)	Yes	Negative in males	Negro Silva (2009)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 79991 (71.6% glyphosate)	Yes	Negative in males	Xu (2009a)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76138 (38.5% glyphosate)	Yes	Negative in males	Xu (2009b)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78910 (30.3% glyphosate)	Yes	Negative in males	Xu (2010) [amended version of Erexson (200 6)]
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	TROP M (Glyphosate 480) (358.4 g/L glyphosate acid; 483.6 g/L glyphosate isopropylamine salt)	Yes	Negative in males and females	Flügge (2010)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate soluble liquid concentrate (A13013Z) (500 g/L)	Yes	Negative in males	Negro Silva (2011)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78239 (36.6% glyphosate)	Yes	Negative in males	Xu (2011) [amended version of Erexson (200 3)]
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate (96.3%)	Yes	Negative in males	Roth (2012)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate TGAI (98.9%)	Yes	Negative in males	Patel (2012)
Micronucleus	Rat bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate technical grade (98.8%)	Yes	Negative in males and females	Flügge (2009 b)
Micronucleus	Rat bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate 75.5 DF (69.1% glyphosate)	Yes	Negative in males and females	Flügge (2010)
Unscheduled DNA synthesis	Rat liver hepatocytes	150–600 mg/kg bw	Glyphosate trimesium ICIA0224 (57.6%)	Yes	Negative in males	Kennelly (19 90)
Sister chromatid exchange	Mouse bone marrow cells	50–200 mg/kg bw	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Intraperitoneal administration						
Chromosomal aberrations	Rat bone marrow cells	1 000 mg/kg bw	Glyphosate (98%)	No	Negative in males and females	Li & Long (1988)
Chromosomal aberrations	Mouse bone marrow cells	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse spermatocytes	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse bone marrow cells	25 and 50 mg/kg bw	Roundup (> 41%)	No	Positive in males	Prasad et al. (2009)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw	Glyphosate (98.6%)	Yes	Negative in males and females	Jensen (1991 c)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	850–3 400 mg/kg bw	Rodeo formulation (40%)	Yes	Negative in males and females	Kier, Flowers & Huffman (1992)
Micronucleus	Mouse bone marrow erythrocytes	100–200 mg/kg bw	Glyphosate isopropylamine salt	No	Negative in combined males and females	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	133 and 200 mg/kg bw as glyphosate isopropylamine salt	Roundup (480 g/L)	No	Negative in combined males and females	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	68–206 mg/kg bw	Glifos (360 g/L glyphosate)	No	Negative in males and females	Zaccaria (1996)
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	450 mg/kg bw; 135 mg/kg as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	188–563 mg/kg bw	Glyphosate technical Nufarm (95%)	Yes	Negative in combined males and females	Carvalho Marques (1999)
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate technical grade	No	Negative in males	Chruscielska et al. (2000b)
Micronucleus	Mouse bone marrow erythrocytes	90 mg/kg bw	Glyphosate formulation Perzocyd 10 soluble liquid concentrate	No	Negative in males	Chruscielska et al. (2000b)
Micronucleus	Mouse bone marrow erythrocytes	50–200 mg/kg bw	Glyphosate (Roundup 69)	No	Negative (sex not specified)	Nascimento & Grisolia (2000)
Micronucleus	Mouse bone marrow erythrocytes	1 008–3 024 mg/kg bw	Glifosato IPA Technico Nufar; glyphosate isopropylamine salt (613 g/kg salt equivalent)	Yes	Negative in males and females	Gava (2000)
Micronucleus	Mouse bone marrow erythrocytes	50–200 mg/kg bw	Roundup (480 g/L)	No	Negative in combined males and females	Grisolia (2002)
Micronucleus	Mouse bone marrow erythrocytes	150–600 mg/kg bw	Glyphosate technical grade (95.7%)	Yes	Negative/equivocal in males	Durward (2006)
Micronucleus	Mouse bone marrow erythrocytes	15.6–62.5 mg/kg bw	Glyphosate technical grade (98%)	Yes	Negative in males and females	Costa (2008)
Micronucleus	Mouse bone marrow erythrocytes	25 and 50 mg/kg bw	Roundup (> 41%)	No	Positive in males	Prasad et al. (2009)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	100–400 mg/kg bw	Glyphosate (analytical grade; 96%)	No	Positive in combined males and females	Manas et al. (2009a)
Micronucleus	Mouse bone marrow erythrocytes	0.148–1.28 mg/kg bw	Roundup	No	Positive (sex not specified)	Rodrigues et al. (2011)
DNA strand breaks	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
DNA strand breaks	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
DNA adducts by ^{32}P-postlabelling	Liver and kidney of mice	130 and 270 mg/kg	Glyphosate isopropylammonium salt	No	Negative in combined males and females	Peluso et al. (1998)
DNA adducts by ^{32}P-postlabelling	Liver and kidney of mice	400–600 mg/kg	Roundup (30.4%)	No	Positive in combined males and females	Peluso et al. (1998)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	600 and 900 mg/kg bw	Glyphosate formulation (30.4%)	No	Negative in males	Heydens et al. (2008)
AMPA						
Micronucleus	Mouse bone marrow erythrocytes	100–1 000 mg/kg bw IP	AMPA (94.4%)	Yes	Negative in males and females	Kier & Stegeman (1993)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw oral route	AMPA (99.2%)	Yes	Negative in males and females	Jensen (1993c)
Micronucleus	Mouse bone marrow erythrocytes	200–400 mg/kg bw IP	AMPA (99%)	No	Positive	Manas et al. (2009b)
***N*-acetyl-AMPA**						
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw (active ingredient, adjusted for purity) oral route	*N*-acetyl-AMPA (72%; IN-EY252)	Yes	Negative in males and females	Donner (2007)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
***N*-Acetyl-glyphosate**						
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw (active ingredient, adjusted for purity) oral route	*N*-acetyl-glyphosate (63%; IN-MCX20)	Yes	Negative in males and females	Donner (2006)
Other related chemicals						
Chromosomal aberrations	Mouse bone marrow cells	10 and 100 mg/kg bw	Series of α-aminophosphonic acids	No	Positive	Naydenova et al. (2007)

AMPA: aminomethylphosphonic acid; bw: body weight; GLP: Good laboratory practice; IP: intraperitoneal; N/S: not stated; 8-OHdG: 8-hydroxy-2'-deoxyguanosine

(c) Non-traditional tests or tests in phylogenetically distant organisms

The results of genotoxicity studies in phylogenetically distant organisms or using non-traditional and generally non-validated assays are presented in Appendix 1. Studies were performed both in vitro and in vivo with most of the tests measuring DNA strand breakage or micronucleus formation. Approximately two thirds of these studies reported positive results. Mixed positive and negative results were seen in mutation studies in *Drosophila*. The reason for the differences in response between these species and those seen in mammals orally administered glyphosate is not known. Surfactants and other components of the glyphosate formulation products have been reported to be toxic to fish and other species, and this may contribute to the observed differences in test results (Howe et al., 2004; Guilherme et al., 2012a; Navarro & Martinez, 2014). For example, the surfactant polyoxyethylene amine, a common component in glyphosate formulations, was shown to induce several indices of toxicity in the neotropical fish *Prochilodus lineatus* at all of the doses tested (Navarro & Martinez, 2014).

(d) Human biomonitoring studies

The association between exposure to glyphosate and increase in micronucleus frequencies in peripheral blood lymphocytes, as well as the persistence of any effects over time, was evaluated over several months in individuals living in three areas of Colombia where glyphosate formulations were aerially sprayed over illicit and legal crops (Bolognesi et al., 2009). Significant increases in micronucleus frequencies were observed several days after spraying, but these increases did not correlate with glyphosate spray rates. Over time, the induced micronucleus frequencies decreased among the people in one area, remained the same among those in another, and increased among those in the third. In addition, in all three communities, the micronucleus frequencies of individuals who reported being directly exposed to glyphosate did not differ from those who reported no glyphosate exposure.

The JMPR committee reviewed the studies and considered the results to be inconclusive or equivocal. It noted that the micronucleus frequencies in the reference population were unusually low and that the frequencies within the glyphosate-exposed communities fall well within the normal range for non-exposed individuals (Bonassi et al., 2001). The results were considered to be inadequate to reach a conclusion on the potential chromosome-damaging properties of glyphosate in humans.

Another study used the Comet assay to determine the frequency of DNA strand breakage in the peripheral blood lymphocytes of individuals living in an Ecuadorian community within 3 kilometres of where glyphosate was sprayed. The frequency of DNA strand breakage was reported to be significantly higher than that of individuals living in a community 80 kilometres away where

glyphosate was not used (Paz-y-Mino et al., 2007). The samples were collected from exposed individuals 2 weeks to 2 months after the spraying had occurred. In reviewing the study, the JMPR committee noted that the study had some major deficiencies; the blood samples of the two groups were collected and processed at different times, a key consideration for an assay that is highly prone to technical artefacts during sample preparation. In addition, the two populations were located at considerable distance from each other, the background frequencies of DNA breakage in these communities was not known, and the median DNA migration values were identical for 20 of the 21 subjects in the control population, a result that was considered to be highly unusual.

The JMPR committee concluded that the study was inconclusive as problems with study design severely limit the conclusions that can be drawn.

In a follow-up study by the same authors, the frequency of structural chromosomal aberrations in peripheral blood lymphocytes was measured in the study population that two years previously had been exposed to glyphosate; the frequencies were found to be normal (Paz-y-Mino, 2011). The study results were considered to be negative but minimally informative as many types of chromosome alterations do not persist for extended periods of time.

In another study, the levels of 8-OHdG, a lesion formed from oxidative damage to DNA, were measured in the peripheral blood lymphocytes of workers spraying glyphosate (Koureas et al., 2014). A modestly elevated but statistically nonsignificant increase was reported.

Summaries of these biomonitoring studies are shown in Table 36.

Table 36. Summary of human biomonitoring studies

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Structural chromosomal aberrations	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Colombia	Glyphosate-containing mixture	No	Negative	Paz-y-Mino et al. (2011)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Narino, Colombia	Herbicide mixtures containing glyphosate and adjuvant	No	Equivocal/inconclusive	Bolognesi et al. (2009)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Putumayo, Colombia	Herbicide mixtures containing glyphosate and adjuvant	No	Equivocal / inconclusive	Bolognesi et al. (2009)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Valle del Cuaca, Colombia	Roundup 47	No	Equivocal / inconclusive	Bolognesi et al. (2009)
DNA strand breaks/Comet	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Colombia	Roundup Ultra (44%)	No	Equivocal/ inconclusive	Paz-y-Mino et al. (2007)
DNA adducts (8-OHdG)	Human peripheral blood cells	Pesticide applicators	Glyphosate	No	Negative	Koureas et al. (2014)

8-OHdG: 8-hydroxy-2'-deoxyguanosine

duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

No adverse effects were noted in the study. The NOAEL for parental, reproductive and offspring toxicity was 30 mg/kg bw per day, the highest dose tested (Schroeder & Hogan, 1981).

In a two-generation reproduction study, glyphosate (purity 97.67%) was administered to Sprague Dawley rats (30/sex per dose) in the diet at concentrations of 0, 2000, 10 000 or 30 000 ppm (equal to 0, 132, 666 and 1983 mg/kg bw per day for males and 0, 160, 777 and 2322 mg/kg bw per day for females). After approximately 11 weeks of treatment, pairs of animals within each dose group were mated on a 1:1 basis to produce the F_1 litters. At weaning, 30 of these F_1 generation rats (referred to as F_{1A} in study report) per sex per dose were similarly exposed (approximately 14 weeks) and mated twice to produce F_{2A} and F_{2B} generations. On day 4 postpartum, litters were standardized (four males and four females when possible). Offspring not selected for mating, F_{2A} and F_{2B} pups, and adult females which had littered were terminated on or after day 21 of lactation. Adult males were terminated after mating. Organs were retained from all parental animals and one pup per sex per litter from F_{2A} and F_{2B}. Tissues from control and high-dose animals were examined microscopically.

The stability and homogeneity of glyphosate in the diet were acceptable. Analytical concentrations were, on the average, 95–96.7% of target levels. No treatment-related adverse effects were observed on mortality, feed consumption, organ weights or histopathological changes for parental animals of either generation. The incidence of soft stools was increased for high-dose adult animals in both generations (Table 37). Reduced body weights were noted in parental animals of both generations at termination: body weights were approximately 8–10% lower than controls for the F_0 generation and 10–13% lower than controls in the F_1 generation (Table 38).

No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Compared to the controls, there was a slight reduction in average litter size for F_0 dams in the highest dose group; an even smaller difference was noted after the first F_1 mating. However, the slight reduction in average litter size was not statistically significant. The F_{1a} adults were re-mated to produce the F_{2b} generation. There was no dose-related decrease in litter size in this second mating. Since the reductions in litter size were neither statistically significant nor consistently observed in all generations, the relationship to treatment could not be conclusively established. Therefore, it was concluded that litter size and viability were not affected by treatment.

No adverse effects were noted for offspring body weights or development. Statistically significant differences in pup body weights compared to controls were observed at mid and high dose, but these differences were small and within biologically variability.

Table 37. Soft stools in two successive generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 ppm	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
F_0 – males				
No. of animals	0	0	0	30/30
No. of occurrences	0	0	0	457
F_0 – females				
No. of animals	0	0	0	22/30
No. of occurrences	0	0	0	116
F_1 – males				
No. of animals	0	0	1/30	30/30
No. of occurrences	0	0	1/30	698
F_1 – females				
No. of animals	0	0	0	29/30

	Incidence per dietary concentration of glyphosate			
	0 ppm	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
Number of occurrences	0	0	0	537

ppm: parts per million; F_0: parental generation; F_1: first filial generation; No.: number

Results presented as number of animals with soft stools / number of animals examined.

Source: Reyna (1990)

Table 38. Terminal body weights in two successive generations of parental rats administered glyphosate

	Weight per dietary concentration of glyphosate			
	0	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
F_0				
Males	549.6 ± 46.8	550.2 ± 80.7	540.0 ± 58.1	503.5 ± 45.7 (↓8%)
Females	296.3 ± 23.6	290.6 ± 19.5	290.7 ± 25.4	265.9 ± 15.4 (↓10%)
F_1				
Males	625.0 ± 53.1	632.1 ± 74.6	591.0 ± 70.1	543.4 ± 58.1 (↓13%)
Females	316.2 ± 37.4	313.7 ± 30.5	312.4 ± 26.7	284.7 ± 18.4 (↓10%)

ppm: parts per million; F_0: parental generation; F_1: first filial generation; no.: number; ↓: decrease

Results presented as mean weight in grams ± standard deviations, with per cent change relative to controls in parentheses for the high-dose group only.

Source: Reyna (1990)

The NOAEL for parental toxicity was 10 000 ppm (equal to 666 mg/kg bw per day) based on decreased body weights and increased incidence of soft stools in rats at 30 000 ppm. As there were no effects on reproductive parameters or offspring measurements, the NOAEL for reproductive and offspring toxicity was 30 000 ppm (equal to 1983 mg/kg bw per day (Reyna, 1990).

In a two-generation reproduction study, 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 concentrations of 0, 1000, 3000 or 10 000 ppm (equal to 0, 66.4, 196.8 and 668.1 mg/kg bw per day for males and 0, 75.3, 226.0 and 752.3 mg/kg bw per day for females) for 70 days before their first mating and until termination. Each generation was mated twice, changing partners for the second mating and avoiding sister/brother matings throughout. On postnatal day 4, litters were standardized (four males and four females, when possible). The remaining pups and those not selected for mating were terminated and underwent gross pathological examinations. Treatment was continued for parental animals until day 21 of weaning of the second litter when animals were terminated for organ weighing, gross pathological examination and histopathological examination. Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analysed when an effect was seen in a tissue at the highest dose.

No treatment-related adverse effects on mortality, clinical signs, body weights, feed consumption, feed efficiency or organ weights were observed for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

Treatment-related histopathological changes were found in the parotid salivary gland of both sexes and submaxillary salivary gland of females in both generations (Table 39). The changes were

described as hypertrophy of acinar cells with prominent granular cytoplasm (minimal severity). Increased incidence of the effects was observed at the highest dose tested.

Table 39. Cellular alterations in salivary glands of two successive generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate							
	Males				Females			
Site of cellular alteration	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**
F_0								
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								
Parotid gland	1/24	0/24	4/23	11/23	0/24	0	4/24	9/23
Submaxillary gland	0/24	–	–	0	0/24	0	0/24	3/23

ppm: parts per million; F_0: parental generation; F_1: first filial generation; –: not examined.

Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analysed when an effect was seen at the highest dose.

Results presented as number of animals with hypertrophy of acinar cells with prominent granular cytoplasm / number of animals examined.

Source: Brooker et al. (1992)

The NOAEL for parental toxicity was 3000 ppm (equal to 196.8 mg/kg bw per day, based on increased incidence of histopathological effects observed in the parotid (males and females) and submaxillary (females only) salivary glands in both generations of rats at 10 000 ppm (equal to 668.1 mg/kg bw per day). As there were no effects on reproductive parameters or offspring measurements, the NOAEL for reproductive and offspring toxicity of glyphosate in rats is 10 000 ppm (equal to 668.1 mg/kg bw per day) (Brooker et al., 1992).

In a two-generation reproduction study, glyphosate (purity 96.8%) was administered to Wistar (30 rats/sex per dose) in the diet at concentrations of 0, 100, 1000 or 10 000 ppm (equivalent to 0, 6.6, 66.0 and 660 mg/kg bw per day) for two successive generations with one litter per generation. The mean daily intake of glyphosate was not reported for all dietary levels; however, the low dose of 100 ppm corresponds to an average of 7.7 mg/kg bw per day according to the original study report. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. On day 4 postpartum, litters were standardized (four males and four females, if possible). At weaning, 30 males and 30 females from each dose group were selected to produce the F_1 generation; these rats were dosed for at least 10 weeks and paired within their dose group to produce F_2 litters. All parental animals, non-selected pups from F_1 and all pups from F_2 were necropsied. Only parental tissue was collected.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

As no adverse effects were noted in the study, the NOAEL for parental, reproductive and offspring toxicity in rats was 10 000 ppm (equivalent to 660 mg/kg bw per day), the highest dose tested (Suresh, 1993b).

In a two-generation reproduction study, glyphosate (purity 94.61%) was administered to 24 Crl:CD(SD) rats/sex per dose at concentrations of 0, 1200, 6000 and 30 000 ppm (equal to 0, 83.6, 417 and 2150 mg/kg bw per day for males and 0, 96.9, 485 and 2532 mg/kg bw per day for females) for two successive generations with one litter per generation. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. On day 4 postpartum, litters were standardized (four males and four females, if possible). At weaning, 24 males and 24 females from each dose group were selected to produce the F_1 generation. Unselected offspring were terminated and underwent gross necropsy. The offspring selected for the F_1 generation were dosed for at least 10 weeks and paired within dose group to produce F_2 litters. At weaning, parental animals and their offspring were terminated and examined macroscopically. Organs were taken from all parental animals for weights and histopathological examination. For offspring, the same organs were taken from one animal per sex per litter at random. The overall calculated mean daily intake of glyphosate was 0, 84, 417 and 2150 mg/kg bw per day for F_0 males; 0, 97, 485 and 2532 mg/kg bw per day for F_0 females; 0, 92, 458 and 2411 mg/kg bw per day for F_1 males; and 0, 105, 530 and 2760 mg/kg bw per day for F_1 females.

There were no treatment-related adverse effects on mortality, body weights, feed consumption, feed efficiency or histopathological changes for parental animals of either generation. The incidence of loose stools was increased for high-dose parental animals in both generations (Table 40). In addition, the incidences of caecum distension were increased in high-dose parental animals in both generations (Table 41). Although increases in liver and kidney weights were noted in the high-dose group, these changes were not considered adverse given the magnitude of the change and/or lack of corresponding histopathological changes in these organs.

Table 40. Loose stools in two generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate											
	Pre-mating				**Mating/gestation**				**Lactation/post-weaning**			
	0 ppm	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**
F_0												
M	0/24	0/24	0/24	3/24	0/23	0/24	0/24	2/24	N/A	N/A	N/A	N/A
F	0/24	0/24	0/24	1/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	6/24
F_1												
M	0/24	0/24	0/24	13/24	0/23	0/24	0/23	0/24	N/A	N/A	N/A	N/A
F	0/24	0/24	0/24	4/24	0/23	0/23	0/21	0/19	0/23	0/23	0/21	2/19

F: female; F_0: parental generation; F_1: first filial generation; M: male; N/A: not applicable; ppm: parts per million

Results presented as number of animals with loose stools / number of animals examined.

Source: Takahashi (1997)

Table 41. Incidence of caecum distension in three generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 ppm	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**
F_0				
Males	0/24	0/24	0/24	21/24
Females	0/24	0/24	0/24	24/24
F_1				
Males	0/24	0/24	0/24	19/24
Females	0/24	0/24	0/24	17/24
Pups	0/136	0/141	0/143	89/141
F_2				
Pups	0/182	0/183	0/164	111/149

ppm: parts per million; F_0: parental generation; F_1: first filial generation; F_2: second filial generation

Results presented as number of animals with caecum distension / number of animals examined.

Source: Takahashi (1997)

No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. Body weights of offspring at high doses were decreased in both generations, starting typically on postnatal day 14 (Table 42). Gross pathological examinations found an increased incidence of caecum distension in high-dose offspring of both generations.

Table 42. Mean body weights of two generations of offspring of rats administered glyphosate

	Mean body weights per dietary concentration of glyphosate							
	F_1 pups – male				**F_2 pups – male**			
PND	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**
0	6.7 ± 0.6	6.8 ± 0.5	6.7 ± 0.4	7.2 ± 0.7*	7.0 ± 0.5	6.9 ± 0.6	7.3 ± 0.7	7.1 ± 0.5
4	11.6 ± 1.2	11.6 ± 1.2	11.7 ± 1.0	11.6 ± 1.2	12.0 ± 1.2	12.1 ± 1.5	12.5 ± 1.5	12.5 ± 1.3
7	19.5 ± 1.7	19.1 ± 2.0	19.5 ± 1.6	19.3 ± 1.2	19.8 ± 1.5	20.0 ± 1.9	20.4 ± 2.2	20.6 ± 1.7
14	39.5 ± 3.2	39.4 ± 2.6	39.3 ± 2.6	36.6 ± 2.6**	40.1 ± 3.0	39.0 ± 2.8	38.7 ± 2.9	39.1 ± 2.8
21	63.9 ± 4.4	63.8 ± 4.1	62.4 ± 3.7	55.1 ± 3.5***	58.6 ± 5.1	59.4 ± 4.4	58.3 ± 4.3	53.1 ± 4.4**
	F_1 pups – female				**F_2 pups – female**			
0	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	6.8 ± 0.6*	6.6 ± 0.5	6.6 ± 0.7	6.8 ± 0.6	6.8 ± 0.6
4	11.1 ± 1.2	11.2 ± 1.1	11.3 ± 0.9	11.3 ± 1.2	11.6 ± 1.2	11.5 ± 1.6	12.0 ± 1.5	12.1 ± 1.1
7	18.6 ± 1.8	18.4 ± 1.9	18.8 ± 1.5	18.3 ± 1.6	18.9 ± 2.0	19.1 ± 2.1	19.6 ± 2.2	19.9 ± 1.4
14	38.4 ± 3.6	37.9 ± 2.6	38.2 ± 2.2	35.4 ± 2.6**	38.7 ± 3.5	38.0 ± 2.2	37.5 ± 2.9	38.1 ± 2.9
21	61.0 ± 4.8	60.6 ± 3.9	59.8 ± 3.1	53.2 ± 4.0***	56.4 ± 5.5	57.1 ± 4.4	56.2 ± 4.5	51.8 ± 4.2*

F_1: first filial generation; F_2: second filial generation; PND: postnatal day; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

Results presented are mean weights in grams ± standard deviation. Statistics from study report.

Source: Takahashi (1997)

The NOAEL for parental toxicity was 6000 ppm (equal to 417 mg/kg bw per day) based on increased incidence of loose stools and caecum distension in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day). As there were no effects on reproductive parameters the NOAEL for reproductive toxicity was 30 000 ppm (equal to 2150 mg/kg bw per day). The NOAEL for offspring toxicity was 6000 ppm (equal to 417 mg/kg bw per day) based on decreased pup body weights and increased incidence of caecum distension in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day) (Takahashi, 1997).

In a two-generation reproduction study, groups of 26 male and female Wistar-derived Alpk:AP$_f$SD rats (aged 5–6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity 97.6%) at concentrations of 0, 1000, 3000 or 10 000 ppm (equal to 0, 99.4, 292.6 and 984.7 mg/kg bw per day for males and 0, 104.4, 322.8 and 1054.3 mg/kg bw per day for females) for 10 weeks before their first mating and until termination. Each generation was mated twice avoiding sister/brother matings throughout. Males were terminated after completion of mating and females on or soon after day 29 of lactation, after which their organs were weighed and gross pathological and histopathological examinations conducted. The offspring not selected for mating were also terminated on day 29 postpartum, with one pup/sex per litter used for organ-weight determination and two pups /sex per litter given macroscopic examinations. All the remaining pups were terminated with no further examination.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment.

The body weights of F_{1A} pups were lower compared to the control group from day 8 onwards, but a similar effect was not seen in the F_{2A} pups. There was no treatment-related effect on total litter weight (Table 43).

Table 43. Mean body weights of two successive generations of offspring of rats administered glyphosate

	Mean body weights per dietary concentration of glyphosate (g)							
	Males				**Females**			
PND	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**
F_{1A}								
1	5.8	6.1	6.0	6.1	5.4	5.8	5.6	5.7
5	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
8	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
15	26.8	26.1	25.8	24.6*	26.1	25.2	24.5	23.8*
22	43.4	42.4	41.4	39.2*	41.9	40.3	39.4	37.7*
29	81.7	79.5	79.6	74.6*	77.1	74.0	74.1	69.9**
F_{2A}								
1	6.3	6.3	6.3	6.2	6.1	5.9	5.9	5.8
5	9.7	9.9	9.3	9.5	9.3	9.6	9.1	9.1
8	14.3	14.7	13.8	14.2	13.8	14.2	13.4	13.7
15	27.4	28.3	26.4	27.5	26.7	27.5	25.8	26.5
22	44.5	46.2	43.1	44.9	42.7	44.8	41.8	42.9

	Mean body weights per dietary concentration of glyphosate (g)							
	Males				Females			
PND	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm
29	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4

F_{1A}: first filial generation, first litter; F_{2A}: second filial generation, second litter; PND: postnatal day; ppm: parts per million; *: $P = 0.05$ (Student *t*-test, 2 sided); **: $P = 0.01$ (Student *t*-test, 2 sided)

Source: Moxon (2000)

As no adverse effects were noted in the study, the NOAEL for parental and reproductive toxicity was 10 000 ppm (equal to 984.7 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 3000 ppm (equal to 292.6 mg/kg bw per day) based on reduced pup weights in the F_{1A} generation seen at 10 000 ppm; equal to 984.7 mg/kg bw per day (Moxon, 2000).

In a two-generation reproduction study, glyphosate (purity 95.7%) was administered in the diet to 28 Crl:CD(SD) IGS BR rats per sex per dose at 0, 1500, 5000 or 15 000 ppm (equal to 0, 104, 351 and 1063 mg/kg bw per day in males and 0, 126, 423 and 1273 mg/kg bw per day in females) for two successive generations with one litter per generation. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. At weaning, 24 males and 24 females from each dose group were selected to produce the F_2 generation. Surviving adult females and males and unselected offspring were terminated on day 21 postpartum. All adult animals and offspring underwent macroscopic examinations and parental organs were weighed. A small subset of organs were taken from one male and one female offspring from the F_0 and F_1 pairings (where available). Tissues from control and high-dose F_0 and F_1 animals underwent histopathological examination. As there were indications of changes in the adrenal glands of F_1 animals, microscopic examination was extended to include all dose groups.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed or feed efficiency, organ weights or histopathological changes in parental animals of either generation. No adverse effects were observed on mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size, viability and offspring body weights were not affected by treatment. Complete preputial separation was delayed by 2.9 days in high-dose F_1 male pups (2.9 days) and body weights were increased by 10% at attainment. There were no treatment-related effects on the age or weight at attainment of vaginal opening.

As there were no effects for parental animals or on reproductive parameters, the NOAEL for parental and reproductive toxicity was 15 000 ppm (equal to 1063 mg/kg bw per day), the highest dose tested. The NOAEL for offspring was 5000 ppm (equal to 351 mg/kg bw per day), based on delayed age and increased weight at attainment of preputial separation at 15 000 ppm (equal to 1063 mg/kg bw per day) (Dhinsa, 2007).

(b) Developmental toxicity

Rats

In a pre-GLP developmental toxicity study, glyphosate (purity 98.7%) suspended in 0.5% aqueous Methocel was administered to 25 copulated CD female rats per dose by oral gavage at concentrations of 0, 300, 1000 or 3500 mg/kg bw per day from gestation day 6 through 19. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

Soft stools, diarrhoea, red nasal discharge, reduced activity and rales (abnormal respiratory noise) were noted in the highest dose group. By gestation day 17, six rats in this group had died. A

Table 44. Skeletal anomalies in fetuses and litters of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 mg/kg bw per day	**300 mg/kg bw per day**	**1000 mg/kg bw per day**	**3500 mg/kg bw per day**
Fetal anomolies[a]	19/155	36/143	46/166	55/142
Litter anomolies[b]	11/23	16/23	19/25	19/22
Fetal skeletal variations (%)[c]	11.7	22.6	28.4*	35.7**
Historical range	21.9–27.2			

bw: body weight; *: $P < 0.05$; **: $P < 0.01$

Kruskal–Wallis H-statistic followed, if significant, by intergroup comparison with control (distribution-free Williams' test).

[a] Results presented as number of fetuses with skeletal anomalies / total number of fetuses.

[b] Results presented as number of litters with skeletal anomalies / total number of litters.

[c] Results expressed as number of fetuses with skeletal variations (with malformed fetuses excluded) as a percentage of the total number of fetuses examined.

Source: Brooker et al. (1991a)

The NOAEL for maternal toxicity was 300 mg/kg per day based on clinical signs and reduced body-weight gain at 1000 mg/kg bw per day and higher. The NOAEL for developmental toxicity was 300 mg/kg per day based on an increased incidence of delayed ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day and higher (Brooker et al., 1991a).

In a developmental toxicity study, glyphosate (purity 95.68%) suspended in a 0.5% aqueous solution of sodium carboxymethylcellulose was administered to 24 copulated Crj:CD(SD) female rats/dose by oral gavage at concentrations of 0, 30, 300 or 1000 mg/kg bw per day from gestation day 6 through 15. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in mortality, body weight, feed consumption or macroscopic findings in dams. An increased incidence of slightly loose stools was observed during the dosing period in 20 of the 22 pregnant females at 1000 mg/kg bw per day. Of these 20 animals, 9 still displayed the effect on the day after the last dosing.

There were no effects on number, growth or survival of fetuses. Any external, visceral or skeletal abnormalities were considered secondary to maternal toxicity; furthermore, the effects were also seen in the control group, incidences of the effects were low and/or there was no dose–response relationship for the effect.

The NOAEL for maternal toxicity was 300 mg/kg bw per day based on the increased incidence of slightly loose stools observed in dams at 1000 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 1000 mg/kg bw per day (Hatakenaka, 1995).

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionized water was administered to 24 time-mated female Alpk:APfSD (Wistar-derived) rats/dose by oral gavage at 0, 250, 500 or 1000 mg/kg bw per day from gestation day 7 through 16. On gestation day 22, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All the fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

than the other treatments groups; however, when taking into account the variability for these measurements, the changes were not considered adverse.

Incidences of external, visceral or skeletal variations/malformations in fetuses in the low- and mid-dose groups did not differ from those of the control group (Table 45). At 500 mg/kg bw per day, incidences of variations/malformations were higher than in the control group, but in many cases the increase was minimal or similar to the 125 and 250 mg/kg bw per day dose groups when evaluated on a litter basis. These increases in incidences of variations/malformation were observed in the presence of severe maternal toxicity. The occurrences of a variety of low-incidence fetal effects (malformations) were slightly increased at higher dose levels. These increases are considered secondary to maternal toxicity.

Table 45. Malformations and variations in fetuses and litters of rabbit administered glyphosate

	Incidence per dietary concentration of glyphosate			
Malformations / variations	**0 mg/kg bw per day**	**125 mg/kg bw per day**	**250 mg/kg bw per day**	**500 mg/kg bw per day**
Number of litters examined	13	14	14	12
Number of fetuses examined	109	113	120	78
Malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	2 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	2 (2)	6 (4)
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle smaller than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal fetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)
Cervical central 1–3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extrathoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra – 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)

Malformations / variations	Incidence per dietary concentration of glyphosate			
	0 mg/kg bw per day	125 mg/kg bw per day	250 mg/kg bw per day	500 mg/kg bw per day
Number of litters examined	13	14	14	12
Number of fetuses examined	109	113	120	78
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	3 (2)	2 (1)	2 (2)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

bw: body weight

Results presented as number of fetuses with malformations and variations and, in parentheses, the number of litters with malformations and variations.

Source: Bhide & Patil (1989)

The NOAEL for maternal toxicity was 250 mg/kg bw per day based on abortions observed at 500 mg/kg bw per day in rabbits. The NOAEL for developmental toxicity was 250 mg/kg bw per day based on increased incidence of variations/malformations observed at 500 mg/kg bw per day in rabbits. It should be noted that individual data, uterine weights, maternal necropsy results and statistical analyses were not provided for this study; therefore, the NOAEL and LOAEL values are based on the available data (Bhide & Patil, 1989).

In a developmental toxicity study, glyphosate acid (purity 98.6%) suspended in a 1% aqueous methylcellulose solution was administered to 19, 19, 16 or 20 New Zealand White rabbits per dose by oral gavage at concentrations of 0, 50, 150 or 450 mg/kg bw per day, respectively, from gestation day 7 through 19. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in body weight, feed consumption or macroscopic findings for dams. One high-dose animal was found dead on day 20 following signs of abortion on day 19 and soft/liquid faeces, a reduction in feed intake and body-weight loss from the start of treatment. The incidence of soft/liquid faeces was increased at the high dose (13/20 animals).

There were no treatment-related adverse effects on the number, growth or survival of fetuses. At termination, 18, 12, 15 and 13 pregnant females were available for evaluation in the control, low, mid and high doses, so evaluation of developmental effects is limited at the low and high doses. Embryo/fetal death and post-implantation loss were increased in all treatment groups; however, there was no dose–response and the values were within or slightly above the historical control range.

Any external, visceral or skeletal abnormalities were not considered treatment related. There was a slightly increased incidence of cardiac malformation (interventricular septal defect) at the high dose (4/13 pregnant animals); however, it was barely outside of the historical control range from studies conducted during the same period, and the number of litters to evaluate this dose was reduced. Furthermore, this effect was considered secondary to the maternal toxicity observed at 450 mg/kg bw per day.

The NOAEL for maternal toxicity was 150 mg/kg bw per day based on clinical signs (soft/liquid faeces) at 450 mg/kg bw per day in rabbits. The NOAEL for developmental toxicity was 150 mg/kg bw per day based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw per day (Brooker et al., 1991b).

implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in body weight, feed consumption or macroscopic findings for dams. One high-dose female was found dead prior to dosing on day 19 and another was terminated in extremis on day 20; one death also occurred in the control group and in the mid-dose group. An increased incidence of diarrhoea was observed at the high dose in 10 of the 16 surviving pregnant females. All other clinical observations were isolated or a dose–response relationship was not observed.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. The increases in late fetal deaths and post-implantation loss noted at the high doses were not considered adverse once the variability in the measurements were taken into consideration. In addition, the increase can mainly be attributed to one animal with nine late-death fetuses. No treatment-related external, visceral or skeletal abnormalities were observed.

The NOAEL for maternal toxicity was 200 mg/kg bw per day based on increased incidence of diarrhoea in dams at 400 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 400 mg/kg bw per day (Coles & Doleman, 1996).

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionized water was given to 20 time-mated New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 100, 175 or 300 mg/kg bw per day from gestation day 8 through 20. On gestation day 30, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in mortality, body weight, feed consumption or macroscopic findings for dams. There was a significant increase in the incidence of either diarrhoea or decreased faecal output at the mid and high doses (no statistical significance was provided) (Table 46). The incidence of staining in the genital area was also increased at the high dose.

Table 46. Clinical signs in pregnant rabbits administered glyphosate by gavage

		No. per dietary concentration of glyphosate		
Clinical sign	**0 mg/kg bw per day**	**100 mg/kg bw per day**	**175 mg/kg bw per day**	**300 mg/kg bw per day**
Few faeces in tray	3	3	9	9
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11

bw: body weight; no. number

Source: Moxon (1996b)

There were no treatment-related adverse effects on the number, growth or survival of fetuses. Although mean fetal weight was reduced at the high dose, this was not considered adverse once the variability in the measurements was taken into account. In addition, the decrease could be attributed to two litters with lower weights. Any external, visceral or skeletal abnormalities were not considered treatment related.

The NOAEL for maternal toxicity was 100 mg/kg bw per day based on increased incidence of clinical signs (decreased faecal output or signs of diarrhoea) in rabbits at 175 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 300 mg/kg bw per day (Moxon, 1996b).

Table 47. Summary of information supporting EDSP data in relation to glyphosate and endocrine end-points

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Estrogen pathway						
EDSP Tier 1 data 2014/2015: In vitro: ER binding; TG 455 ER STTA and Hela Assay In vivo: mammalian assays, i.e. uterotrophic and female pubertal assays and mammalian toxicity studies	Glyphosate Purity: 85.1–95.93% Concentration range: 10^{-10} to 10^{-3} mol/L	USEPA validated assays In vitro assays are well-characterized and OECD TGs ER STTA: uses HeLa cell line which has ER α not ER β. ER α perturbation is more strongly associated with adverse outcomes	There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies, however decreases in offspring body weight were observed in one avian reproduction study	High	Negative	USEPA (2015)
In vitro: ER agonism in estrogen-dependent T47D human breast cancer cells	Glyphosate Purity > 98%) Accustandard Concentration range: 10^{-12} to 10^{-6} mol/L	Validated assay	Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, and not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} mol/L in estrogen withdrawal condition, which was reported to be confirmed by the inhibitory effect of the ER antagonist ICI 182780. The T47D cell line contains both ERα and ERβ. While the use of ICI 182780 can exclude the possibility of dioxin-like interference of coformulant contaminant 1,4-dioxane with AhR interactions affecting the ER, this study is confounded because it was not tested with an ER*α*-specific antagonist, such as methylpiperidino pyrazole (CAS No. 289726-02-9). This would determine the relative activities of each ER (Evans, Gray & Wilson, 2012) The luciferase reporter system was then also used with combinations of genistein, an isoflavone in soy. Phytoestrogens such as genistein are known to overstimulate luciferase, and also are stronger ligands for ERβ. Non-receptor–mediated luminescence signals have	Low	Positive	Thongprakaisang et al. (2013)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			been reported at phytoestrogen concentrations higher than 1 µmol/L due to the over-activation of the luciferase reporter gene (Kuiper et al., 1998; Escande et al., 2006). While the dose–response curve indicates that true activation of the ER system occurs at lower concentrations, luciferase expression obtained at high concentrations of phytoestrogens or similar compounds suspected of producing phytoestrogen-like over-activation of the luciferase reporter gene needs to be examined carefully in stably transfected ERTA assay systems. (See Annex 2 of OECD TG 455)			
In vitro: hERα and hERβ (ant)agonism in reporter gene–transfected CHO cells	Glyphosate (> 95–100%); whether this is a formulation is not specified in the paper. Concentrations for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.		Concentrations of pesticides that tested negative, which included glyphosate, are not reported; only the results of those that tested positive are provided. Concentration of positively testing chemicals ranged from 10^{-6} to 10^{-12} mol/L Concentration of the test chemicals showing 20% of the agonistic activity of 10^{-10} mol/L 17β-estradiol, and is given as REC 20(mol/L)	Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hERα and hERβ transient transfection into human hepatocarcinoma HepG2 cells	Glyphosate formulations and glyphosate parent chemical. Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line	Formulations reduced transcription of *ERα* and *ERβ* in HepG2 cells transiently transfected with ERE, but glyphosate parent did not	Med	Parent-negative Formulations-positive	Gasnier et al. (2009)
In vivo: FSTRA		In this validated assay, the non-treatment-responsive decrease (only significant at mid-treatment) in VTG was seen in isolation in the absence of any treatment-related effects in the other estrogen-related end-points such as gonado-somatic		Med	Negative	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
		index, gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology				
In vivo: Rainbow trout VTG assay	Glyphosate and glyphosate plus surfactants; measured concentration of glyphosate 0.11 mg/L for 7 days	VTG induction in fish is a standard measure for estrogenicity in environmental regulatory toxicology that also considers the relevance to humans (e.g. USEPA FIFRA SAP 2009a,b, 2012). Glyphosate did not increase plasma VTG in levels in juvenile rainbow trout, glyphosates plus surfactants were only marginally greater than the controls, no trend, no significance		Med	Negative	Xie et al. (2005)
Overall conclusion: No convincing evidence of a potential interaction with the estrogen pathway. The one in vitro study that is positive has not been reproduced by another laboratory.						
Androgen pathway						
EDSP Tier 1 data 2014/2015: In vitro: negative; both for androgen-receptor binding assay and the aromatase assay In vivo mammalian assays: Hershberger and male pubertal assays	Glyphosate	Standardized and validated assays	Androgen-receptor binding assay is not a validated OECD TG but other validated androgen receptor assays not available in 2014/2015 Aromatase assay: highest soluble test concentration of glyphosate was 10^{-3} mol/L The in vivo Tier 1 FSTRA and mammalian assays (i.e. Hershberger and male pubertal assays) were negative in the absence of overt toxicity. The only treatment-related effects observed in the Part 158 mammalian studies in the absence of overt toxicity were decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial	High	Negative, but sperm count and delay in preputial separation effects seen at very high doses, > 1 000 mg/kg bw per day	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			separation at 1 234 mg/kg bw per day in the post-1998 two-generation reproduction study in rats (the EDSP Tier 2 study). Both effects were observed at a dose that was above the limit dose (1 000 mg/kg bw per day) for those studies. No androgen-related effects were seen in the wildlife Part 158 studies (decreases in offspring body weight observed in one avian reproduction study)			
In vitro: hAR transactivation assay in CHO cells	Glyphosate (> 95–100%) formulation not specified in the paper Concentrations given for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.			Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hAR transient transfection into human HepG2 cells, aromatase evaluation within the HepG2 cells, and MDA-MB453-kb2 cells	Glyphosate and formulations Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line. Method for aromatase activity evaluation is also part of OECD TG 456 for steroidogenesis.	MDA-MB453-kb2 cell line has a high content of glucocorticoid receptors in addition to androgen receptors The characterization of the cell line and discussion of such confounding factors is not considered in the paper. While glyphosate and formulations reduced AR transcription in this cell line, there appears to have been no control with androgen-specific responses to exclude glucocorticoid-specific responses	Low	Positive	Gasnier et al. (2009)
Steroidogenesis In vitro: Transformed and human aromatase–transfected cDNA in human embryonic kidney 293 cells and placental-	Glyphosate and formulations 0.01% (with 210 µmol/L glyphosate) to 2% glyphosate/glyphosate formulation	Relevant cell models, but limited characterization provided in the paper	Inhibition of aromatase noted in two different species by both parent compound and formulations The aromatase assay may be subject to variability, e.g. due to degradation of the enzyme, and therefore performance criteria are specified in guideline OPPTS 890.1200 to	Low–Med	Positive	Benachour et al. (2007)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
derived JEG3 cells Ex vivo: normal human placenta and equine testis			demonstrate that the assay is functioning correctly. This is addressed in the EDSP data, but is not evident in the Séralini lab. papers (Benachour et al., 2007; Gasnier et al., 2009), although OECD GD 150 is cited. An adequate response with the proficiency chemicals econazole, fenarimol, nitrofen (inhibitors) and atrazine (non-inhibitor) should be demonstrated and the inhibitor 4-hydroxyandrostenedione (formestane) used as a positive control chemical in each experiment. While the correct positive control was used, proficiency testing is not reported Compliance with the performance criteria should be checked before evaluating results from this assay. A positive result in GD OPPTS 890.1200 requires demonstration of inhibition of aromatase activity that fits a 4-parameter nonlinear regression model such that the concentration response curve crosses 50% inhibition. The concentration response curve allows the determination of potency, i.e. IC_{50}. In some cases, variability may be due to limited solubility of a chemical			
Steroidogenesis In vitro: Placenta-derived JEG3 cells	Glyphosate and formulation ingredients Top dose: 100 ppm		The coformulants were each tested independently and were reported to inhibit aromatase activity at concentrations 20–67% below the no-observed-effect concentration, at which levels glyphosate alone did not significantly inhibit aromatase. (See also comment above regarding proficiency testing of the assay)	Low–Med	Positive	Defarge et al. (2016)
In vitro: BLTK1 murine Leydig cells	300 µmol/L	characterized Leydig cell model				(2012)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Steroidogenesis In vitro: StAR in a mouse MA-10 Leydig tumour cell	Glyphosate formulation (containing 180 g/L glyphosate)	Relevant and well-characterized cell model	Statistically significant reduction ($P < 0.01$) of $(Bu)_2$cAMP with the glyphosate formulation was observed after 2 hours of treatment. Statistical significance ($P < 0.01$) was also [illegible] for the conversion of cholesterol to pregnenolone and for initiating the synthesis of all steroid hormones	Med–High	Positive	Walsh et al. (2000)
Overall conclusion: There is no convincing evidence of a potential interaction between glyphosate and the androgen-receptor pathway. Decreases in sperm count in the subchronic rat study (1 678 mg/kg bw per day; USEPA 2015) and a delay in preputial separation at 1234 mg/kg bw per day in the two-generation reproduction study in rats (the EDSP Tier 2 study) were observed at a dose that was above the limit dose (1000 mg/kg bw per day) and therefore of low physiological relevance. However, there is plausible evidence that glyphosate and glyphosate coformulants affect the steroidogenesis pathway, via P450scc and StAR. Further investigation is needed.						
Thyroid						
EDSP Tier 1 data 2014/2015: In vitro: No assays conducted. In vivo test battery: There were no treatment-related effects on T4 and TSH, thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. No thyroid-related effects were observed in the female pubertal assay. There were no developmental effects or alterations in thyroid histopathology in the amphibian metamorphosis assay. No thyroid-related effects were noted in any of the Part 158 studies.	Glyphosate	Relevant and validated test methods	No convincing evidence of potential interaction of glyphosate	High	Negative	USEPA 2015

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Overall conclusion: There is no convincing evidence of a potential interaction with the thyroid pathway for glyphosate						
Other endocrine mechanisms						
Retinoid system In vivo *Xenopus laevis* embryo model and chicken embryos	360 pg and 5 000 pg of glyphosate (Sigma)	Whole vertebrate models, two species	Experimental design and hypothesis based on medical observations of craniofacial defects with malformations observed in humans residing in areas chronically exposed to glyphosate formulations. Suspected to be resulting from a dysfunctional retinoic-acid or Sonic hedgehog pathway. Further investigation is needed	Med–High	Positive: increase in endogenous retinoic-acid activity	Paganelli et al. (2010)
Cortisol In vivo fish study *Rhamdia quelen* fingerlings	Glyphosate formulation 360 g/L	Stress response of *Rhamdia quelen* fingerlings acute exposure at 45, 90, 135 and 180 days	Stress responses important but difficult variable to control for, as stress is induced from handling, etc. This study included appropriate controls for stress confounders	Med	Negative for impaired cortisol release, but impaired growth and survival	Koakoski et al. (2014)
Hypolipidaemia and peroxisome proliferation In vivo rat	Glyphosate formulation 300 mg/kg single daily dose for 2 weeks, 5 animals/dose per group		No increase in number or size or peroxisomes	Med	Negative	Vainio et al. (1983)
AhR induction In vitro: Mouse hepatoma Hepa1c1c7 cells AhR Luciferase reporter gene transcriptional assay	Glyphosate (95–100% purity) Assay performed at concentrations of $\leq 10^{-5}$ mol/L	Relevant and recognized assay		Med	Negative	Takeuchi et al. (2008)
In vitro mPPARα, mAhR, hPXR	Glyphosate		Review, insufficient detail given. Concentration tested not given for negative test chemicals	Low	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
Overall conclusion: Suggestion of adverse effect upon retinoic-acid pathways. Further investigation required.						

AhR: aryl hydrocarbon receptor; AR: androgen receptor; CAS: Chemical Abstracts Service; CHO: Chinese hamster ovary; EDSP: Endocrine Disruptor Screening Program; ER: estrogen receptor; ERTA: estrogen receptor transcriptional activation; FSTRA: fish short-term reproduction assay; GD: guideline; hAR: human androgen receptor; HepG2: hepatocellular carcinoma; IC_{50}: median inhibitory concentration; no.: number; OECD: Organisation for Economic Co-operation and Development; PPAR: peroxisome proliferator-activated receptor; PXR: pregnane X receptor; rhCG: recombinant human chorionic gonadotrophin; StAR: steroidogenic acute regulatory protein; T4: thyroxine; TG: test guideline; TSH: thyroid-stimulating hormone; VTG: vitellogenin

[a] High: line of evidence could be sufficient on its own to be almost sure of entry (approaching 100% likelihood); Med: contributes importantly towards increasing likelihood; Low: minor contribution towards increasing likelihood.

(g) Microbiological effects

Bacteria

The herbicidal action of glyphosate is generated by chelating manganese required in the reduction of the flavin mononucleotide cofactor 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) (Cerdeira & Duke, 2006). Since bacteria have EPSPS and produce amino acids via the shikimate pathway, there is potential for glyphosate residues to disrupt microbes in the human gastrointestinal tract. However, no studies have specifically addressed whether glyphosate affects the microbiota in the human gastrointestinal tract or in mouse and rat animal models. What is known is that selected bacterial pathogens and probiotic bacteria from dairy cows and poultry can be affected differently by residual levels of glyphosate.

The minimum inhibitory concentration (MIC) of glyphosate on the growth and viability of poultry microbiota and pathogens was determined in triplicate in 24-well microtitre plates. Just 100 µL of the tested bacteria (105 colony-forming units [cfu] per mL) was added to 900 µL broth media containing different concentrations of glyphosate (0.075, 0.15, 0.30, 0.60, 1.20, 2.40 or 5.0 mg/mL). Plates containing glyphosate and bacteria were incubated at 37 ºC. MIC values were determined by quantitative analysis of bacteria on agar plates.

Clostridium perfringens, Salmonella gallinarum, S. typhimurium and S. enteritidis were highly resistant to glyphosate (MIC of 5 mg/mL*). Lactobacillus casei, L. buchneri, L. harbinensis, Staphylococcus aureus, S. lentus* and *S. haemolyticu*s were moderately resistant to glyphosate (MIC 0.60–0.30 mg/mL). All other tested bacteria including *Enterococcus faecalis, E. faecium, Bacillus badius, B. cereus* and *Bifidobacterium adolescentis* were highly sensitive to glyphosate, with MIC values ranging from 0.15 to 0.075 mg/mL (Table 48). Pathogenic *E. coli* and *E. coli* 1917 strain Nissle were also found to be resistant to glyphosate (MIC of 1.2 mg/mL).

In summary, most of the tested pathogenic bacteria were highly resistant to glyphosate; however, most other tested bacteria were moderate to highly susceptible (Shehata et al., 2013b).

Table 48. Inhibitory effects of glyphosate on different bacteria

		Bacterial count [a]	
Genus/species	**MIC (mg/mL)**	**Treated with glyphosate at MIC**	**Not treated with glyphosate**
Bacillus badius	0.15	2.24 ± 0.49	8.90 ± 0.44
B. cereus	0.3	2.75 ± 0.68	8.08 ± 0.12
Bacteriodes vulgatus	0.6	3.54 ± 0.31	7.37 ± 0.10
Bifidobacterium adolescentis	0.075	3.87 ± 0.50	8.67 ± 0.48
Campylobacter coli	0.15	3.07 ± 0.50	9.00 ± 0.70
C. jejuni	0.15	3.90 ± 0.50	9.54 ± 0.97
Clostridium perfringens	5.0	3.37 ± 0.89	8.30 ± 0.28
C. botulinum type A	1.2	4.00 ± 0.50	8.16 ± 0.32
C. botulinum type B	1.2	3.56 ± 0.45	7.60 ± 057
E. coli	1.2	3.15 ± 0.24	8.00 ± 0.34
E. coli 1917 strain Nissle	1.2	2.35 ± 0.24	7.26 ± 0.21
Enterococcus faecalis	0.15	2.00 ± 0.45	8.49 ± 0.58
E. faecium	0.15	2.01± 0.34	7.06 ± 0.95
Lactobacillus buchneri	0.6	4.00 ± 0.88	8.00 ± 0.34
L. casei	0.6	4.74 ± 0.56	8.28 ± 0.35
L. harbinensis	0.6	5.30 ± 0.44	8.40 ± 0.32

Genus/species	MIC (mg/mL)	Bacterial count [a]	
		Treated with glyphosate at MIC	Not treated with glyphosate
Riemerella anatipestifer	0.15	4.00 ± 0.50	7.88 ± 0.50
Salmonella enteritidis	5.0	2.35 ± 0.26	8.28 ± 0.16
S. gallinarum	5.0	2.15 ± 0.33	8.68 ± 0.20
S. typhimurium	5.0	2.75 ± 0.68	8.03 ± 0.16
Staphylococcus aureus	0.3	5.74 ± 0.58	9.00 ± 0.10
S. haemolyticus	0.3	5.74 ± 0.32	8.08 ± 0.16
S. lentus	0.3	3.90 ± 0.44	8.08 ± 0.14

MIC: minimum inhibitory concentration; SD: standard deviation

[a] Mean of $n = 3$ quantitative bacterial counts expressed as reciprocal $\log_{10} \pm$ SD.

Source: Shehata et al. (2013b)

An evaluation of the effects of Roundup and its glyphosate ingredients on the growth and viability of three food-associated microorganisms widely used as starters in traditional and industrial dairy technologies found that glyphosate inhibited the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* at a concentration of 1 mg/mL and *Lactococcus lactis* subsp. *cremoris*, which was more sensitive to glyphosate, with an MIC of 0.312 mg/mL (Table 49). The fungus *Geotrichum candidum* was more sensitive, with an MIC of 0.100 mg/mL (Clair et al., 2012).

Table 49. Effect of Roundup on three food-associated microorganisms

Microorganism strain	Concentration of glyphosate in Roundup (g/L)	MIC (ppm)	MMC (ppm)
G. candidum ATCC 204307	400	100	1000
	450	625	1000
L. lactis subsp. *cremoris* ATCC 19257	450	312	625
L. delbrueckii subsp. *bulgaricus* CFL1	450	1000	1250

MIC: minimum inhibitory concentration; MMC: minimum microbicidal concentration; ppm: parts per million

MIC and MMC measured after 24-hour incubation in growth media supplemented with Roundup or equivalent amount of glyphosate.

Source: Clair et al. (2012)

The minimal agricultural use of the herbicide is 10 000 ppm.

In a study of the impact of glyphosate on poultry microbiota and the production of botulinum neurotoxin during ruminal fermentation, ruminal microbiota were characterized by fluorescence in situ hybridization technique using 16S rRNA/23S rRNA-targeted oligonucleotide probes. After incubation with 0, 1, 10 or 100 µg/mL glyphosate in rumen fluids from donor cows, the cell counts of *Ruminococcus albus* and *R. flavefaciens* were significantly lower in the presence of 1 µg/mL glyphosate; *Streptococcus* spp. cell counts were significantly lower with 100 µg/mL glyphosate, and cell counts of the phylum Euryarchaeota were significantly lower on exposure to 10 and 100 µg/mL. In contrast, cell counts of *Clostridium histolyticum* and *Lactobacilli* and *Enterococci* were significantly higher with 100 µg/mL glyphosate. The study authors noted that more bacterial species were inhibited when cows were fed a crude fibre-rich diet than a lower-fibre diet, indicating a possible inhibitory effect on the microbiota responsible for fibre degradation (Ackermann et al., 2015).

In a study of the toxicity of glyphosate to the most prevalent *Enterococcus* spp. in the gastrointestinal tract, the lowest concentration of glyphosate and Roundup to show bactericidal or bacteriostatic effects was determined in 96-well microtitre plates. Serial dilutions of glyphosate from
10–0.001 mg/mL were made in nutrient broth. *Enterococcus* isolates were added at a final concentration of 10^4 cfu/mL, and the test plates with diluted glyphosate and *Enterococcus* incubated overnight at 37 °C before plating aliquots on citrate azide tween carbonate agar. Bacterial growth on each agar plate was evaluated.

Glyphosate and Roundup at 0.1–10 mg/mL inhibited the growth of *E. faecalis* but not of *C. botulinum* or the production of botulinum neurotoxin (Table 50). The study authors proposed that glyphosate may be a significant factor in the observed increased risk of *C. botulinum* infection in cattle in Germany over the past 10 to 15 years (Krüger et al., 2013). Glyphosate toxicity to *Enterococcus* spp. leads to an imbalance in the gut favouring overgrowth of *Clostridium* spp. because the common, beneficial bacteria, *Enterococcus* spp., suppress *Clostridium* growth in the gastrointestinal tract (Krüger et al., 2013; Shehata et al., 2013a,b).

***Table 50. Effect of glyphosate and Roundup on the growth of* C. botulinum *type B and* E. faecalis**

	Glyphosate			Roundup formulation		
Herbicide concentration (mg/mL)	***C. botulinum* type B (cfu/mL) [a]**	**BoNT (ng/mL) [b]**	***E. faecalis* (cfu/mL) [c]**	***C. botulinum* type B (cfu/mL) [a]**	**BoNT (ng/mL)**	***E. faecalis* (cfu/mL)**
0	6.9 ± 0.34	300 ± 47	8.2 ± 0.87	6.9 ± 0.34	270 ± 120	8.2 ± 0.87
0.1	5.3 ± 0.78	312 ± 20	0	5.1 ± 0.78	337 ± 50	0
1	5.4 ± 0.45	319 ± 60	0	3.3 ± 0.80	0	0
10	3.2 ± 0.43	0	0	3.0 ± 0.65	0	0

BoNT: botulinum neurotoxin; cfu: colony-forming unit; ELISA: enzyme-linked immunosorbent assay; SD: standard deviation

[a] *C. botulinum* type B (10^4/mL) cultured anaerobically in reinforced clostridial medium containing different concentrations of glyphosate or herbicide formulation for 5 days. *C. botulinum* quantified using the most probable number estimation method. Data express as reciprocal $\log_{10}$.

[b] *C. botulinum* type B quantified by ELISA.

[c] *E. faecalis* cultured aerobically in reinforced clostridial medium containing different concentrations of glyphosate or herbicide formulation for 8 hours and quantified on citrate-acid-tween-carbonate agar. Data expressed as reciprocal $\log_{10}$ ± SD.

Source: Krüger et al. (2013)

The neutralization ability of the antimicrobial effect of glyphosate by different humic acids was investigated by determining the MIC of glyphosate for different bacteria in different concentrations (0.25, 0.5 and 1.0 mg/mL) of humic acid. The MIC values of glyphosate for *E. faecalis, B. badius and B. adolescentis* were 0.3, 0.3 and 0.15 mg/mL, respectively. Humic acids neutralized the antimicrobial effect of glyphosate in different patterns. The WH67/2, WH67/4/3 and WH67/4 humic acids at 1 mg/mL showed the highest degree of neutralization of the antimicrobial effect of glyphosate. The MIC values of glyphosate for *E. faecalis, B. badius* and *B. adolescentis* in the presence of 1 mg/mL WH67/2, WH67/3, and WH67/4 humic acids were more than 2.4 mg/mL, while the MIC values in the presence of other humic acids ranged from 0.3 to 0.6 mg/mL (Shehata et al., 2014). Sorption of the glyphosate to humic acids varied, depending upon their macromolecular structure, but overall, these compounds neutralized the antimicrobial effect of glyphosate (Piccolo et al., 1995, 1996).

In a 90-day toxicity study, groups of 10 male and 10 female Sprague Dawley rats were administered AMPA (purity 99.2%; in CMC) at a concentrations of 0, 10, 100 or 1000 mg/kg bw per day by gavage for 13 weeks. Blood samples were taken from all animals during week 13 for investigation of haematology and clinical chemistry parameters. An ophthalmoscopic examination was undertaken on all animals during pre-trial and on all control and high-dose animals during week 12. All surviving animals were necropsied at termination as were all pre-terminal decedents. Histological examination was carried out on selected tissues from all control and high-dose animals and all pre-terminal decedents and on the kidneys, liver, lungs, submaxillary salivary gland, sublingual salivary gland and parotid salivary gland of all other animals.

There was no treatment-related effect on mortality, clinical signs, body weight, body-weight gain, feed consumption, water consumption, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights, macroscopic findings and histological examination. The NOAEL in this 90-day gavage toxicity in rats with AMPA was 1000 mg/kg bw per day (Strutt et al., 1993).

Table 51. Summary of acute toxicity studies of AMPA

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	(Crj:CD-1)	M + F	Oral	99.33	> 5 000	Komura (1996)
Rat	Alpk:AP$_f$SD, Wistar	M + F	Oral	100 (assumed)	> 5 000	Leah (1988)
Rat	Sprague Dawley	M + F	Oral	99.2	> 5 000	Cuthbert & Jackson (1993a)
Rat	Sprague Dawley	M + F	Dermal	99.2	> 2 000	Cuthbert & Jackson (1993b)
Rat	CD/Crl:CD	M + F	Dermal	98.0	> 2 000	Leuschner (2002a)
Guinea pig	Dunkin Hartley	F	Sensitization (Magnusson–Kligman Maximization Test)	99.2	Negative	Cuthbert & Jackson (1993c)
Guinea pig	Dunkin Hartley	M	Sensitization (Magnusson–Kligman Maximization Test)	98.0	Negative	Leuschner (2002b)

LD_{50}: median lethal dose

(c) Genotoxicity of AMPA

A much smaller number of studies have been conducted on the glyphosate metabolite, AMPA, as well as the plant metabolites, *N*-acetyl-glyphosate and *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo studies (Jensen, 1993c; Kier & Stegeman, 1993; Manas et al., 2009b; see Table 35) investigated the ability of AMPA to induce micronuclei in the bone marrow erythrocytes of mice and have largely been negative although a modest positive response was reported by Manas (2009b) when AMPA was administered by intraperitoneal injection to male mice.

Studies by other investigators using the more relevant oral route of administration did not show an increase in micronuclei in either male or female mice.

In the in vitro studies, increases in mutation in bacteria were not seen for AMPA or the acetylated metabolites. Both positive (Manas et al., 2009b) and negative (Jensen, 1993b,c; Roustan et al., 2014) results were reported in studies of chromosome aberrations and DNA damage for AMPA. AMPA was negative in two studies of unscheduled DNA synthesis in isolated rat hepatocytes (Bakke,

Publication bias

A formal analysis of publication bias was not undertaken because the number of studies (risk estimates from non-overlapping study populations) available were few and funnel plot tests for asymmetry should be used only where there are at least 10 studies because otherwise statistical power is insufficient to distinguish true asymmetry from chance (Higgins & Green, 2011; Sterne et al., 2011). Other formal objective statistical tests require an even larger number of studies, typically at least 30, to achieve sufficient statistical power (Lau et al., 2006). As a result, publication bias cannot be fully excluded. However, given the very considerable resources invested in these types of (large, difficult exposure assessment) studies, it is unlikely that results would go unpublished.

Summary of evidence for an association between glyphosate and NHL

This evaluation considered several aspects of each study and of all the studies combined, including factors which decrease the level of confidence in the body of evidence, including risk of bias, unexplained inconsistency, and imprecision, and factors which increase the level of confidence, including large magnitude of effect, a dose–response relationship, residual confounding and consistency (Guyatt et al., 2008; Morgan et al., 2016).

The risk estimates findings for each study are summarized in Table 52, and findings for non-quantitative exposure assessment (predominantly ever- vs never-use) are shown in the forest plot below.

Table 52. Results of Tier 1 evaluation and summary of publications by glyphosate/cancer site

Study/ Location	Glyphosate / NHL	Reference
Meta-analysis	Qualitative exposure only – ever-/never-use of glyphosate Meta risk ratio: 1.5 (95% CI: 1.1–2.0) Meta-analysis includes McDuffie et al. (2001); Hardell et al. (2002); De Roos et al. (2003, 2005a); Eriksson et al. (2008); and Orsi et al. (2009). *N*s for each meta-analysis not presented	Schinasi & Leon (2014)
Agricultural Health Study	Quantitative exposure (cumulative exposure days; intensity-weighted cumulative exposure days [years of use × days/year × estimated intensity level]: in tertiles) Risk estimates – aRR (95% CI) Ever-use 1.1 (0.7–1.9) LED 1–20.0 1.0 (ref.) LED 21–56 0.7 (0.4–1.4) LED 57–2678 0.9 (0.5–1.6) *P* for trend 0.73 IW-LED 0.1–79.5 1.0 (ref.) IW-LED 79.6–337.1 0.6 (0.3–1.1) IW-LED 337.2–18241 0.8 (0.5–1.4) *P* for trend = 0.99 Total *N* = 54 315 (49 211/36 823, depending on the analysis), with 92 incident NHL cases (for ever-use; and 61 for analysis based on tertiles of exposure)	De Roos et al. (2005)
United States Midwest case–control studies	The study population overlaps with that of De Roos et al. (2003). See comment below Qualitative – ever/never (analysis stratified by asthmatics vs non asthmatics) Risk estimates – aRR (95% CI) Non-asthmatics: 1.4 (0.98–2.1) Asthmatics: 1.2 (0.4–3.3) Total *N* = 3208 (872 NHL cases, 2336 controls). *N* = 53/91 glyphosate-exposed NHL cases/controls for non-asthmatics and 6/12 glyphosate-exposed NHL cases/controls for asthmatics	Lee et al. (2004)

Study/ Location	Glyphosate / NHL	Reference
	The study population overlaps with Lee et al. (2004) and total *N* is smaller, but as an exception this study was not excluded in the assessment of consistency of risk estimates as it provides overall risk estimates which are comparable with other studies, while Lee et al. (2004) only provides risk estimates stratified by asthma diagnosis Qualitative (ever/never) Risk estimates – aOR (95% CI) From a logistic regression model: Exposed 2.1 (1.1–4.0) From the hierarchical regression model: Exposed 1.6 (0.9–2.8) Both adjusted for other pesticides Total *N* =2 583 (650 NHL cases, 1 933 controls). *N* = 36 exposed cases; *N* = 61 controls	De Roos et al. (2003)
	Excluded – as this study is pooled in De Roos et al. (2003) and Lee et al. (2004) Qualitative exposure only – ever-/never-use of glyphosate Risk estimates – OR (95% CI) Ever-use = 1.1 (0.7–1.9) Total *N* = 1867 (622 cases, 1245 controls) *N* = 26 exposed cases	Cantor et al. (1992)
Cross-Canada Study of Pesticides and Health	Quantitative exposure – days of use per year (3 categories – cutpoints are given). Risk estimates – OR (95% CI) Ever-use: 1.2 (0.83–1.74) Unexposed 1.0 (ref.) >0–<=2 days/year 1.0 (0.63–1.57) > 2 days/year 2.12 (1.20–3.73) *P* trend = NR Total *N* = 2 023 517 cases, 1 506 controls (overall) *N* = 51 exposed cases, 133 exposed controls	McDuffie et al. (2001)
Sweden – note that there is some overlap between Eriksson et al. (2008), Hardell et al. (2002) and Hardell & Eriksson (1999)	Quantitative exposure – days of use per year (2 categories – cutpoints are given). Risk estimates – aOR (95% CI) Ever-use: 2.02 (1.10–3.71) Risk estimates – aOR (95% CI) Non-farmers: 1.0 (ref.) ≤ 10 days/year: 1.69 (0.7–4.07) > 10 days/year: 2.36 (1.04–5.37) *P* trend = NR Total N = 1926 (910 cases, 1016 controls) *N* = 29 exposed cases; *N* = 18 exposed controls	Eriksson et al. (2008)
	Qualitative exposure only – ever-/never-use of glyphosate. A pooled analysis of Nordström et al. (1998) (NHL subtype only, not evaluated separately here) and Hardell & Eriksson (1999) Risk estimates – aOR (95% CI) Ever-use: 1.85 (0.55–6.20) Total *N* = 1 656 (515 cases, 1 141 controls) *N* = 8 exposed cases; *N* = 8 exposed controls.	Hardell et al. (2002)
	Exclude as this study is pooled in Hardell et al. (2002). Qualitative exposure only – ever-/never-use of glyphosate	Hardell & Eriksson (1999)

Study/ Location	Glyphosate / NHL	Reference
France	Qualitative – ever-/never-use of glyphosate Risk estimates – aOR (95% CI) Ever-use: 1.0 (0.5–2.2) *N* = 12 exposed cases; *N* = 24 exposed controls (The researchers report evaluating quantitative duration with respect to median duration of exposure among exposed controls as never exposed; duration < median; duration > median, but neither the median cutpoint nor ORs/test for trend results are presented in the paper, so this study cannot contribute any information for quantitative risk assessment.)	Orsi et al. (2009)

aOR: adjusted odds ratio; aRR: adjusted risk ratio; CI: confidence interval; IW-LED: intensity-weighted lifetime exposure days, defined as number of years of use × number of days used per year × personal protective equipment use reduction factor × intensity level score (a unit-less score which reflects a combination of self-reported pesticide exposure modifiers, e.g. pesticide mixing status, application method, equipment repair activities); LED: lifetime exposure days, defined as number of years of use × number of days used per year; NHL: non-Hodgkin lymphoma; *N*: sample size; NR: not reported; OR: odds ratio; ref.: reference

The maximally adjusted risk estimates were extracted.

The Glyphosate / NHL evaluation included seven studies (McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Lee et al., 2004; De Roos et al., 2005; Eriksson et al., 2008; Orsi et al., 2009) and one meta-analysis (Schinasi & Leon, 2014). Three studies used quantitative exposure metrics, although, the units differed: lifetime exposure days and intensity-weighted lifetime exposure days (De Roos et al., 2005) and days of use per year (McDuffie et al., 2001; Eriksson et al., 2008). The AHS found no evidence of elevated risk of NHL or exposure–response associated with glyphosate exposure (De Roos et al., 2005). Elevated risks were reported in various case–control studies. De Roos et al. (2003) reported significant elevated risk of NHL associated with ever- versus never-use of glyphosate (OR: 2.1 [1.1–4.0] and a borderline nonsignificant OR (1.6 [0.9–2.8]) with an alternative Bayesian hierarchical model) from the United States Midwest pooled case–control studies. There was no evidence of effect modification by asthma diagnosis in the United States Midwest pooled case–control studies (Lee et al., 2004). Ever-use of glyphosate was not associated with risk of NHL in the Cross-Canada Case–control Study of Pesticides and Health, but using glyphosate for longer than 2 days per year was associated with a significant elevated risk (OR: 2.12; 95% CI: 1.20–3.73), although there was no indication of an exposure–response relationship across exposure categories (McDuffie et al., 2001). Eriksson et al. (2008) reported significant elevated risk of NHL associated with ever-use (OR: 2.02 [1.10–3.71]) and use of glyphosate for longer than 10 days per year (OR: 2.36 [1.04–5.37]) and indicate an exposure–response relationship. A pooled study of two Swedish case–control studies reported a nonsignificant elevated risk of NHL for ever-use of (OR: 1.85 [0.55–6.2]); however, with only eight exposed cases, this study had limited power to detect associations (Hardell et al., 2002). Orsi et al. (2009) found no evidence of association. Schinasi & Leon (2014) reported a meta risk ratio of 1.5 (95% CI: 1.1–2.0) for ever- versus never-use of glyphosate. The meta-analysis included the AHS (De Roos et al., 2005) and five out of the six case–control studies included in this evaluation (McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Eriksson et al., 2008; Orsi et al., 2009).