WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

DISTR.: RESTRICTED
DISTR.: RESTREINTE

2016 JOINT FAO/WHO MEETING ON PESTICIDE RESIDUES
Geneva, 9 - 13 May 2016

GLYPHOSATE
(Draft 06/05/2016)

The contents of this restricted doc ument may not be divulged to persons other than those to whom it has been originally addressed. It may not be further distributed nor reproduced in any manner and should not be referenced in bibliographical matter or cited.

Le contenu du présent document à distribution restreinte ne doit pas être divulgué à des personnes autres que celles à qui il était initialement destiné. Il ne saurait faire l'objet d'une redistribution o u d'une reproduction quelconque et ne doit pas figurer dans une bibliographie ou être cité.

GLYPHOSATE

First draft prepared by

P.V. Shah[1] , Carl Cerniglia, David Eastmond, Miriam Jacobs, Rachel Smith, and Jurg Zarn

[1] Office of Pesticide Programs, Environmental Protection Agency, Washington, DC, United States of America (USA)

Explanation x
Evaluation for acceptable intakex
1. Biochemical aspects x
1.1 Absorption, distribution and excretion x
(a) Oral administration x
(b) Other routes of administration x
1.2 Biotransformation x
(a) Oral administration x
(b) Other routes of administration x
2. Toxicological studies x
2.1 Acute toxicity x
(a) Oral administration x
(b) Dermal application x
(c) Exposure by inhalation x
(d) Dermal irritation x
(e) Ocular irritation x
(f) Dermal sensitization x
(g)
Phototoxicity x
2.2 Short-term studies of toxicity x
(a) Oral administration x
(b) Dermal application x
2.3 Long-term studies of toxicity and carcinogenicity x
2.4 Genotoxicity x
2.5 Reproductive and developmental toxicity x
(a) Multigeneration studies x
(b) Developmental toxicity x
2.6 Special studies x
(a) Neurotoxicity x
(b) Immunotoxicity x
(c) Effects on salivary gland x
(d) Gastrointestinal tract irritation x
(e) Endocrine disruption x
(f) Intestinal microflora effects
(g) Epidemiological studies
2.7 Studies on metabolites x
2.8 Studies on formulants (POEA)
3. Observations in humans x
Comments x
Toxicological evaluation x
References x

Explanation

Glyphosate is the International Organization for Standardization (ISO) –approved common name for N-(phosphonomethyl) glycine (International Union of Pure and Applied Chemistry ; IUPAC), with Chemical Abstracts Service number 1071-83-6. It is a broad spectrum systemic herbicides. Glypphosate related salts are: glyphosate isopropyl amine salt (N -

EPA-HQ-2018-002024_0004804

(phosphonomethyl)glycine-isopropylamine; CAS No. 38641-94-0); glyphosate-potassium salt (potassium N-[(hydroxyphosphinato)methyl]glycine; CAS no. 70901-20-1); glyphosate-ammonium salt (ammonium N-[(hydroxyphosphinato)methyl]glycine; CAS No. 40465-66-5) and Glyphosate-dimethylammonium salt (N-(phosphonomethyl)glycine-dimethylamine (1:1); CAS No. 34494-04-7).

Glyphosate was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1986, 1997 (aminomethylphosphonic acid (AMPA), 2004 and 2011 (new plant metabolites in genetically modified maize and soy beans). In 1986, when the Meeting established an acceptable daily intake (ADI) of 0–0.3mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 31mg/kg bw per day, the highest dose tested in a 26-month study of toxicity in rats. In 1997, the Joint Meeting evaluated aminomethylphosphonic acid (AMPA), the major metabolite of glyphosate, and concluded that AMPA was of no greater toxicological concern than its parent compound. A group ADI of 0–0.3mg/kg bw was established for AMPA alone or in combination with glyphosate. In 2004, when the Meeting established a group ADI for glyphosate and AMPA of 0-1.0mg/kg bw on the basis of the NOAEL of 100mg/kgbw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. The 2004 JMPR concluded that it was not necessary to establish an acute reference dose (ARfD) for glyphosate. In 2011, the Meeting concluded that the group ADI of 0-1 mg/kg bw established by the 2004 JMPR for glyphosate and AMPA may also be applied to N-acetyl-glyphosate and N-acetyl-AMPA, as the available toxicological data showed that these plant metabolites have no greater toxicity than the parent glyphosate.

In March 2015, the International Agency for Research on Cancer (IARC) classified diazinon, glyphosate and malathion as "probably carcinogenic to humans" and on this basis the WHO established an ad hoc expert taskforce to determine whether these three compounds need to be re-evaluated by the JMPR. The current re-evaluation of glyphosate was undertaken at the recommendation of this taskforce.

All unpublished studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and that complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the European Union or United States Environmental Protection Agency, unless otherwise indicated. Minor deviations from these protocols were not considered to affect the reliability of the studies.

The studies evaluated by the previous JMPR (2004 and 2011) are adopted in this assessment and they are re-evaluated according current policies and procedures of the JMPR. Relevant new studies submitted since 2004 and 2011 are included in this assessment.

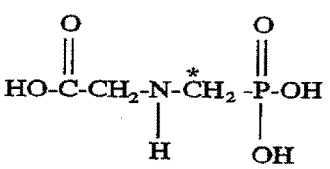
Evaluation for acceptable intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of glyphosate were 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 (IV) and intraperitoneal (IP) in rats and intramuscular (IM) administration in Rhesus monkeys.

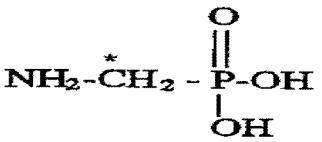
Fig. 1 shows the structure of radiolabelled glyphosate

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 administration

The summary of excretion and residue levels from various studies following single oral dose or repeated oral administration in rats and rabbits is shown in the table 1.

Table 1. Total elimination and residues (%) of administered radioactivity after a single or repeated oral administration

Dose administered	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-hr	Rat	14-16	35-43	81-85	49-55	0.14-0.65	0.83-1.02	Colvin[a], 1973a
10 mg/kg bw, single dose 24/48-hr	Rat	17.9/34.0	12.8/12.5	59.3/60.5	80.3/91.2	ND	ND	Davies, 1996
10 mg/kg bw, single dose,72-hr	Rat	13	10.6	88.5	88.7	0.59	0.49	Davies, 1996
10mg/kg bw, single dose 7-day	Rat	28.6	22.5	62.4	69.4	0.44	0.31	Ridley, 1988
10 mg/kg bw, repeated dosing, 72-hr	Rat	10.6	10.7	86.6	90.7	0.46	0.41	Davies[b], 1996
10 mg/kg bw, repeated dosing, 7-day	Rat	30.9	23.1	61.0	70.9	0.54	0.35	Ridley[b], 1988
30 mg/kg bw, single dose, 168-hr	Rat	29.04	30.71	58.84	56.53	0.62	0.64	Powles, 1992
30 mg/kg bw, repeated dosing 168-hr	Rat	34.28	34.63	49.64	46.73	0.96	0.83	Powles, 1992
1000 mg/kg bw, single dose 72-hr	Rat	16.7	17.5	89.6	84.5	0.52	0.58	Davies, 1996
1000 mg/kg bw, single dose 168-hr	Rat	30.55	22.41	53.27	60.37	0.47	0.40	Powles,P 1992

1000 mg/kg bw, single dose 7-day	Rat	17.8	14.3	68.9	69.4	0.28	0.24	Ridley, 1988
10 mg/kg bw, single dose, 168-hr	Rat	22.5	19.4	74.6	84.3	0.33	0.27	McEwen, 1995[c]
10 mg/kg bw, single dose, 168-hr	Rat	30.3	29.5	74.7	74.2	0.31	0.39	McEwen, 1995[c]
1 mg/kg bw, single dose, 168-hr	Rat	18.4	27.2	72.6	62.4	0.8	1.0	Knowles and Mookherjee 1996[c]
100 mg/kg bw, single dose, 168-hr	Rat	39.4	43.1	41.2	42.4	0.8	1.0	Knowles and Mookherjee 1996[c]
5.7-8.8 mg/kg bw, single dose 120-hr	Rabbit	7-11	ND	80-97	ND	0.1-1.2	ND	Colvin [a], 1973c

ND – not determined
[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 rats were given 14 consecutive daily oral doses of 10 mg unlabelled glyphosate/kg bw followed by a single oral dose 10 mg [^{14}C]-glyphosate/kg bw.
[c]The value represent for residual activity in carcass only

The summary of excretion and residue levels from various studies following single intraperitoneal (IP), intravenous (IV) or intramuscular (IM) single administration in rats and Rhesus monkeys is shown in Table 2

Table 2 Total elimination and residues (%) of administered after an intraperitoneal (IP), intravenous (IV) or intramuscular (IM) single administration

Dose administered	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 – IP 120-hr	Rat	82-90	ND	6-14	ND	≤1	ND	Colvin [a], 1973
10 mg/kg bw, single dose – IV 7-day	Rat	79.0	74.5	4.65	8.3	1.27	1.09	Ridley, 1988
30 mg/kg bw, single dose – IV 168-hr	Rat	85.98	84.18	3.42	1.48	1.35	1.09	Powles, 1992
4 mg single dose – IM 7-day	Monkey	89.9	ND	ND	ND	ND	ND	Maibach, 1983

ND – not determined
[a] Glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon

EPA-HQ-2018-002024_0004807

In a pre -GLP study, g lyphosate ^{14}C-labelled at the methylene carbon, at the C1 -glycine carbon and at the C2 -glycine carbon was dissolved in water and administered to Wistar rats by gavage. The radiochemical purity of the labelled materials used were ≥ 95% for ^{14}C-methylene glyphosate, ^{14}C-C1-glycine glyphosate and ^{14}C-C2-glycine glyphosate. F or 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 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 three male rats each were dosed separately 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). In a third series of experiments designed to determine t he gross distribution of plant derived metabolites of glyphosate, aqueous extracts of soybeans grown in hydroponic solutions of ^{14}C-glyphosate were administered orally to rats. The extracts were obtained from soybean plants, which had been cultured for fo ur weeks in separate hydroponic media containing the three forms of ^{14}C-glyphosate. Treatment groups composed of three male rats each for each type of radiolabelled material were dosed separately with the aqueous extracts of the roots of soybeans. In ad dition, a fourth treatment group of three male rats was also dosed with the aqueous extract of the aerial portion of soybean plants grown in hydroponic media containing ^{14}C-methylene glyphosate.

^{14}C-glyphosate orally administered to male rats was excreted in urine and faeces (94-98% of the administered dose) within 48-hours post -administration. Approximately 15% of the oral dose administered to male rats was excreted in the urine in 120 hours post-administration, with most of the remainder of administered radioactivity being excreted in the faeces (81-85%). Of the ^{14}C-glyphosate absorbed from the gut, only very small amounts were catabolized in the male rat. The percentage of administered radioactivity recovered as expired $^{14}CO_2$ was 0.5%. Tissue reten tion at 120 hours post - administration was less than 1% of the dose for the three ^{14}C-labelled forms of glyphosate. When ^{14}C-glyphosate is orally administered to female rats, total excretion of radioactivity was 82 -84% and 91 - 93% of the administered dose, r espectively, at 48 and 120 hours post -administration. Female rats excreted 34-40% of the administered dose in the urine in 120 hours post-administration, compared to only 14-16% by males, with most of the remainder of administered radioactivity being excreted in the faeces (49-55%). The levels of exhaled $^{14}CO2$ were also slightly higher for female rats than males, as were carcass retentions. For female rats, the percentage of administered radioactivity recovered as expired $^{14}CO2$ was 0.72%. Tissue retenti on at 120 hours post -administration was approx. 1% for the three ^{14}C -labelled forms of glyphosate. For both sexes, the order of retention of radioactivity in tissues 120 days post-administration of a single oral dose of ^{14}C -glyphosate was similar, altho ugh in all cases the female tissues contained higher concentrations. The highest concentrations of radioactivity were found in the tissues of the liver, kidney, and gut, but were in all cases ≤0.20 ppm on a fresh weight basis.

^{14}C -glyphosate administered to male rats via intraperitoneal injection , about 74-78% of the administered dose was excreted in the urine within the first 12 hours post-administration. At 96 hours post-administration, total urinary excretion ranged from 81 to 90% of the administered dose. Faecal excretion ranged from 6 to 14% of the administered radioactivity by 96 hours post-administration, and strongly suggests that ^{14}C -glyphosate is also eliminated via the bile. The percentage of administered radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that observed following oral administration, but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater than in male rats after oral administration, but was in all cases ≤1% of the administered dose.

When the extracts of soybeans grown in hydroponic solutions of ^{14}C -glyphosate are orally administered to male rats, 96-99% of the administered radioactivity is excreted in the faeces and urine 120 hours post -administration, except for the rats dosed with extracts of soybean roots from plants treated with ^{14}C -C2-glycine glyphosate, for which only 76% of the administered dose was found in the excreta. The relatively high tissue retention (5.19% and 1.86% of the administered dose) and $^{14}CO_2$ expiration (3.67% and 3.49% of the administered dose) by rats administered extracts of roots from plants treated with ^{14}C -C2-glycine glyphosate and the extracts of the aerial portion of plants

treated with ^{14}C -methylene glyphosate was attributed t o the metabolism of natural plant products since the radioactivity contained in these extracts was due to 30% and 10% natural products, respectively. The radioactivity remaining in rat tissues following oral dosing with extracts of plants treated with ^{14}C -glyphosate (Colvin and Miller, 1973a).

In a pre -GLP study, t he accumulation and depletion of glyphosate was investigated by the daily administration of feed containing 0, 1 ppm, 10 ppm, and 100 ppm, respectively, of ^{14}C-glyphosate, to Wistar rats (15/se x/dose) for 14 days, followed by a 10 day depuration period on control ration. Determinations of tissue residues were made after 2, 6, 10, and 14 days on dosed feed, and 1, 3, 6, and 10 days after withdrawal from dosed feed. The excretion of ingested ^{14}C-glyphosate in faeces and urine were determined daily.

Determination of body weights and organ weights indicated that the continuous administration of feed containing 1 ppm, 10 ppm, and 100 ppm of glyphosate for 14 days had no detrimental effect on the gro wth 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 six days, indicating that a plateau had been reached. By day four of dosing, combined excretion of radioactivity in the urine and 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 ppm, 10 ppm, 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, thereby 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 c hronic ^{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 fresh or dry -weight basis, indicating a very low prope nsity for accumulation. The residues which were observed in the tissues were reversibly bound and began to deplete as soon as medicated feed was withdrawn (Colvin and Miller, 1973b).

Seven different test groups of rats (Crl:CD®, Sprague Dawley BR rats), each containing an equal number of males and females, were dosed with ^{14}C -glyphosate labelled in the methylene position between the nitrogen and phosphorous atoms. The radiochemical purity was 98% or greater. Single oral doses were administered by gastr ointubation (10 mg/kg bw and 1000 mg/kg bw) and the intravenous doses were administered by injection into the lateral tail vein (10 mg/kg bw). A further group of five male and five female rats received unlabelled glyphosate as 14 consecutive oral doses at 10mg/kg bw per day followed by ^{14}C -labelled glyphosate as a single oral dose at 10mg/kg bw. Blood samples, urine and faeces were collected at various time points. At the end the study, animals were killed and various tissues including carcass was analysed for radioactivity.

The distribution of radioactivity in the excreta, and collected tissue samples for various groups are summarized in Tables 3.

Table 3 Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C -labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)							
	10 (single dose, IV)		10 (single dose, oral)		10 (repeated dose, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	79.0	74.5	28.6	22.5	30.9	23.1	17.8	14.3
Faeces	4.65	8.30	62.4	69.4	61.0	70.9	68.9	69.4
Organs/Tissues	0.09	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*	86.0	85.3	92.8	94.2	93.3	96.3	90.9	92.1

* Total recovery is the mean of individual animal data.
From Ridley and Mirly (1988)

The major route of elimination of an oral dose of ^{14}C -glyphosate at 10 mg/kg body bw was faeces . The faeces contained 62.4% and 69.4% of the administered dose for the males and females, respectively, after a 7 -day elimination period. The majority of the remaining radioactivity was excreted in the urine, 28.6 and 22.5% of the dose, respectively, fo r the males and females. A greater percentage of the administered dose remained in the organs, tissues, and residual carcasses for the males than for the females, although the overall amount of retained radioactivity was very low (<0.5 of the administered dose). The tissue with the highest concentrations of radioactivity was the bone, where 0.552 ppm and 0.313 ppm were found for the males and females, respectively.
For the test group orally dosed at 1000 mg/kg bw, 68.9 and 69.4% of the administered dose was excreted in the faeces and 17.8 and 14.3% was excreted in the urine of the male and female rats, respectively. Very low levels (<0.4%) of the administered dose remained in the gastrointestinal contents, residual carcasses, organs, and tissues at 7 days after dosing. The tissues showing more than 1.0 ppm of radioactivity were the liver, kidney, spleen, lung, stomach, small intestines, bone, and residual carcass. The bone had the greatest amount of radioactivity, 30.6 ppm and 19.7 ppm for the males and females, respectively.
For the test group that received 14 daily doses of non -labelled glyphosate at 10 mg/kg prior to receiving a single oral dose of labelled glyphosate at 10 mg/kg bw, males excreted 61.0 and 30.9% of the dose in the faeces and urine, r espectively. In the females, 70.9 and 23.1% of the dose was excreted in the faeces and urine, respectively. Very low levels (<0.7%) of the administered dose remained in the gastrointestinal contents, residual carcasses, organs and tissues at 7 days after dosing. Again, bone was the tissue with the highest concentration of radioactivity. The bone contained 0.748 and 0.462 ppm glyphosate equivalents for the males and females, respectively.
The half -lifes of the α and β elimination phases were 5.9 to 6.2 hours and 79 to 106 hours, respectively, following a single oral dose of 10 mg/kg bw. In 1000 mg/kg bw dosed group, the α phase was comparable to 10 mg/kg bw group, but the β phase was found to be 181 to 337 hours. Comparison of the area under the curves of plots of radioactivity levels in the blood versus time for the two groups indicated that the orally administered glyphosate was 30 -35% absorbed. These values are in good agreement with the absorption values of 30 -36% found by dividing the percent urinary excretion of administered radioactivity for the group dosed orally at 10 mg/kg bw by the percent urinary excretion of administered radioactivity from the group dosed intravenously at 10 mg/ kg bw. The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated after a single oral dose at 10 or 1000mg/kg bw (Ridley & Mirly, 1988).

In a preliminary study of absorption and distribution, male Sprague -Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.6%; radiochemical purity, 94.3 –97.4%) as a single oral dose at 30mg/kg bw in 0.9% saline by gavage. Blood samples were taken from the tai l vein of three animals at various times between 0.5 and

48h after dosing. Additional animals were killed 4, 10 and 24h after dosing and the tissue distribution of radioactivity was investigated by whole body autoradiography.

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations (Cmax) reached within 4 h were 1.769, 1.137, and 0.705 µg equivalent/mL for three animals. Thereafter plasma levels decayed exponentially to non-detectable levels at 12 h post-dose. The elimination half-lives were 6.196 h and 12.35 h for two animals. A value could not be obtained for the third animal. The concentration of radioactivity was highest after 10 hours. At that time the highest concentration of radioactivity was present in bone, bone marrow, cartilage, parts of the gastrointestinal tract, kidney, urinary tract and nasal mucosa. The highest concentrations within bone were associated with the epiphyses. Lower concentrations were found in a number of other tissues. Twenty-four hours after dosing tissue concentrations of radioactivity were negligible in all tissues except bone, bone marrow, parts of the gastrointestinal tract, bladder and kidney cortex (Powles, 1992a).

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

Following administration of a single intravenous dose (30 mg/kg) more than 84% of the dose was eliminated in urine, mostly within 8 hours post-dosing. Faecal elimination accounted for less than 3.5% of the administered radioactivity. Only a very small proportion of the radioactivity was eliminated in exhaled air and less than 1.4% was present in tissues and the residual carcass when the animals were sacrificed. In contrast faeces were the major route of elimination when (^{14}C)-glyphosate was given by the oral route. Approximately 56-59% of an oral dose of 30 mg/kg was excreted in faeces; most of this was eliminated in the 12-36 hour period after dosing. Urinary elimination of the 30 mg/kg oral dose was slower than for the intravenous dose, 29-31% was eliminated, mainly within 36 hours of dosing. Excretion was unaffected by administration of unlabelled glyphosate for 14 days prior to the administration of (^{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 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 present in bone with lower concentrations in bone marrow, kidney, liver, lungs and the residual carcass (Powles, 1992b).

In a study of absorption, distribution, excretion and metabolism study, groups of five male and five female Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.9%; radiochemical purity, >98%) as a single dose at 10 or 600mg/kg bw by gavage in water. For excretion study, urine and faeces (5/sex) were collected at selected time intervals for 168 hours. Animals were killed at 168 hours post-dosing and radioactivity in blood and selected tissues were analysed. For plasma concentration study, blood samples (total 9/sex/dose) were withdrawn at selected intervals up to 168 hours. For tissue distribution study, 12 rats (6 male, 6 female) received single oral doses of either 10 or 600 mg/kg bw per day by gavage. The animals were divided into two groups of six (3 per sex) and sacrificed by cervical dislocation 6 and 18 h (low dose) or 3 and 9 h (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 TLC or HPLC.

During the 7 days observation period, about 23% and 19% of the administered dose was excreted in the urine in male and female rats, respectively, at 10 mg/kg bw dose (Table 4). Slightly

higher percentages, about 30% and 29% (male/female), of total administered radioactivity were detected in urine of the high dose group. In both dose groups about 75% of the administered radioactivity could be detected in the faec es of males and females within 7 days (75% and 84%, 10 mg/kg bw; 75% and 74%, 600 mg/kg bw).

After a single oral dose of 10 mg/kg bw to rats, peak mean concentrations of radioactivity in plasma occurred at 6 and 2 h in males (0.22 µg equiv./mL) and females (0.28 µg equiv./mL), respectively (Table 5). After a single oral dose of 600 mg/kg bw to rats, peak mean concentrations of radioactivity in plasma occurred at 3 h in males (26 µg equiv./mL) and females (29 µg equiv./mL), respectively. The area under the c oncentration versus time curve (AUCt) was calculated at 400 and 355 µg equiv./mL*h in males and females, respectively. These values were around 120 fold higher than the AUCt obtained in the low dose group.

Table 4 Excretion (% of the administered dose) up to 168 hours in rats treated at 10 mg/kg bw and 600 mg/kg bw

Excretion intervals	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Urine 0-6	2.63	3.25	11.55	9.08
Urine 6-24	15.85	12.69	13.85	13.36
Urine 24-48	2.82	2.41	2.33	4.40
Urine 48-72	0.54	0.44	0.59	1.07
Urine 72-96	0.24	0.19	0.30	0.40
Urine 96-120	0.15	0.13	0.21	0.24
Urine 120-144	0.09	0.07	0.17	0.17
Urine 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
Faeces 24-48	11.72	7.56	13.41	22.87
Faeces 48-72	1.18	1.34	1.36	3.83
Faeces 72-96	0.29	0.36	0.35	0.47
Faeces 96-120	0.17	0.27	0.36	0.23
Faeces 120-144	0.35	0.08	0.08	0.12
Faeces 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

From: McEwen, A.B. (1995)

Table 5 Pharmacokinetic parameters of total plasma radioactivity following oral administration of single doses of 10 and 600 mg/kg bw of glyphosate in male and female rats

Parameter	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
Cmax (µg equiv./mL)	0.2219	0.2789	25.97	28.84
Tmax (h)	6.00	2.00	3.00	3.00
AUCt (µg equiv./mL.hour)	3.20	3.70	399.90	355.30
AUC (µg equiv./mL.hour)	3.80	4.20	419.00	*
Terminal rate constant (h^{-1})	0.0840	0.0887	0.1174	*
Terminal half-life (h)	8.30	7.80	5.90	*
Absorption rate constant (h^{-1})	0.2963	0.4239	0.2845	0.4477

* Could not be calculated accurately due to the data at later times being at or close to the limit of reliable measurement; AUCt is the area under the plasma curve calculated up to the last detectable sample. In this study calculations were performed up to 24 hours.; AUC is the area under the plasma curve calculated to infinity; From: McEwen, A.B. (1995).

There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract (GIT) the stomach, muscles and the kidneys, the organs of excretion contained higher concentrations of radioactivity than the plasma (Table 6). High levels of radioactivity were

detected in the content of stomach and GIT. At 7 day poist dosing, the radioactivity in most tissues had decreased to around the limit of detection.

Table 6 Radioactivity in tissues after single oral dose of 10 mg/ kg bw (in mean % of applied dose, except bone expressed as % of applied dose/g)

Tissue	Male			Females		
	6 h	18 h	168h	6 h	18 h	168 h
Bone	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 (GIT)	19.05	10.04	0.01	16.47	5.41	0.01
GIT 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

N=3 From: McEwen, A.B. (1995).

The major urinary component was unchanged glyphosate accounting for 18 -27% of the administered dose. A minor component was also observed in urine, account ing for 0.1 -0.3% of the administered dose, and this was shown to co -chromatograph, using normal phase TLC and reverse phase HPLC to aminomethyl phosphonic acid. The major component in faecal extract was unchanged glyphosate accounting for 65 -78% of the adm inistered dose. Two minor metabolites were also observed in faecal extract accounting for 0.3 -1.6% of the administered dose. One of these two metabolites was shown to co-chromatograph with aminomethyl phosphonic acid (McEwen, 1995).

Five male and five f emale Alpk:APfSD rats were each given a single oral dose of 10 mg/kg bw [^{14}C]-phosphonomethyl labelled glyphosate ((radiochemical purity >98%) in de-ionised water. The excretion was measured over 72 hours. Animals were killed at 72 hours after treatment an d the radioactivity in blood and selected tissues including residual carcass was analysed.

Excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces within 24 hours. Males excreted 1 3.0% and 88.5% of the administered dose in urine and faeces, respectively. Females excreted 10.6% and 88.7% of the administered dose in urine and faeces, respectively.

At the termination of the experiment, tissue concentrations of radioactivity were very l ow and accounted for 0.6% of the administered dose in males and 0.5% of the administered dose in females. The highest concentrations were present in bone (0.5 and 0.4 µg equivalents glyphosate/g for males and females respectively). All other tissue concentrations were 0.07 µg equiv/g or lower. No marked sex difference was seen in the tissue distribution of radioactivity (Davies, 1996a).

Five male and five female Alpk:APfSD rats were each given a single oral dose of 1000 mg [^{14}C]-phosphonomethyl labelled glyphosate /kg bw (radiochemical purity >98%) in de -ionised water. The excretion was measured over 72 hours. Animals were killed at 72 hours after treatment and the radioactivity in blood and selected tissues including residual carcass was analysed.

Excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces within 24 hours. Males excreted 16.7% and 89.6% of the administered dose in urine and faeces, respectively. Females excreted 17. 5% and 84.5% of the administered dose in urine and faeces, respectively.

At the termination of the experiment, tissue concentrations of radioactivity were very low and accounted for 0.5% of the administered dose in males and 0.6% of the administered dose i n females. The amount present in the intestinal tract plus contents were 0.2% of the administered dose in both sexes. The highest concentrations were present in bone (50 and 45 µg equivalents glyphosate/g for males and females respectively). All other tis sue concentrations were 7 µg equiv/g or lower. No marked sex difference was seen in the tissue distribution of radioactivity (Davies, 1996b).

EPA-HQ-2018-002024_0004813

Five male and five female Alpk:APfSD rats were each given a single oral dose of 10 mg [^{14}C]-phosphonomethyl labelled glyphosate /kg bw (radiochemical purity >98%) in de-ionised water. These rats had previously received 10 mg/kg bw of unlabeled glyphosate (purity 99.2%) for 14 days. The excretion was measured over 72 hours. Animals were killed at 72 hours after treatment and the radioactivity in blood and selected tissues including residual carcass was analysed.

Excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces within 24 hours. Males excreted 10.6% and 86.6% of the administered dose in urine and faeces, respectively. Females excreted 10.7% and 90.7% of the administered dose in urine and faeces, respectively.

At the termination of the experiment, tissue concentrations of radioactivity were very low and accounted for 0.5% of the administered dose in both sexes. The amount present in the intestinal tract plus contents were 0.12% of the administered dose in both sexes. The highest concentrations were present in bone (0.36 and 0.35µg equivalents glyphosate/g for males and females respectively). All other tissue concentrations were 0.07 µg equiv/g or lower. No marked sex difference was seen in the tissue distribution of radioactivity. Comparison of the results with those obtained in a separate study where [^{14}C]-glyphosate was administered at the same dose level, but without pre-administration of the unlabelled test substance, shows that pre-dosing of rats with unlabelled glyphosate has no significant effect on either the routes or rates of elimination of a single dose of the radiolabelled test substance (Davies, 1996c).

Two male and two female Alpk:APfSD rats were each given a single oral dose of 10 mg [^{14}C]-phosphonomethyl labelled glyphosate /kg bw (radiochemical purity >96%) in de-ionised water. At intervals of 24 and 48 hours after dosing one rat of each sex were killed and rapidly frozen for whole body autoradiography, to investigate the distribution of radioactivity. The excretion was measured during the study.

Twenty four hours after dosing, male rats excreted 22.3% and 55.5% of the administered dose in the urine and faeces, respectively. Female rats excreted 11.9% and 83.8% of the administered dose in the urine and faeces in 24 hours, respectively. Forty-eight hours after dosing, the remaining male rats excreted 34.0% and 60.5% of the administered dose in the urine and faeces, respectively. Female rats excreted 12.5% and 91.2% of the administered dose in the urine and faeces, respectively, in 48 hours.

The whole body autograms showed no marked differences in the tissue distribution of radioactivity between male and female rats. After 24 hours, whole body autoradiograms showed high levels of radioactivity in the gastrointestinal tract, which was consistent with faeces representing the predominant route of elimination; accordingly, this had declined markedly by 48 hours. The greatest intensity of tissue radiolabelling at both time points was apparent in bone. Some radioactivity was present in the kidney after 24 hours, but had declined by 48 hours. No significant levels of radioactivity were apparent in other tissues (Davies, 1996d).

In a study of absorption, distribution, excretion and metabolism study, groups of five male and five female Sprague-Dawley (Crl:CD BR) rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 95.3% and 96.0% (two batches); radiochemical purity, >99%) as a single dose at 1 or 100mg/kg bw by gavage in water. For excretion study, urine and faeces (5/sex/dose) were collected at selected time intervals for 168 hours. Animals were killed at 168 hours post-dosing and radioactivity in blood and selected tissues were analysed. For pharmacokinetic study, blood samples were withdrawn from (5/sex/dose) at selected intervals up to 72 hours post dosing. For tissue distribution study, 12 male and 12 female rats received single oral dose of either 10 or 100 mg/kg bw per day by gavage. The treated animals were divided into four groups (3 per sex) and were killed at 4, 12, 24 and 72 hours post dosing. For biliary excretion study, 7 males and 7 female cannulated rats received a single oral gavage dose of 1 mg/kg bw. Urine, faeces and bile were collected at various time intervals upto 48 hours post-dosing. Samples of urine and faecal extracts from male and female rats in excretion study were pooled and analysed directly by TLC or HPLC.

Following a single oral dose of 1 mg/kg bw, the major route of elimination after oral dosing was in the faeces with 72.62% and 62.40% recovered in males and females, respectively, with most of the radioactivity being excreted within the first 24 h after dosing (63.93% and 49.69% in males and

females, respectively), suggesting this proportion of the dose was not systemically absorbed. During the 7 days 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 male and 7.71% in female), cage debris (0.03% in male and 0.58% in female) and carcass (0.75% in male and 0.98% in female).

Following a single oral 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 compared 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 male and 11.96% in female), 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 bile cannulated rats dosed at a single oral dose of 1 mg/kg bw, the majority of the dose recovered in faeces (55.33% in male and 60.97% in female) in 48 hours. Renal elimination accounted for 27.45% in male and 24.21% in female, of the administered dose. 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 h and 8.07 h with corresponding AUC of 0.319 and 0.340 µequiv./mL*h in males and females respectively (Table 7). As the elimination halflives could not be calculated for several animals of the high dose group mean AUC0-24 (0.257 and 0.338 µg quiv./mL*h in males and females) were calculated to compare the results of both groups. Following a single oral dose of 100 mg/kg bw, mean AUC0-24 were 58.2 and 50.7µg equiv./mL*h in males and females, respectively.

Table 7: Kinetic parameters in plasma after single oral dose of 1 or 100 mg/kg bw (n = 5)

Kinetic parameters	1 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
Cmax (µg equivalent/mL)	0.016	0.037	8.909	7.634
Tmax (h)	3.900	8.000	3.600	4.000
AUC0-24 (µg equivalent/mL/hr)	0.257	0.338	58.200	50.700
AUC (µg equivalent/mL/hr)	0.319	0.340	*	*
Terminal half life (h)	10.860	8.065	*	*

From: Knowles, S.L. and Mookherjee, C.R. (1996)

After administration of 1 mg/kg bw radioactivity concentrations were detected in all tissues by 4 h post-dose indicating rapid absorption and distribution in the body. Apart from the gastrointestinal tract (and content) and carcass, the kidney was the only tissue with a notable content of radioactivity throughout the observation period. By 72 h, post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with carcass containing most of the remaining radioactivity. After administration of 100 mg/kg bw all tissues were exposed to radiolabelled material by 4 h post-dose. Again, only gastrointestinal tract, carcass and kidney contained significant amounts of radioactivity. By 72 h post-dose concentrations had decreased or plateaued to less than 2 % of the administered dose in all tissues of either sex, with 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 ofglyphosate was very low with greater than 90% of the administered dose eliminated unchanged in the urine and faeces. Elimination was essentially completed by 48 hours, the majority of the radioactivity was recovered in faeces and is likely to be unabsorbed dose (Knowles and 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 rabbits fasted for three hours. In the two replicate experiments conducted three rabbits received ^{14}C-methylene glyphosate, two rabbits received ^{14}C-C1-glycine glyphosate and two rabbits received ^{14}C-C2-glycine glyphosate. All 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 a period of 120 hours. The exhalation of $^{14}CO_2$ was less than 1% of the dose. Aproxamately, 1.2%, 0.7% and 0.1% of the dose was retained in the tissues (excluding gut contents) for ^{14}C-C2-glycine, ^{14}C-C1-glycine and ^{14}C-methylene glyphosate, respectively. The radioactivity retained in the tissues differed by 4-5 fold between ^{14}C-C2-glycine and ^{14}C-C1-glycine but the ranking was similar: liver ≥ kidney ≥ spleen ≥ heart, muscle and gonads. Only ^{14}C-C2-glycine radioactivity got incorporated in the fat (Colvin and Miller, 1973b).

(b) Other routes of administration

Intraperitoneal

In a previously described study by Colvin and Miller (1973c), three treatment groups of three male Wistar rats each were dosed separately via intraperitoneal (IP) 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). ^{14}C -glyphosate administered to male rats via IP injection is 74 -78% excreted in the urine within the first 12 hours post -administration. At 96 hours post -administration, total urinary excretion ranged from 81 to 90% of the administered dose. Faecal excretion ranged from 6 to 14% of the administered radioactivity by 96 hours post-administration, and strongly suggests that ^{14}C -glyphosate is also eliminated via the bile. The percentage of administered radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that observed following oral administration, but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater than in male rats after oral administration, but was i n all cases ≤1% of the administered dose (Colvin and Miller, 1973c).

^{14}C -glyphosate with a radiochemical purity of 98% was administered by the IP route to nine male and nine female Sprague -Dawley rats at a dose level of 1150 mg/kg bw. After dosing the r ats were housed in metabolism cages and blood samples were collected from three to six rats at approximately 0.25, 0.50, 1, 2, 4, 6 and 10 hours. At approximately 0.5, 4 and 10 hours after dosing, three animals of each sex were killed and the femoral bone marrow was isolated. The plasma and bone marrow samples were analysed for radioactivity by LSC.

Peak levels of radioactivity were observed in plasma and bone marrow at approximately 0.5 hours after dosing. When expressed as glyphosate acid equivalents t he peak values for bone marrow and plasma in males and females combined were approximately 340 and 1940 ppm, respectively. The radioactivity in plasma decreased rapidly while it remained more constant in bone marrow over the experimental period of 10 hours. The analysis of the first order elimination rates indicated a half -life of elimination from the plasma of approximately 1 hour for both males and females. The elimination from the bone marrow was slower with a half -life of 4.2 hours for the females and 7.6 hours for the males (Ridley, 1983).

Intravenous

In a previously described study by Ridley and Mirly (1988), groups of male and female rats (Crl:CD®, Sprague Dawley BR rats) received single intravenous (IV) doses of 10 mg/kg bw administered by injection into the lateral tail vein. Urine and faeces at various intervals for 7 days and animals were killed and various tissues and carcass was analysed for radioactivity.

Following IV dosing with ^{14}C -glyphosate at 10 mg/kg bw, the majority of the dose was excreted in the urine following a 7 day elimination period: 79.0% in males and 74.5% in females. Faecal excretion was 4.65% and 8.30% of the administered dose, respectively, for males and females which suggestive of elimination of glyphosate via the bile . Very little of the administered dose was found in the tissues and organs following a 7 -day elimination period (<0.1%). An IV dosing resulted in significantly higher levels of radioactivity in the residual carcasses than found following oral dosing, with the highest concentrations being found in the bone. For male rats, 1.48 ppm glyphosate

EPA-HQ-2018-002024_0004816

equivalents were found in the bone, and 1.59 ppm was found in the bone for the females (Ridley and Mirly, 1988).

In a previously described study by Powles (1992b), absorption, distribution and excretion, groups of five male and five female Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 96.8%; radiochemical purity, >98%) as a single dose at 30 or 1000mg/kg bw by gavage in saline, or intravenously as a single dose at 30mg/kg bw.

Following administration of a single intravenous dose (30 mg/kg bw) more than 84% of the dose was eliminated in urine, mostly within 8 hours post-dosing. Faecal elimination accounted for less than 3.5% of the administered radioactivity. Only a very small proportion of the radioactivity was eliminated in exhaled air and less than 1.4% was present in tissues and the residual carcass when the animals were sacrificed. In contrast faeces were the major route of elimination when (^{14}C)-glyphosate was given by the oral route (Powles, 1992b).

Intramuscular

^{14}C -glyphosate was mixed with isopropylamine and unlabelled glyphosate isopropylamine salt and diluted in water to produce a solution of 4 mg glyphosate/mL. One mL of this solution was injected into the thigh muscle of each of four male Rhesus monkeys. Urine samples were collected at various intervals up to 7 days. For the dermal penetration phase, 25 microliter of ^{14}C glyphosate containing 8.9 mg glyphosate was applied to previously shaved abdomen (7.9 square centimeter area) of six male Rhesus monkeys. After 24 hours each abdomen was swabbed twice with water, twice with acetone and again twice with water to remove the residual glyphosate. Urine samples were collected at various time intervals up to 7 days post application.

During the seven-day collection period following intramuscular injection, an average of 89.9% of the applied radioactivity was excreted in the urine. The overall urinary elimination half-life time ($t_{1/2}$) was 19.7 hours. There were two distinct phases to the elimination kinetics, a rapid phase with a $t_{1/2}$ of 6.9 hours (over the first 24 hours) and a slow phase with a $t_{1/2}$ of 35.1 hours.

The washing procedure removed 14.2% of the applied ^{14}C label of the glyphosate. Following topical application of ^{14}C -glyphosate, a mean total of 1.8% of the applied dose was recovered in the urine during the seven-day collection period. Glyphosate penetrated the monkey skin slowly as only 0.4% of the topically applied dose appeared in the urine after 24 hours. The half-time for dermal penetration and elimination via the kidneys of topically applied glyphosate was 59 hours (Maibach 1983).

In Vitro Dermal absorption

The absorption of glyphosate acid (purity 95.93) from a dried glyphosate wet cake preparation, through abraded rabbit whole skin was measured *in vitro* over 24 hours. The dose was applied to the abraded skin at a nominal rate of 79.8 mg/cm^2 (48.3 mg glyphosate acid/cm^2) which was calculated to be equivalent to the 5000 mg/kg bw per day dose received by rabbits in an in vivo dermal toxicity study (Johnson, 1982), based upon a dose per unit area of skin. The diffusion cell was left unoccluded for an exposure period of 6 hours, at which time the surface of the skin was decontaminated with a sponge wash. Physiological saline was used as the receptor fluid.

The total recovery of the individual cells was in the range of 87.3 % to 98.2 %, with an overall mean recovery of 93.3 % of applied dose. The majority of the applied glyphosate acid (mean 87.9 %) was washed off the skin at 6 hours, with a further 2.38 % washed off at 24 hours. A small proportion (0.041 %) of the dose applied was recovered from the epidermis, with 0.243 % remaining in the dermis. The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24 hour experimental period was 1177 µg/cm2, corresponding to 2.42% of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis was 2.66 %. The results of this

EPA-HQ-2018-002024_0004817

in vitro study indicate the dermal absorption of glyphosate through abraded rabbit skin is slow (Hadfield, 2012a).

The penetration of glyphosate from a glyphosate 360 Isopropyl amine (IPA) salt of glyphosate formulation concentrate, containing a nominal 360 g glyphosate/L and a 1/133 w/v aqueous dilution of the concentrate, containing a nominal 2.7 g glyphosate/L, through human epidermis was measured *in vitro* over 24 hours. The doses were applied to the epidermal membranes at a rate of 10 µL/cm2 and left unoccluded for an exposure period of 8 hours.

Penetration of glyphosate was fastest between 0-2 hours (0.914 µg/cm²/h). The mean penetration rate slowed to 0.074 µg/cm²/h between 2-24 hours. The mean amount penetrated over the entire 24 hour exposure period was 3.51 µg/cm^2, corresponding to 0.096% of the applied dose (Hadfield, 2012b).

The absorption and distribution of glyphosate from an SL formulation (MON 79545) was measured in vitro through human epidermis. The doses were applied as the concentrate formulation (450 g glyphosate/L) and as 1/15.6 v/v and 1/188 v/v (nominally 28.8 and 2.4 g glyphosate /L) aqueous spray strength dilutions of the formulation. [^{14}C]-radiolabeled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to the epidermal membranes at a rate of 10 µL/cm2 and left unoccluded for an exposure period of 24 hours.

The mean total amount of absorbed glyphosate in 24 hours was 0.573 µg/cm2 (0.012% of applied dose) from the 450 g/L concentrate formulation. From the 1/15.6 v/v and 1/188 v/v aqueous dilutions of the formulation, the mean total amounts of absorbed glyphosate in 24 hours were 0.379 and 0.021 µg/cm2 (0.129% and 0.082% of applied dose), respectively (Ward, 2010a).

The absorption and distribution of glyphosate from an SL formulation (MON 79351) was measured in vitro through human epidermis. The doses were applied as the concentrate formulation (480 g glyphosate/L) and as 1/16.7 v/v and 1/200 v/v (nominally 28.7 and 2.4 g glyphosate /L) aqueous spray strength dilutions of the formulation. [^{14}C -radiolabeled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to the epidermal membranes at a rate of 10 µL/cm2 and left unoccluded for an exposure period of 24 hours.

The mean total amount of absorbed glyphosate in 24 hours was 0.342 µg/cm2 (0.0070% of applied dose) from the 480 g/L concentrate formulation. From the 1/16.7 v/v and 1/200 v/v aqueous dilutions of the formulation, the mean total amounts of absorbed glyphosate in 24 hours were 0.0.553 and 0.015 µg/cm2 (0.182% and 0.0488% of applied dose), respectively (Ward, 2010b).

The absorption and distribution of glyphosate from a 360g/L SL formulation was measured in vitro through human epidermis. The doses were applied as the concentrate formulation (360 g glyphosate/L) and as a 3/200 v/v aqueous spray strength dilutions of the formulation. [^{14}C]-radiolabeled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The actual concentrations achieved were 364 and 6.70 g glyphosate for the concentrate and the spray dilution, respectively. The doses were applied to the epidermal membranes at a rate of 5 µL/cm2 and left unoccluded for an exposure period of 24 hours.

For the concentrate, the mean rate of absorption in 24 hours was 0.02 µg/cm2/h. For the 3/200 v/v aqueous dilution, the mean rate of absorption in 24 hours was 0.001 µg/cm2/h. For the concentrate, mild skin washing at 6 and 24 hours removed practically all of the applied dose from the surface of epidermal membrane. For the 3/200 v/v spray dilution skin washing at 6 and 24 hour removed 90.8% and 87.9% of the applied dose, respectively (Davies, 2003).

1.2 Biotransformation

(a) Oral administration

Seven different test groups of rats (Crl:CD®, Sprague Dawley BR rats), each containing an equal number of males and females, were dosed with N-(Phosphono[^{14}C] methyl)glycine (glyphosate labelled in the methylene position between the nitrogen and phosphorous atoms). The radiochemical purity was 98% or greater. Single oral doses were administered by gastrointubation and the intravenous doses were administered by injection into the lateral tail vein. . The number of animals, average body weight at dosing, average dose level, and method of dosing for each test group is summarized in Table 8.

Table 8: Number of animals, average body weight at dosing, average dose level, and method of dosing

Test group	Number of males	Number of females	Mean body weight (g)	Mean dose level (mg/kg bw)	Method of dosing
#1	3	3	males: 242 females: 192	males: 9.93 females: 10.1	oral
#2	3	3	males: 215 females: 189	males: 10.2 females: 10.6	oral
#3	5	5	males: 272 females: 201	males: 10.7 females: 10.6	IV
#4	5	5	males: 229 females: 172	males: 1118 females: 1153	oral
#5	5	5	males: 210 females: 154	males: 9.41 females: 9.28	oral
#6*	5	5	males: 312 females: 235	males: 10.2 females: 10.3	oral
#7	3	3	males: 259 females: 207	males: 10.7 females: 11.0	IV

* Group #6 animals were preconditioned with nonlabelled glyphosate at 10 mg/kg /day for 14 days followed by one oral dose of ^{14}C-glyphosate at the average dose levels listed above.

Comparison of the areas under the curves for radioactivity levels in whole blood after oral and iv administration of radiolabeled glyphosate (Groups 2 and 7, respectively) indicated that absorption of the oral dose of glyphosate at the10 mg/kg bw dose level was 30.4% for males and 35.4% for the females. Glyphosate was isolated as the predominant radioactive fraction in urine (overall recovery of 81.3%) and faeces (overall recovery of 99.2%) and was positively identified in each case by various analytical methods. The minimum glyphosate content as a percent of either urine or faecal extract -contained radioactivity in all of the individual rat excreta samples was 97.46%. HPLC analyses further indicated that glyphosate in the excreta accounted for 98.50 -99.33% of the administered ^{14}C -glyphosate.

In test groups 5 and 6, there was evidence for formation of 0.2 -0.3% and 0.4% AMPA, respectively, from metabolism of glyphosate in vivo. The remainder of the excreta -contained radioactivity was due to low -level impurities present in the dosing material or due to materials that were formed during storage of the excreta samples (Howe, Chott and McClanahan, 1988).

Urine and faeces samples from the previously described study by Powel (1992b) were analysed for identification of glyphosate metabolites. Briefly, groups of five male and five female Sprague -Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 96.8%; radiochemical purity >98%) as a single dose at 30 or 1000 mg/kg bw by gavage in saline, or intravenously as a single dose at 30 mg/kg bw. A further group of five male and five female rats received unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 30 mg/kg bw.

The recovery of radioactivity during the extraction of urine and faecal samples was generally greater than 90%. For all dose groups only one major region of radioactivity was detected when extracts

were analysed by either LC or TLC and this co -chromatographed with a glyphosate standard. The identity of the major component as glyphosate was confirmed by comparison of its FT -IR spectrum with a glyphosate standard. Small amounts of other components were detected but no radiolabelled metabolites were identified (Powles, 1992b).

Urine and faeces samples from the previously described study by McEwen (1995) were analysed for identification of glyphosate metabolites. Briefly, groups of five female Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.9%; radiochemical purity, >98%) as a single dose at 10 or 600mg/kg bw by gavage in water. Urine and faeces were collected for 7 days and analysed for metabolites.

The major urinary component was unchanged glyphosate accounting for 18 -27% of the administered dose. A minor component was also observed in urine, accounting for 0.1 -0.3% of the administered dose, and this was shown to co -chromatograph, using normal phase TLC and reverse phase HPLC to aminomethyl phosphonic acid. The major component in faecal extract was unchanged glyphosate accounting for 65 -78% of the administered dose. Two minor metabolites were also observed in faecal extract accounting for 0.3 -1.6% of the administered dose. One of these two metabolites was shown to co-chromatograph with aminomethyl phosphonic acid (McEwen, 1995).

The biotransformation of glyphosate was investigated in male and female rats administered a single oral dose of [^{14}C]-test substance, either as a single 10 mg/kg dose or following repeated oral doses of 10 mg unlabeled glyphosate/kg or as a single 1000 mg/kg bw dose. The excreta from Davies studies (Davies, 1996a, 1996b, and 1996c) were subjected to metabolite identification. In addition, a single oral dose of 1000 mg [^{14}C]-glyphosate/kg (97.8 radiochemical purity) was also administered to male and female Alpk:APfSD rats fitted with a bile duct cannula. The structural identification of metabolites isolated from urine, bile and faeces, collected over 48 hours (biliary study) or a 72 hour period, was characterised by various analytical methods.

Biliary excretion of radioactivity over 48 hours was negligible, 0.055% and 0. 062% of the administered dose for male and female rats, respectively. The greater percentage of excreted dose was present in faeces in both male (39.1%) and female rats (30.5%). Urinary excretion accounted for 20.8% of the administered dose in male rats and 16.3% of the administered dose in female rats. In bile cannulated rats, the excreted radioactivity (including cage wash) after 48 hours accounted for 62.5% and 52.0% of the administered dose in male and female rats, respectively.

Table 9: Quantification of glyphosate metabolites expressed as percentages of a single oral dose (10 mg/kg), a single oral [^{14}C]-dose after 14 unlabelled doses (10 mg/kg) or a single oral dose (1000 mg/kg) to rats

Sample	Analyte	Low dose study 10 mg/kg		Repeat dose study 10 mg/kg		High dose study 1000 mg/kg	
		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

The main urinary metabolite was unchanged glyphosate, which accounted for virtually the entire radioactivity present, with minor amounts of AMPA, which represented less than 1% of the dose in each study. Solvent extraction of faeces, collected from the various excretion and tissue distribution studies, resulted in the extraction of 61- 89% of the radioactivity present. In each case the extracts contained a single peak, which corresponded to unchanged glyphosate (Macpherson, 1996).

Urine and faeces samples from the previously described study by Knowles and Mookherjee (1996) were analysed for identification of glyphosate metabolites. Briefly, five female Sprague -

EPA-HQ-2018-002024_0004820

Dawley (Crl:CD BR) str ain rats were given [^{14}C]phosphonomethyl-labelled glyphosate as a single dose at 1 or 100mg/kg bw by gavage in water. For excretion study, urine and faeces (5/sex/dose) were collected at selected time intervals for 168 hours.

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

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with glyphosate (including skin and eye irritation and dermal sensitization studies) are summarized in Table 10-15.

Table 10. Results of studies of acute oral toxicity of glyphosate

Species	Strain	Sex	Route	Purity %	Results LD50 (mg/kg bw)	Reference
Mouse	ICR	Male Female	Oral	96.7	>10,000	Shirasu and Takahashi (1975)
Mouse	NMRI	Male Female	Oral	98.6	>2000	Dideriksen (1991)
Mouse	ICR Crj:CD-1	Male Female	Oral	95.68	>5000 (m) >5000 (f) >5000 (combined)	Komura (1995b)
Rat	Sprague-Dawley	Female	Oral	Two analyses: 96.40 & 96.71	>5000	Komura (1995a)
Rat	HanRcc: WIST	Female	Oral	96.66	>2000	Simon (2009a)
Rat	CD / Crl:CD(SD)	Female	Oral	97.52	>2000	Haferkorn (2009)
Rat	Sprague-Dawley	Female	Oral	96.40 and 96.71	>5000	You (2009a)
Rat	CD / Crl:CD(SD)	Female	Oral	95.23	>2000	Haferkorn (2010a)
Rat	CD / Crl:CD(SD)	Female	Oral	97.3	>2000	Haferkorn (2010b)
Rat	Sprague-Dawley derived	Female	Oral	97.23	>5000	Merkel (2005)
Rat	Wistar Hannover	Female	Oral	98.05	>2000	Do Amaral Guimaraes (2008), with addendum dated 2010
Rat	HanRcc: WIST(SPF)	Female	Oral	95.1	>2000	Talvioja, 2007
Rat	Sprague-Dawley	Male Female	Oral	97.76	>5000 (m) >5000 (f) >5000 (combined)	Reagan & Laveglia (1988a)
Rat	Wistar	Male Female	Oral	99	5600 (combined)	Heenehan, Rinehart & Braun (1979a)
Rat	Sprague-Dawley	Male Female	Oral	85.5	>5000	Blaszcak, 1988a)

Rat	Sprague-Dawley	Male Female	Oral	98.6	>5000	Cuthbert and Jackson (1989a)
Rat	Alpk:AP_fSD (Wistar-derived)	Male Female	Oral	95.6	>5000 (male) >5000 (female) >5000 (combined)	Doyle (1996a)
Rat	WIST(SPF)	Female	Oral	96.1	>5000	Arcelin (2007a)
Rat	RjHan:WI	Female	Oral	96.3	>5000	Tavaszi (2011a)
Rat	Wistar	Male Female	Oral	99%	56005	Heenehan (1979)
Mouse	Crj:CD-1(ICR)	Male Female	Oral	62.34% isopropyl amine salt of glyphosate	>5000	Enami & Nakamura (1995)
Rat	Sprague-Dawley derived	Male Female	Oral	62% isopropyl amine salt of glyphosate	>5000	Moore (1999)

(a) *Oral administration*

Mice

Groups of 10 ICR mice of each sex were given glyphosate as a single dose at 1000, 5000 or 10000mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Inactiveness of the behaviour was observed in all mice at a dose level ≥ 5,000 mg/kg bw. Lethality was found in 2/10 males and l/10 females at 10,000 mg/kg bw. The other mice recovered to normal within 2 days after dosing. No abnormalities were found at sacrifice (14 days).

The acute oral LD50 in mice was >10,000 mg/kg bw (Shirasu and Takahashi, 1975).

Groups of five male and five female Bom:NMRI mice were given glyphosate as a single dose at 2000mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination (day 14). Toxicological signs included piloerection and sedation in all mice on day 1. No macroscopic abnormalities were observed at necropsy.

The acute oral LD50 in mice was >2,000 mg/kg bw (Dideriksen, 1991).

In an acute oral toxicity study, 5 male and 5 female ICR (strain Crj:CD-1) mice were orally dosed with 5000 mg/kg bw glyphosate (purity 95.68%). The test material was administered as a 25% suspension in 0.5% carboxymethyl cellulose (CMC) sodium solution at 20 mL/kg bw. Signs of toxicity observed at 1 and/or 3 hours after administration included decreased spontaneous activity in one female and one male, and another male had sedation and a crouching position. One male lost a slight amount of weight days 0-7, but all mice had weight gains over the 14-day observation period. There were no observed abnormalities at necropsy.

The oral LD_{50} of technical glyphosate in male and female mice was > 5 000 mg/kg bw (Komura, 1995b).

Rats

In an acute oral toxicity study, 5 male and 5 female SD (strain Crj:CD) rats were orally dosed with 5000 mg/kg bw glyphosate (purity 95.68%). The test material was administered as a 25% suspension in 0.5% CMC sodium solution at 20 mL/kg bw. There was no mortality. There was

decreased spontaneous motor activity in 5 male and 3 females, and one male had salivation. All rats gained weight days 0-7 and 7-14. There were no observed abnormalities at necropsy.

The oral LD_{50} of technical glyphosate in male and female rats was > 5000 mg/kg bw (Komura, 1995a).

In an acute oral toxicity study, three female albino Sprague -Dawley rats were orally gavaged with 5000 mg/kg bw glyphosate (purity 96.40 and 96.71%). The test material was mixed with deionized water and administered as a 40% suspension at 12.5 mL/kg bw. There was no mortality. One rat showed slight to moderate signs of salivation, piloerection, diarrhoea, polyuria, and activity decrease, with recovery by day 8. The other two rats showed no indications of toxicity. All rats gained weight days 0-7 and 7-14. There were no observed abnormalities at necropsy.

The oral LD_{50} of technical glyphosate in female rats was > 5000 mg/kg bw (You, 2009a).

In an acute oral toxicity study, 2 groups, each containing 3 female HanRcc:WIST rats were orally dosed with 2000 mg/kg bw technical (96.66%) glyphosate. The test material was administered as a 20% suspension in purified water at a dose volume of 10 mL/kg. All rats survived. There were no signs of toxicity, body weight gain was normal, and no macroscopic lesions were observed at necropsy.

The oral LD_{50} of technical glyphosate was >2000 mg/kg bw (Simon, 2009).

In an acute oral toxicity study, 2 groups, each consisting of 3 female CD / Crl:CD(SD) rats were orally dosed with 2000 mg/kg bw technical (97.52%) glyphosate. The test material was administered as a 20% suspension in 0.8% aqueous hydroxypropylmethylcellulose gel at a dose volume of 10 mL/kg. All rats survived. There were no signs of toxicity, body weight gain was normal, and no pathological findings were noted at necropsy.

The oral LD_{50} of technical glyphosate was >2000 mg/kg bw (Haferkorn, 2009).

In an acute oral toxicity study, 2 groups, each consisting of 3 female CD / Crl:CD(SD) rats were orally dosed with 2000 mg/kg bw technical (95.23%) glyphosate. The test material was administered as a 20% suspension in 0.8% aqueous hydroxypropylmethylcellulose gel at a dose volume of 10 mL/kg. All rats survived. There were no signs of toxicity, body weight gain was normal, and no pathological findings were noted at necropsy.

The oral LD_{50} of technical (95.23%) glyphosate was >2000 mg/kg bw (Haferkorn, 2010a).

In an acute oral toxicity study, 2 groups, each consisting of 3 female CD / Crl:CD(SD) rats were orally dosed with 2000 mg/kg bw technical (97.3%) glyphosate. The test material was administered as a 20% suspension in 0.8% aqueous hydroxypropylmethylcellulose gel at a dose volume of 10 mL/kg. All rats survived. There were no signs of toxicity, body weight gain was normal, and no pathological findings were noted at necropsy.

The oral LD_{50} of technical (97.3%) glyphosate was >2000 mg/kg bw (Haferkorn, 2010b).

In an acute oral toxicity study, 3 female Sprague-Dawley derived albino rat were orally dosed with 5000 mg/kg bw technical (97.23%) glyphosate. The test material was administered as a 50% w/v suspension in distilled water (specific gravity: 1.252 g/mL). All rats survived. Clinical signs (all rats) were diarrhoea, ano-genital and facial staining and/or reduced fecal volume, with recovery by day 4. All rats gained weight days 0-7 and again days 7-14. There were no gross abnormalities at necropsy.

The oral LD_{50} of technical (97.23%) glyphosate was >5000 mg/kg bw (Merkel, 2005).

In an acute oral toxicity study, 2 groups, each consisting of 3 female Wistar Hannover rats were orally dosed with 2000 mg/kg bw technical (98.05%) glyphosate. The test material was mixed with deionized water to form a dosing mixture containing 200 mg technical glyphosate/mL. All rats survived. There were no signs of toxicity, all gained weight days 0-7 and 7-14, and there were no specific signs at necropsy.

The oral LD_{50} of technical (98.05%) glyphosate was >2000 mg/kg bw (Do Amaral Guimaraes, 2008, with an addendum dated 2010).

In an acute oral toxicity study, 2 groups, each consisting of 3 female HanRcc:WIST(SPF) rats were orally gavaged with 2000 mg/kg bw technical (95.1%) glyphosate. The test material was diluted in PEG 300 to 0.2 g/mL and administered at a dosing volume of 10 mL/kg. All rats survived. All rats had slightly ruffled hair at 1-3 or 2-3 hours after dosing. No other clinical signs were observed. All gained weight days 1-8 and 8-15. There were no macroscopic signs at necropsy.

The oral LD_{50} of technical (95.1%) glyphosate was >2000 mg/kg bw (Talvioja, 2007).

In an acute oral toxicity study, 5 male and 5 female Sprague-Dawley rats were orally dosed with 5000 mg/kg bw of technical (97.76%) glyphosate. The test material was administered as a 50% w/v aqueous suspension. All rats survived. All had diarrhoea, with recovery by day 4. In addition 3/5 males and 2/5 females had wet abdomen ("apparent urinary incontinence") and one male and one female had hair loss on the abdomen at termination. All gained weight days 1-8 and 8-15. No internal abnormalities were observed at necropsy.

The oral LD_{50} of technical (97.76%) glyphosate was >5000 mg/kg bw (Reagan and Laveglia, 1988a).

In an acute oral toxicity study, groups of 5 male and 5 female Wistar strain albino rats were dosed at 2.5, 3.5 5.0, 7.0 and 9.9 g/kg of technical (99%) glyphosate, administered as a 25% "solution" (suspension?) in distilled water. At 2.5 g/kg 1/5 males and 0/5 females died; at 3.5 g/kg 1/5 males and 0/5 females died; at 5.0 g/kg 0/5 males and 3/5 females died; at 7.0 g/kg 5/5 males and 3/5 females died; at 9.9 g/kg 5/5 males and 5/5 females died. Signs of toxicity included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and fecal staining of the abdomen. The rats which died following dosage at 2.5 g/kg (Day 5) and 3.5 g/kg (Day 8) had considerable weight losses. At 7 and 9.9 g/kg all deaths occurred on Day 1, except for one 9.9 g/kg male which died on Day 12. At necropsy, the male which died on Day 5 after dosing at 2.5 g/kg had urinary and fecal staining of the abdomen, bright red lungs, stomach containing dark red fluid, upper intestines containing dark grey fluid, lower intestines distended with air and containing yellow fluid. The male which died on Day 8 after dosing at 3.5 g/kg had white lungs. Almost all the surviving rats at 2.5 and 3.5 g/kg had red spots on the lungs, and mottled or purple livers. Surprisingly, most of the surviving rats at 5.0 g/kg had no observable abnormalities.

The oral LD_{50} (combined sexes) of technical (99%) glyphosate was calculated to be 5.6 g/kg with 95% confidence limits of 4.9 to 6.3 g/kg (Heenehan, Rinehart and Braun, 1979a).

Groups of five male and five female fasted CD (Sprague-Dawley derived) rats were given glyphosate as a single dose at 5000mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. One of the five females exhibited a weight loss on day 7 but gained weight between days 7 and 14. Toxicological signs included wet rales, faecal staining, urinary staining and soft stool. Some animals had decreased food consumption after dosing; this continued in one animal through day 2. No gross post mortem abnormalities were found at sacrifice (day 14).

The oral LD50 of glyphosate in rats was >5000 mg/kg bw (Blaszcak, 1988a).

Groups of five male and five female fasted Sprague-Dawley rats were given glyphosate as a single dose at 5000mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination (day 14). Toxicological signs included piloerection, reduced activity and ataxia through day 9. No gross post mortem abnormalities were found at sacrifice.

The oral LD50 of glyphosate in rats was >5000 mg/kg bw (Cuthbert and Jackson, 1989a).

In an acute oral toxicity study, 5 male and 5 female Alpk:APfSD (Wistar-derived) rats were dosed at 5000 mg/kg bw with technical (95.6%) glyphosate, administered as a 0.5 g/mL suspension in deionized water. None of the rats died and there were no signs of toxicity. All gained weight Days 1-8 and 8-15. At necropsy 2 males and 2 females had mottled or red areas on the lungs, and one male had red areas on the thymus.

The oral LD_{50} of technical (95.6%) glyphosate was >5000 mg/kg bw (Doyle, 1996a).

In an acute oral toxicity study, 3 female HanRcc:WIST(SPF) rats were dosed at 5000 mg/kg bw with technical (96.1%) glyphosate, administered as a 0.5 g/mL suspension in purified water. None of the rats died. All had slightly ruffled fur (persisting in one rat through Day 3) and all had hunched posture from 1-5 or 2-5 hours after dosage. All gained weight Days 1-8 and 8-15. There were no macroscopic findings at gross necropsy.

The oral LD_{50} of technical (96.1%) glyphosate was >5000 mg/kg bw (Arcelin, 2007a).

In an acute oral toxicity study, 3 female RjHan:WI rats were dosed at 5000 mg/kg bw with technical (96.3%) glyphosate, administered as a 0.5 g/mL suspension in 0.5% Carboxymethylcellulose (CMC). None of the rats died and there were no signs of toxicity. All rats gained weight from Days 0-7 and 7-14. At necropsy, no observations (abnormalities?) were recorded.

The oral LD_{50} of technical (96.3%) glyphosate was >5000 mg/kg bw (Tavaszi, 2011a).

In an acute oral toxicity study groups of 5 male and 5 female Wistar strain albino rats were orally dosed with glyphosate technical (99%) at 2.5, 3.5, 5.0, 7.0 and 9.9 g/kg bw. The test material was administered as a 25% w/v solution in distilled water.

The following were the incidences of mortality: 2.5 g/kg: 1/10; 3.5 g/kg: 1/10; 5.0 g/kg: 3/10; 7.0 g/kg: 8/10; and 9.9 g/kg: 10/10. Signs of toxicity included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and fecal staining of the abdomen.

The oral LD50 in rats was 5.6 g/kg with 95% confidence limits of 4.9 to 6.3 g/kg (Heenehan, 1979).

In an acute oral toxicity study 5 male and 5 female Crj:CD-1(ICR) mice were dosed with a formulation (described as a light viscous solution with a specific gravity of 1.23) containing 62.34% isopropyl amine salt of glyphosate. The test material was administered undiluted. None of the mice died and there were no signs of toxicity. There was a slight retardation in mean body weight gain in the males from Day 0 to 7 as compared with their controls (5000 mg/kg bw: 32.8 to 35.1 g; controls: 32.6 to 37.3 g). No gross pathological abnormalities were observed at gross necropsy.

The mouse oral LD_{50} of a formulation containing 62.34% isopropyl amine salt of glyphosate was >5000 mg/kg bw (Enami and Nakamura, 1995).

In an acute oral toxicity study 5 male and 5 female Sprague-Dawley derived albino rats were orally dosed with a formulation (described as a clear viscous amber liquid with a specific gravity of 1.214 g/mL) containing 62% isopropylamine glyphosate. There was no mortality. There were no signs of toxicity in the males; 4/5 females had anogenital staining, with diarrhoea in one of these females and soft faeces in another. These signs were gone by Day 3. All rats gained weight Days 0-7 and 7-14. There were no gross abnormalities at necropsy.

The rat oral LD_{50} of a formulation containing 62% isopropylamine glyphosate was >5000 mg/kg bw (Moore, 1999).

(b) Dermal application

Table 11. Results of studies of acute dermal toxicity of glyphosate

Species	Strain	Sex	Route	Purity %	Results LD50 or (mg/kg bw)	Reference
Rat	Sprague-Dawley	Male Female	Dermal	Not reported	>2000	Cuthbert & Jackson (1989)
Rat	Sprague-Dawley	Male Female	Dermal	Two analyses: 96.40 & 96.71	>5050	You (2009b)
Rat	SD(Crj:CD)	Male Female	Dermal	95.68	>2000	Komura (1995c)
Rat	HanRcc: WIST(SPF)	Male Female	Dermal	96.66	>2000	Simon (2009b)
Rat	CD/Crl: CD(SD)	Male Female	Dermal	97.52	>2000	Haferkorn (2009b)
Rat	CD/Crl: CD(SD)	Male Female	Dermal	95.23	>2000	Haferkorn (2010e)
Rat	CD/Crl: CD(SD)	Male Female	Dermal	96.6	>2000	Haferkorn (2010f)
Rat	Sprague-Dawley	Male Female	Dermal	97.23	>5000	Merkel (2005b)
Rat	Wistar Hannover	Male Female	Dermal	98.05	>2000	Do Amaral Guimaraes (2008b),
Rat	HanRcc: WIST(SPF)	Male Female	Dermal	95.1	>2000	Talvioja (2007e)
Rat	Alpk:AP$_f$SD (Wistar-derived)	Male Female	Dermal	95.6	>2000	Doyle (1996b)
Rat	HanRcc: WIST(SPF)	Male Female	Dermal	96.1	>5000	Arcelin (2007b)
Rat	RjHan (WI) Wistar	Male Female	Dermal	96.3	>5000	Zelenak (2011a)
Rabbit	New Zealand White	Male Female	Dermal	85.5	>5000	Blaszcak (1988)
Rabbit	New Zealand White	Male Female	Dermal	97.76	>5000	Reagan (1988)
Rabbit	New Zealand White	Male Female	Dermal	99	>5000	Heenehan et al. (1979)

Rats

In an acute dermal toxicity study, 5 male and 5 female Sprague -Dawley rats were dermally dosed with 2000 mg technical glyphosate (purity not reported)/kg, with 24 -hour exposure. The test material was moistened with an unspecified amount of water before application. There was no mortality. Clinical signs during exposure consisted of piloerecti on and reduced activity. All rats gained weight Days 0 -7; all gained weight Days 7 -14 except for one female that lost 30 g. No abnormalities were detected at necropsy.

The rat dermal LD_{50} of technical (purity not reported) glyphosate was >2000 mg/kg bw (Cuthbert and Jackson, 1989).

In an acute dermal toxicity study, 5 male and 5 female Sprague -Dawley albino rats were dermally dosed with 5050 mg technical (two analyses: 96.40 and 96.71%) glyphosate, with 24 -hour

EPA-HQ-2018-002024_0004826

exposure. The test material was moistened with 0.284 mL deionized water/g test material. There was no mortality. There were no clinical signs. All rats gained weight Days 0 -7; except for one female that lost 3 g all gained or maintained weight Days 7 -14. There were no observable abnormalities at necropsy.

The rat dermal LD_{50} of technical (two analyses: 96.40 and 96.71%) glyphosate was >5050 mg/kg bw (You, 2009b).

In an acute dermal toxicity study, 5 male and 5 female SD (Crj:CD) rats were dermally exposed to 2000 mg technical (95.68%) glypho sate/kg. Appropriate amounts of finely ground test material were applied to a shaved 4x5 cm skin site on each rat, which was then covered with a filter paper moistened with 0.5 mL deionized water. There was 24 -hour exposure. A control group of 5 males and 5 females was similarly treated without the test material. There was no mortality. There were no clinical signs. All gained weight Days 0-7 and 7-14, and weight gains were similar in the glyphosate-exposed rats and their controls. There were no abnormalities at necropsy.

The rat dermal LD_{50} of technical (95.68%) glyphosate was >2000 mg/kg bw (Komura, 1995c).

In an acute dermal toxicity study, 5 male and 5 female HanRcc:WIST(SPF) rats were dermally exposed to 2000 mg technical (96.66%) glyphosate, with 24-hour exposure. The test material was formulated in purified water at a concentration of 0.5 g/mL, and this formulation was applied at a volume dose of 4 mL/kg. There was no mortality. There were no clinical signs. There was no dermal irritation in males. Dermal irritation (slight erythema, scaling, scabs) was seen in 4 females from Day 4, persisting to Day 12 at the latest. All males had weight gains Days 1 -8 and 8-15. Two females had slight (0.6 and 1.7 g) weight losses Days 1-8, but all females had good weight gains Days 8-15. No macroscopic findings were observed at necropsy.

The rat dermal LD_{50} of technical (analysis: 96.66%) glyphosate was >2000 mg/kg bw (Simon, 2009b).

In an acute dermal toxicity study, 5 male and 5 female CD/Crl:CD(SD) rats were dermally exposed to 2000 mg technical (analysis: 97.52%) glyphosate, with 24 -hour exposure. The test material was suspended (0.2 g/mL) in *aqua ad iniectabilia*. This suspension was applied to 8 layers of gauze, which in turn was applied to a n intact dermal skin site measuring 5 cm x 6 cm. The gauze was covered with a plastic sheet which was then secured with adhesive plaster. There was no mortality. There were no signs of toxicity. All rats gained weight Days 0 -8 and 8 -15. No skin irritation was observed. No pathological changes were observed at necropsy.

The rat dermal LD_{50} of technical (analysis: 97.52%) glyphosate was >2000 mg/kg bw (Haferkorn, 2009b).

In an acute dermal toxicity study, 5 male and 5 female CD/Crl:CD(SD) rats were d ermally exposed to 2000 mg technical (analysis: 95.23%) glyphosate, with 24 -hour exposure. The test material was suspended (0.2 g/mL) in *aqua ad iniectabilia*. This suspension was applied to 8 layers of gauze, which in turn was applied to an intact dermal skin site measuring 5 cm x 6 cm. The gauze was covered with a plastic sheet which was then secured with adhesive plaster. There was no mortality. There were no signs of toxicity. All rats gained weight Days 0 -8 and 8 -15. No skin irritation was observed. No pathological changes were observed at necropsy.

The rat dermal LD_{50} of technical (analysis: 95.23%) glyphosate was >2000 mg/kg bw (Haferkorn, 2010e).

In an acute dermal toxicity study, 5 male and 5 female CD/Crl:CD(SD) rats were dermally exposed t o 2000 mg technical (analysis: 966 g/kg) glyphosate, with 24 -hour exposure. The test material was suspended (0.2 g/mL) in *aqua ad iniectabilia*. This suspension was applied to 8 layers of gauze, which in turn was applied to an intact dermal skin site meas uring 5 cm x 6 cm. The gauze

was covered with a plastic sheet which was then secured with adhesive plaster. There was no mortality. There were no signs of toxicity. All rats gained weight Days 0-8 and 8-15. No skin irritation was observed. No pathological changes were observed at necropsy.

The rat dermal LD_{50} of technical (analysis: 966 g/kg) glyphosate was >2000 mg/kg bw (Haferkorn, 2010f).

In an acute dermal toxicity study, 5 male and 5 female Sprague-Dawley derived albino rats were dermally exposed to 5000 mg technical (97.23%) glyphosate, with 24-hour exposure. The test material was mixed with distilled water to form a dry paste (70% w/w mixture in distilled water). An appropriate amount of this paste was applied to a 2 x 3 inch 4-ply gauze pad, which was placed on a skin area of 2 x 3 inches. The gauze pad and trunk of the rat was then wrapped with Durapore tape. There was no mortality. There were no signs of toxicity. All rats gained weight Days 0-7 and 7-14. There were no abnormalities at necropsy.

The rat dermal LD_{50} of technical (97.23%) glyphosate was >5000 mg/kg bw (Merkel, 2005b).

In an acute dermal toxicity study, 5 male and 5 female Wistar Hannover rats were dermally exposed to 2000 mg technical (980.5 g/kg) glyphosate, with 24-hour exposure. The test material was placed on a porous gauze dressing which had been moistened with deionized water. The gauze dressing was applied to the skin with a non-irritating tape. The test site and trunk of the animal were further covered with adhesive tape. There was no mortality. There were no signs of toxicity. All rats gained weight Days 0-7; all rats with the exception of two females (one lost 2 g, the other maintained weight) gained weight Days 7-14. There were no specific findings at necropsy.

The rat dermal LD_{50} of technical (980.5 g/kg) glyphosate was >2000 mg/kg bw (do Amaral Guimaraes, 2008b).

In an acute dermal toxicity study, 5 male and 5 female HanRcc:WIST (SPF) rats were dermally exposed to 2000 mg technical (95.1%) glyphosate, with 24-hour exposure. The test item was diluted in PEG 300 to a concentration of 0.33 g/mL and this dilution was administered at a volume dosage of 6 mL/kg. The dilution was applied on an intact shaved skin site which was covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. There was no mortality. No clinical signs were observed. All rats gained weight Days 1-8 and 8-15 except for one female that maintained weight Days 8-15. There were no macroscopic findings at necropsy.

The rat dermal LD_{50} of technical (95.1%) glyphosate was >2000 mg/kg bw (Talvioja, 2007e).

In an acute dermal toxicity study, 5 male and 5 female Alpk:APfSD (Wistar-derived) rats were dermally dosed with 2000 mg technical (95.6%) glyphosate acid, with 24-hour exposure. The appropriate amount of test material was weighed onto a plastic weighing boat and was then moistened to a dry paste with 0.6-0.8 mL deionized water. Approximately half of the 10 cm x 5 cm clipped skin area was covered by the paste, and the estimated amount of test material applied per unit area of exposed skin was 20.0-21.9 mg/cm^2 for males and 16.2-17.3 mg/cm^2 for females. The paste was covered by a 4-ply gauze patch (~7 cm x 7 cm) which was kept in contact with the skin for 24 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film which was held in place using an adhesive bandage (~25 cm x 7 cm) which was secured by two pieces of PVC tape (~2.5 cm x 20 cm).

None of the animals died and there were no significant signs of systemic toxicity. It is reported that signs of urinary incontinence were seen in some rats, but it is also stated that this is a common finding in dermal toxicity studies as a result of bandaging and is not considered to be of any toxicological significance. The skin of all rats was stained cream by the test substance for up to 8 days. There were practically no signs of skin irritation. One male had slight erythema on Days 2-3

and one female had small scabs on Days 3-8. All gained weight Days 1-8, and, with the exception of one female (which lost 2 g) all gained weight Days 8-15. At necropsy, the only finding was that one female had red mottled lungs. It is stated that this is a common spontaneous finding in rats of this age and strain and is not considered to be treatment-related

The rat dermal LD_{50} of technical (95.6%) glyphosate acid was >2000 mg/kg bw (Doyle, 1996b).

In an acute dermal toxicity study, 5 male and 5 female HanRcc:WIST (SPF) rats were dermally exposed to 5000 mg technical (96.1%) glyphosate acid, with 24-hour exposure. The appropriate amount of test item was weighed out in a plastic weighing boat and moistened to a dry paste with 0.5-0.6 mL purified water. The dry paste was applied evenly on an intact 8 cm^2 clipped skin site which was covered with tape. There was no mortality. No clinical signs were observed. All rats gained weight Days 1-8 and 8-15. There were no macroscopic findings at necropsy.

The rat dermal LD_{50} of technical (95.6%) glyphosate acid was >5000 mg/kg bw (Arcelin, 2007b).

In an acute dermal toxicity study, 5 male and 5 female Rj:Han (WI) Wistar rats were dermally exposed to 5000 mg technical (96.3%) glyphosate, with 24-hour exposure. Sufficient water to moisten the test material was used to ensure good contact with the skin. The test material suspension was applied uniformly at the dermal site. Gauze pads were placed over the application site, and these were covered with a hypoallergenic plaster. The entire trunk of the rat was then wrapped with semi-occlusive plastic wrap for 24 hours. There was no mortality. No clinical signs were observed. There was no treatment-related dermal irritation. All rats gained weight Days 0-7 and 7-14. There were no macroscopic observations at necropsy.

The rat dermal LD_{50} of technical (96.3%) glyphosate acid was >5000 mg/kg bw (Zelenak, 2011a).

In an acute dermal toxicity study, 5 male and 5 female New Zealand White rabbits were dermally exposed to 5000 mg glyphosate (85.5%)/kg, with 24-hour exposure. The test material was applied dry to a strip of 8-ply gauze, and was moistened with ~15 mL 0.9% saline. The gauze strip was then applied to the application site. All rabbits survived the 14-day observation period, with little or no change in body weights. No clinical signs were observed. There was no dermal irritation. Nothing remarkable was observed at gross necropsy.

The rabbit dermal LD50 of glyphosate was >5000 mg/kg bw (Blaszcak, 1988).

In an acute dermal toxicity study, 5 male and 5 female New Zealand White rabbits were dermally exposed to 5000 mg glyphosate (97.76%)/kg, with 24-hour occluded exposure. The test material was moistened with 0.9% saline (~1 mL/g of test material). An appropriate amount of this mixture was then applied to each application site. One female rabbit died at 14 days, but this death was attributed to exposure to mucoid enteropathy and not to exposure to the test material. Other signs were anorexia, diarrhea and soft stools. Most rabbits gained slight amounts of weight in the 14-day observation period. At necropsy, one male rabbit had a white caseous substance adhering to the lungs (not ascribed to exposure to the test material); otherwise, there was nothing remarkable.

The rabbit dermal LD50 of glyphosate (97.76%) was >5000 mg/kg (Reagan, 1988).

In an acute dermal toxicity study, 2 male and 2 female New Zealand White rabbits were dermally exposed (on abraded skin) to 5000 mg technical glyphosate (99%) /kg, with 24-hour occluded exposure. The test material was applied as a 25% w/v solution in physiological saline. All rabbits survived. All rabbits had a clear nasal discharge, which was gone by Day 6. One male lost weight over the 14-day observation period. At 24 hours there was well-defined erythema in 2 rabbits, and very slight erythema in the other 2; very slight edema was present in 2 rabbits. At necropsy there were no internal or external abnormalities.

The rabbit dermal LD50 of technical glyphosate was >5000 mg/kg (Heenehan, 1979).

(c) Exposure by inhalation

Table 12 Results of studies of acute inhalation toxicity of glyphosate

Species	Strain	Sex	Route	Purity %	Results LC50 (mg/L)	Reference
Rat	CD/Crl: CD(SD)	Male Female	Inhalation (nose-only)	96.6	>5.18	Haferkorn (2010a)
Rat	F344/DuCrj SPF	Male Female	Inhalation (whole-body)	97.56	>5.48	Koichi (1995)
Rat	HsdRcc Han™	Male Female	Inhalation (nose-only)	96.66	>5.04	Griffiths (2009)
Rat	CD/Crl:CD(SD)	Male Female	Inhalation (nose-only)	97.52	>5.12	Haferkorn (2009c)
Rat	CD/Crl:CD(SD)	Male Female	Inhalation (nose-only)	95.23	>5.02	Haferkorn (2010g)
Rat	Sprague-Dawley	Male Female	Inhalation (nose-only)	Two analyses: 96.40 & 96.71	>2.24	Carter (2009)
Rat	Sprague-Dawley	Male Female	Inhalation (nose-only)	97.23	>2.04	Merkel (2005c)
Rat	Not reported	Male Female	Inhalation (nose-only)	98.05	>5.21	Dallago (2008)
Rat	HanRcc: WIST(SPF)	Male Female	Inhalation	95.1	>3.252	Decker (2007)
Rat	Alpk:APfSD (Wistar-derived)	Male Female	Inhalation	95.6	>4.43	Rattray (1996)
Rat	Wistar RjHan (WI)	Male Female	Inhalation	96.9	>5.04	Nagy (2011)
Rat	Sprague-Dawley	Male Female	Inhalation	62% isopropyl amine salt of glyphosate	>2.08	Wnorowski (1999)
Rat	Hsd:Sprague-Dawley	Male Female	Inhalation	47.2% glyphosate acid equivalent	>5.27	Bonnette (2004)

Rats

In an acute inhalation toxicity study with glyphosate TC (966 g/kg), 5 male and 5 female CD/Crl:CD(SD) rats were exposed (nose -only) for 4 hours to a mean concentration (HPLC - determined) of 5.18 mg/L (5.05 mg/L as measured gravimetrically). There was no mortality. Following exposure all rats showed tremors and dyspnea which were still present in all at 3 hours (last observation on Day 1) after exposure; these signs were no long er present on test day 2 (the day following exposure). All rats gained weight Days 0-8 and 8-15. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of glyphosate TC (966 g/kg) was >5.18 mg/L (Haferkorn, 2010a).

In an acute inhala tion toxicity study with technical (97.56%) glyphosate, 5 male and 5 female F344/DuCrj SPF rats were exposed (whole -body) for 4 hours to a mean concentration (determined

EPA-HQ-2018-002024_0004830

analytically) of 5.48 mg test material/L. There was no mortality. Following exposure, there was wetted fur in the perioral and periocular regions, and red adhesive materials in the periocular and nasorostral regions, which disappeared by Day 4 in males and by Day 5 in females. All rats gained weight Days 0-7 and 7-14. No abnormalities were detected at necropsy.

The rat inhalation LC_{50} of technical (97.56%) glyphosate was >5.48 mg/L (Koichi, 1995).

In an acute inhalation toxicity study with technical (96.66%) glyphosate, 5 male and 5 female HsdRccHan™ rats were exposed (nose-only) to a mean concentration (gravimetrically determined) of 5.04 mg test material/L. There was no mortality. Following exposure all rats showed an increased respiratory rate, hunched posture, pilo-erection and wet fur, with the signs still present at 1 hour after exposure. These signs were gone the following day. All rats gained weight days 0-7 and all gained or maintained weight days 7-14. There were no macroscopic observations at necropsy.

The rat inhalation LC_{50} of technical (96.66%) glyphosate was >5.04 µm (Griffiths, 2009).

In an acute inhalation toxicity study with technical (analysis: 97.52%), 5 male and 5 female CD/Crl:CD(SD) rats were exposed (nose -only) to 5.12 mg/L (determined by HPLC). There was no mortality. Following exposure all rats had slight dyspnea and ataxia which was still present at 1 hour, but was gone at 3 hours. All rats gained weight Days 0 -8 and 8 -15. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (97.52%) glyphosate was >5.12 mg/L (Haferkorn, 2009c).

In an acute inhalation toxicity study with glyphosate TC (95.23% active), 5 male and 5 female CD/Crl:CD(SD) rats were exposed (nose -only) for 4 hours to a mean concentration (HPLC - determined) of 5.02 mg/L (4.99 mg/L as measured gravimetrically) . There was no mortality. Following exposure all rats showed slight ataxia, slight tremors and slight dyspnea which were still present in all at 3 hours (last observation on Day 1) after exposure; these signs were no longer present on test day 2 (the day following exposure). All rats gained weight Days 0-8 and 8-15. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of glyphosate TC (95.23% active) was >5.02 mg/L (Haferkorn, 2010g).

In an acute inhalation toxicity study with gl yphosate (two analyses: 96.40 and 96.71%), 5 male and 5 female Sprague-Dawley rats were exposed (nose -only) for 4 hours to a mean concentration of 2.24 test substance/L (nominal concentration: 7.89 mg/L). There was no mortality. All rats showed piloerection and activity decrease starting at 4.5 hours after exposure began and continuing until Day 4. All rats gained weight Days 0-7 and 7-14. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate (two analyses: 96.40 and 96.71%) was >2.24 mg/L (Carter, 2009).

In an acute inhalation toxicity study with glyphosate acid technical (97.23%), 5 male and 5 female Sprague-Dawley rats were exposed (nose -only) for 4 hours to a gravimetrically determined mean concentration of 2.0 4 test substance/L (nominal concentration: 8.99 mg/L). There was no mortality. There were no signs of toxicity. All rats gained weight Days 0-7 and 7-14. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate acid technical (97.23%) was >2.04 mg/L (Merkel, 2005c).

In an acute inhalation toxicity study with glyphosate acid technical (980.5 g/kg), 5 male and 5 female rats (strain not reported: "healthy young adults were supplied by BIOAGRI'S rearing house") were exposed (nose-only) for 4 hours to a gravimetrically determined mean concentration of 5.211

test substance/L. There was no mortality. There were no signs of toxicity. All rats gained weight Days 0-7 and 7-14. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate acid technical (980.5 g/kg) was >5.211 mg/L (Dallago, 2008).

In an acute inhalation toxicity study with technical (95.1%) glyphosate, 5 male and 5 female HanRcc:WIST(SPF) rats were exposed (nose-only) for 4 hours to a gravimetrically-determined concentration of 3.252 mg/L (nominal: 6.304 mg/L) test material.

There was no mortality. Two males had salivation and rales following exposure, and another male had rales. Two females had rales. All signs were gone two days after exposure. All gained weight Days 1-8 and 8-15. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (95.1%) glyphosate was >3.252 mg/L (Decker, 2007).

In an acute inhalation toxicity study with glyphosate acid (95.6%), 5 male and 5 female Alpk:AP_fSD (Wistar derived) rats were exposed (nose-only) for 4 hours to a particulate concentration of 4.43 mg/L; the chemical concentration was 4.27 mg/L. Two males and 2 females exposed to 4.43 mg/L were found dead or were terminated *in extremis* on days 5, 6 or 9. Clinical signs seen in all rats included decreased activity, irregular breathing, hunched posture and piloerection. Signs observed in some rats included splayed gait, reduced stability, signs of urinary incontinence, gasping, and vocalization. Hunched posture persisted in some females to day 13. All surviving males and females lost weight days 1-8, but gained weight days 8-15. The two males found dead had dark lungs; one of the females was found dead and another was killed. The lungs of the decedent females were normal and the report states that the dark lungs in the males were probably the result of agonal congestion.

Because of the mortality at 4.43 mg/L, a second group of 5 male and 5 female rats was exposed to a particulate concentration of 2.47 mg/L (the chemical concentration was measured to be 2.43 mg/L). No mortality occurred in this group. Clinical signs seen in all rats included hunched posture, piloerection and salivation. All males and 4/5 females had abnormal respiratory noise, which was still present in one male on day 15. All rats gained weight days 1-8 and 8-15. At necropsy one female had dark lungs and another had a few red spots on the lung. These were probably incidental observations,

The rat inhalation LC_{50} of glyphosate acid (95.6%) was greater than 4.43 mg/L, although mortality (in 4/10 rats) occurred at this concentration. No mortality occurred at 2.47 mg/L, although there were signs of toxicity (Rattray, 1996).

In an acute inhalation toxicity study with glyphosate technical (96.9%), 5 male and 5 female Wistar RjHan (WI) rats were exposed (nose-only) for 4 hours to a gravimetrically-determined concentration of 5.04 mg/L (nominal: 7.71 mg/L). The percentage of the aerosol that was less than 4 µm (considered to be the inhalable portion) was 54.4%. One male was found dead on Day 4. Clinical signs (all rats) included labored and noisy respiration, respiratory rate increase, gasping, sneezing, decreased activity, thin body appearance. The majority of the rats (all except the one found dead on Day 4) had recovered by Day 3; the one male which subsequently died had slight noisy respiration, slight labored respiration, and a wasted appearance on Day 3 (this animal had lost 47 g from Day 0 to Day 3). It is stated that a specific cause of death was not determined for this one male. All survivors gained weight Days 0-7 except for one male which had lost 9 g; all gained weight Days 7-14. At necropsy the male decedent had lungs with dark discoloration, red diffuse all lobes. No observations were noted for the surviving rats.

The rat inhalation LC_{50} of glyphosate technical (96.9%) was greater than 5.04 mg/L, with 1/10 rats dying following exposure to that concentration (Nagy, 2011).

In an acute inhalation toxicity study with NUP5a99 62% glyphosate MUP [described as a clear viscous liquid containing 62% isopropylamine glyphosate, 31% other ingredients, which adds up to

93%], 5 male and 5 female Sprague -Dawley derived albino rats we re exposed (whole -body) for 4 hours to a gravimetrically -determined concentration of 2.08 mg/L [nominal value: 18.38 mg/L]. There was no mortality. In -chamber observations included ocular and nasal discharge, huched posture and hypoactivity, but the rats recovered quickly on removal from the chamber and the only finding at 1 hour post-exposure was test substance on the fur. All rats gained weight Days 0 -7 and 7-14. There were no gross abnormalities at necropsy.

The inhalation LC_{50} of NUP5a99 glyphosate MUP (62% isopropylamine glyphosate) was > 2.08 mg/L (Wnorowski, 1999).

In an acute inhalation toxicity study with MON 78623 (47.2% glyphosate acid equivalent; 57.8% potassium salt of glyphosate), two groups of 5 male and 5 female Hsd: Sprague -Dawley® SD® rats were exposed for 4 hours to either 2.21 or 5.27 mg test material/L.

There was no mortality at either 2.21 or 5.27 mg/L. At 2.21 mg/L there was congested breathing and dark material around the eyes and/or nose, with clearing by Day 8. At 5.27 mg/ L there was congested breathing, with few faeces in 2 females on Day 1. All signs were gone by Day 3. At 2.21 mg/L all rats gained weight Days 0 -7 and 7-14. At 5.27 mg/L all males gained weight Days 0 -7 and 7-14, while two females (the ones with few faeces on Day 1) lost 2 and 6 g on Days 0 -7; another female lost 6 g on Days 7-14; otherwise females gained weight Days 0-7 and 7-14. At both 2.21 and 5.27 mg/L all tissues were within normal limits at necropsy.

The inhalation LC_{50} of MON 78623 (47.2% glyphosate acid equivalent; 57.8% potassium salt of glyphosate) was > 5.27 mg/L (Bonnette, 2004).

(d) Dermal irritation

Table 13: Results of studies of primary dermal irritation with glyphosate

Species	Strain	Sex	Route	Purity %	Results	Reference
Rabbit	New Zealand White	Male Female	Dermal Irritation	95.1	Non-irritating	Talvioja (2007d)
Rabbit	Himalayan	Male	Dermal Irritation	95.23	Non-irritating	Leuschner (2009a)
Rabbit	New Zealand White	Female	Dermal Irritation	97.56	Non-irritating	Hideo Ueda (1995a)
Rabbit	Himalayan	Male	Dermal Irritation	97.52	Non-irritating	Leuschner (2009c)
Rabbit	Himalayan	Male	Dermal Irritation	96.6	Non-irritating	Leuschner (2010a)
Rabbit	New Zealand White	Male Female	Dermal Irritation	96.71	Non-irritating	You (2009c)
Rabbit	New Zealand White	Male	Dermal Irritation	97.23	Non-irritating	Merkel (2005d)
Rabbit	New Zealand White	Female	Dermal Irritation	98.05	Non-irritating	Canabrava Frossard de Faria (2008a)
Rabbit	New Zealand White	Male Female	Dermal Irritation	97.76	Non-irritating	Reagan & Laveglia (1988b)
Rabbit	New Zealand White	Male Female	Dermal Irritation	99	Slightly irritating	Heenehan (1979)
Rabbit	New Zealand White	Female	Dermal Irritation	95.6	Non-irritating	Doyle (1996c)
Rabbit	New Zealand White	Male Female	Dermal Irritation	96.1	Non-irritating	Arcelin (2007c)
Rabbit	New Zealand White	Male-	Dermal Irritation	96.3	Mildly irritating	Zelenak (2011b)
Rabbit	New Zealand White	Male Female	Dermal Irritation	85.5	Slightly irritating	Blaszcak (1988)

In a dermal ir ritation study, 3 male and female New Zealand White rabbits were dermally exposed for 4 hours to NUP 05068 (Glyphosate technical, 95.1%). "According to information provided by the Sponsor the pH of a 1% w/w formulation of Glyphosate Technical (NUP 05068) in water is 2.2…" 0.5 g test material was moistened with ~0.5 mL purified water and this mixture was placed on a ~4 cm x 4 cm gauze patch [Note: the usual protocol calls for application to a 6 cm^2 area] which was then placed on the application site. The patch was covered with a semi-occlusive dressing, which was wrapped around the abdomen and anchored with tape.

All irritation scores were zero. The PDII = 0.00. 4 -hour semi-occluded exposure to Glyphosate Technical (95.1%) over a skin area of ~16 cm^2 (rather than the usual 6 cm^2) resulted in no dermal irritation (Talvioja, 2007d).

In a dermal irritation study, 3 male Himalayan rabbits were dermally exposed for 4 hours to Glyphosate TC (analysis: 95.23%). 1000 or 2000 mg of the test item were mixed with 0.5 or 1.0 mL *aqua ad iniectabilia,* respectively. 750 mg of this paste (containing 500 mg test item) were applied to a 6 cm^2 skin area on each of 3 rabbits. The paste was covered with a gauze patch which was held in contact with the skin with non-irritating tape during the exposure period.

All irritation scores (at 1, 24, 48 and 72 hours) were zero. The PDII = 0.00. 4 -hour dermal exposure to Glyphosate TC (95.23%) resulted in no dermal irritation (Leuschner, 2009a).

In a dermal irritation study, six female New Zealand White rabbits were dermally exposed for 4 hours to HR-001 (97.56% active). Before application, the test material was finely ground in a mortar. A dose of 0.5 g was applied to each 2.5 x 2.5 cm dose site. A 2.5 x 2.5 cm gauze patch moi sted with 0.5 mL water was then applied over the test material. The patch was held in place with a polyethylene sheet and non-irritating occlusive tape during the exposure period.

All irritation scores (at 1, 24, 48 and 72 hours) were zero. The PDII = 0 .00. 4-hour exposure to HR-001 (97.56% active) resulted in no dermal irritation (Hideo Ueda, 1995).

In a dermal irritation study, 3 male Himalayan rabbits were dermally exposed for 4 hours to Glyphosate TC (analysis: 97.52%). 1000 or 2000 mg of the test item were mixed with 0.5 or 1.0 mL *aqua ad iniectabilia,* respectively. 750 mg of this paste (containing 500 mg test item) were applied to a 6 cm^2 skin area on each of 3 rabbits. The paste was covered with a gauze patch which was held in contact with the skin with non-irritating tape during the exposure period.

All irritation scores (at 1, 24, 48 and 72 hours) were zero. The PDII = 0.00. 4 -hour dermal exposure to Glyphosate TC (97.52%) resulted in no dermal irritation (Leuschner, 2009c).

In a dermal ir ritation study, 3 male Himalayan rabbits were dermally exposed for 4 hours to Glyphosate TC (analysis: 966 g/kg). 1000 or 2000 mg of the test item were mixed with 0.5 or 1.0 mL *aqua ad iniectabilia,* respectively. 750 mg of this paste (containing 500 mg test item) were applied to a 6 cm^2 skin area on each of 3 rabbits. The paste was covered with a gauze patch which was held in contact with the skin with non-irritating tape during the exposure period.

All irritation scores (at 1, 24, 48 and 72 hours) were zero. The PDII = 0.00. 4 -hour dermal exposure to Glyphosate TC (966 g/kg) resulted in no dermal irritation (Leuschner, 2010a).

In a dermal irritation study, one male and two female New Zealand white rabbits were dermally exposed for 4 hours to Glyphosa te technical grade (96.71%). 500 mg test material moistened with 0.2 mL deionized water was applied to each test site which was then covered with a 2.5 x 2.5 cm gauze patch. Each patch was secured in place with a strip of non -irritating adhesive tape. The entire trunk of each rabbit was loosely wrapped with a semi -permeable orthopedic stockinette which was secured on both edges with strips of tape.

All irritation scores (at 1, 24, 48 and 72 hours) were zero. The PDII = 0.00. Four hour exposure to Glyphosate technical grade (96.71%) resulted in no dermal irritation (You, 2009c).

In a dermal irritation study, 3 male New Zealand white rabbits were dermally exposed for 4 hours to Glyphosate Acid Technical (97.23% active). The test material was applied as a 70% w/w mixture in distilled water. 0.71 g aliquots of the resulting paste were placed on 1 inch x 1 inch 4 -ply gauze pads which were applied to one 6 cm^2 intact skin site on each of the rabbits. The pad and entire trunk of each rabbit were then wrapped with semi-occlusive 3-inch Micropore tape.

At 1 hour one site scored "1" for erythema; all other scores were zero. All scores were zero at 24, 48 and 72 hrs. The PDII = 0.08. Four hour exposure to Glyphosate Acid Technical (97.23%) resulted in very slight dermal irritation (Merkel, 2005d).

In a dermal irritation study, 3 female New Zealand white rabbits were dermally exposed for 4 hours to Glyphosate Technical (980.5 g/kg). A moistened gauze pad containing 0.5 g test material was applied to a 6 cm^2 skin area. After application the gauze was held in place on the test site by an adhesive and non-irritating tape.

All irritation scores (at 1, 24, 48 and 72 hours) were zero. The PDII = 0.00. Four hour dermal exposure to Glyphosate Technical (980.5 g/kg) resulted in no dermal irritation (Canabrava Frossard de Faria, 2008a).

In a dermal irritation study in 3 male and 3 female New Zealand white rabbits with 97.76% Glyphosate, 0.5 g test material (moistened with 0.5 mL physiological saline) was applie d to 2 intact test sites/rabbit. The test sites were semi -occluded with a 1 inch x 1 inch gauze patch held in place with Micropore® Tape. Exposure was for 4 hours.

All irritation scores (at 0.5, 24, 48 and 72 hours) were zero. The PDII = 0.00. Four ho ur dermal exposure to 97.76% Glyphosate resulted in no dermal irritation (Reagan and Laveglia, 1988b).

In a dermal irritation study in 3 male and 3 female New Zealand White rabbits with 99% Glyphosate technical, the test material was applied as a 25% w/v solution in distilled water. 0.5 mL of the test material (the 25% solution?) was applied underneath an 8 -ply 1 inch x 1 inch surgical gauze pad at 4 sites (2 intact, 2 abraded) on each of 6 albino rabbits, with 24 -hr occluded exposure. Scoring was at 24 and 72 hours after application.

At 24 hours one rabbit scored "1" for erythema at an intact site and "1" for erythema and "1" for edema at an abraded site. Another rabbit scored "1" for erythema at an abraded site. All other scores at 24 hours were zero. All scores for irritation at 72 hours were zero (Heenehan, 1979).

In a dermal irritation with Glyphosate Acid (95.6%), 500 mg of test material was moistened with 0.5 mL to form a dry paste, which was applied to a 2.5 cm x 2.5 cm test site on the left f lank of each of 6 female New Zealand white rabbits. The treated area was covered with an 8 -ply 2.5 cm x 2.5 cm surgical gauze pad, which was secured by two strips of surgical tape. This was covered by impermeable rubber sheeting which was wrapped once ar ound the trunk of the animal and secured with adhesive polyethylene tape. Exposure was for 4 hours.

No irritation was observed at ½ -1 hour, 1, 2, and 3 days. All irritation scores were zero. The PDII was 0.00 (Doyle, 1996c).

In a dermal irritation stu dy with Glyphosate Technical (96.1% Glyphosate Acid), 0.5 g moistened with ~0.5 mL purified water was placed on a 2.5 cm x 2.5 cm 8 -ply gauze surgical patch which was applied to an intact skin site on the left flank of each of 3 male and 3 female New Zealand White rabbits. Each patch was covered with a semi -permeable dressing. The dressing was wrapped around the abdomen and held in place with tape. Exposure was for 4 hours.

No irritation was observed a 1, 24, 48 or 72 hours. All irritation scores were zero. The PDII was 0.00 (Arcelin, 2007c).

In a dermal irritation with Glyphosate Technical (96.3%), 0.5 g (with sufficient water added to dampen the material) was placed on a ~2.5 cm x 2.5 cm surgical gauze pad, which was kept in contact with the skin by a patch with a surrounding hypoallergenic plaster. The entire trunk was wrapped with plastic wrap held in place with an elastic stocking. Three male New Zealand White rabbits were exposed for 4 hours.

One rabbit had grade 1 erythema at 1 and 24 hours. All other irritation scores were zero. The PDII = 0.17 (Zelenak, 2011b).

In a dermal irritation with Glyphosate wet cake (85.5%), 0.5 gram aliquots of test material moistened with 0.5 mL 0.9% saline were applied to 6 rabbits beneath 2.5 cm x 2.5 cm gauze squares (2/rabbit), with 4-hour occluded exposure.

Five of the 6 rabbits showed grade 1 erythema at one or both sites at 0.5, 24 and/or 48 hours. All scores were zero at 72 hours. The PDII=0.31 (Blaszcak, 1988).

(e) Ocular irritation

Table 14 Results of studies of primary eye irritation with glyphosate

Species	Strain	Sex	Route	Purity %	Results	Reference
Rabbit	New Zealand White	Male Female	Eye Irritation	95.1	Mildly irritating	Talvioja (2007b)
Rabbit	Himalayan	Male	Eye Irritation	95.23	Moderately irritating	Leuschner (2009b)
Rabbit	New Zealand White	Female	Eye Irritation	97.56	Severly irritating	Hideo Ueda (1995b)
None	n/a	-	Eye Irritation (pH testing)	Not stated	pH of a 1% solution in water was 1.93. Test item not tested because pH < 2.	Simon (2009c)
Rabbit	Himalayan	Male	Eye Irritation	97.52	Mildly irritating	Leuschner (2009d)
Rabbit	Himalayan	Male	Eye Irritation	96.6	Mildly irritating	Leuschner (2010b)
Rabbit	New Zealand White	Male Female	Eye Irritation	96.40 & 96.71	Moderately irritating	You (2009d)
Rabbit	New Zealand White	Male	Eye Irritation	97.23	Moderately irritating	Merkel (2005e)
Rabbit	New Zealand White	Male Female	Eye Irritation	98.05	Severly irritating	Canabrava Frossard de Faria (2008b)
Rabbit	New Zealand White	Not reported	Eye Irritation	97.76	Severly irritating	Reagan & Laveglia (1988c)
Rabbit	New Zealand White	Female	Eye Irritation	95.6	Mildly irritating	Johnson (1997)
Rabbit	New Zealand White	Male Female	Eye Irritation	96.1	Mildly irritating	Arcelin (2007d)
Rabbit	New Zealand White	Male	Eye Irritation	96.3	Severly irritating	Tavaszi (2011b)
Rabbit	New Zealand White	Male Female	Eye Irritation	85.5%	Moderately irritating	Blaszcak (1988)
Rabbit	New Zealand White	Male Female	Eye Irritation	46.6% glyphosate	Non-irritating	Blaszcak (1998)

Species	Strain	Sex	Route	Purity %	Results	Reference
Rabbit	New Zealand White	Male Female	Eye Irritation	57.8%	Mildly irritating	Bonnette (2001)
Rabbit	New Zealand White	Male Female	Eye Irritation	Not reported	Non-irritating.	Branch (1981) MON 0139, an amber liquid
Rabbit	New Zealand White	Not specified	Eye Irritation	90.8%	Mildly irritating.	Busch (1987a) MON 8750
Rabbit	New Zealand White	Not specified	Eye Irritation	70.7%	Mildly irritating	Busch (1987b) MON 8722
Rabbit	New Zealand White	Not specified	Eye Irritation	99%	Moderately irritating	Heenehan (1979)
Rabbit	New Zealand White	Not specified	Eye Irritation	97.76%	Severely irritating	Reagan (1988)

Rabbits

In an eye irritation study with Glyphosate Technical (95.1%), 0.1 g was instilled into the conjunctival sac of the left eye of each of 3 male and 3 female New Zealand White rabbits.

There was no iridial irritation (all scores zero). Corneal opacity was present in all 3 rabbit eyes along with positive conjunctival irritation (grade 2-3 redness and/or grade 2-3 chemosis) at 1, 24 and 48 hours) and in 2/3 rabbit eyes (with grade 2 redness) at 72 hours. On Day 7 all scores for corneal opacity were zero; 3/3 eyes scored 1 (not considered a positive irritation effect) for conjunctival redness. All scores were zero on Days 10 and 14. The test item was considered to have caused significant but reversible damage to the rabbit eye (Talvioja, 2007b).

In an eye irritation study with Glyphosate TC (95.23%), 100 mg was instilled into the conjunctival sac of the right eye of each of 3 male Himalayan rabbits. "1 hour after instillation the eyes were rinsed with 20 mL NaCl solution."

The maximum score for corneal opacity was 1. This was present in 3/3 eyes at 24, 48 and 72 hours, in 2/3 eyes on Day 4, and in 1/3 eyes on Days 5, 6 and 7, with complete clearing by Day 8. The maximum score for iritis was 1, which was observed in 3/3 eyes at 24 hours, in 2/3 at 48 hours, and in 1/3 eyes at 72 hours, and in 0/3 eyes on Day 4 and subsequently. The maximum score for conjunctival redness was 1, as was the maximum score for chemosis. All scores for conjunctival effects were zero by Day 5. The fluorescein test at 24 hours showed corneal staining of ½ to ¾ of the surface in 2 eyes, and in ¼ to ½ of the surface in one eye. The fluorescein test on Day 7 showed corneal staining in one eye (up to ¼ of the surface). The report (Leuschner, 2009b) states that: "Glyphosate TC was non-irritating to eyes, hence, no labelling is required."

In an eye irritation study with HR-001 (97.56%), 0.1 g test substance was placed in the conjunctival sac of the left eye of each of 12 female New Zealand White rabbits. Six rabbits (Group A) did not receive an eyewash. Three rabbits (Group B) had their eyes washed out starting at 30 seconds after instillation, and three rabbits (Group C) had their eyes washed out starting at 2 minutes after instillation.

All 6 rabbits in Group A had corneal opacity through Day 4. On Day 7, 5/6 had corneal opacity, and on Day 21 3/6 still had corneal opacity while 3/6 had completely cleared (all scores were zero) by that date. In Group B 3/3 rabbits had corneal opacity at 24 and 48 hours, but all eyes had completely cleared (all scores were zero) by Day 7. In Group C 1/3 rabbits was positive for corneal opacity at 24 hours, and none of the rabbits had corneal opacity at 48 hours. One Group C rabbit was positive for conjunctival effects at 72 hours; the other two rabbits had completely cleared (all eye irritation scores were zero). None of the Group C rabbit eyes was positive for irritation on Day 4.

The report states that it was concluded that the test substance had severely irritating potential for the eye mucosa of rabbits and that irrigation at 30 seconds or 2 minutes after application was effective for reduction of eye irritation and for recovery (Hideo Ueda, 1995b).

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 teste d 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." (Simon, 2009c).

In an eye irritation study with Glyphosate TC (97.52%), 100 mg was instilled into the conjunctival sac of the right eye of 3 male Himalayan rabbits. One hour after instillation the eyes were washed out with 20 mL NaCl solution.

Fluorescein testing at 24 hours showed corneal staining in 2/3 eyes. Two eyes had corn eal opacity at 24 and 48 hours, and one of these eyes also had corneal opacity at 72 hours. All eyes had completely cleared (all eye irritation scores were zero) by Day 4. "Glyphosate TC was non -irritating to eyes, hence, no labelling is required." (Leuschner, 2009d).

In an eye irritation study with Glyphosate TC (966 g/kg%), 100 mg was instilled into the conjunctival sac of the right eye of 3 male Himalayan rabbits. One hour after instillation the eyes were washed out with 20 mL NaCl solution.

All 3 eyes had corneal opacity at 24, 48 and 72 hours. Two eyes had corneal opacity at 4 days, and one of these eyes also had corneal opacity on day 5. All eyes had completely cleared (all eye irritation scores were zero) by Day 7. "Glyphosate TC was non -irritating to eyes, hence, no labelling is required." (Leuschner, 2010b).

In an eye irritation study with Glyphosate Tech Grade (two analyses: 96.40 and 96.71%), 0.1 mL (93.2 mg) was placed into the conjunctival sac of the right eye of each of 2 male and one female New Zealand White rabbits.

Two of the 3 eyes had corneal opacity at 24, 48 and 72 hours and at day 4. One eye had corneal opacity on day 7. All eyes had cleared by Day 10. "The test substance is rated moderately irritating and assigned to Toxicity Category II." (You, 2009d).

In an eye irritation study with Glyphosate Acid Technical (97.23%), 0.1 mL (0.06 g) was instilled into the conjunctival sac of the right eye of 3 male New Zealand White rabbits. Prior to testing the test substance was ground with a mortar and pestle. The pH of a 1% solution is reported as 2.5.

All 3 eyes were positive for corneal opacity through day 7, and for iritis and conjunctivitis through day 4 (one eye was also positive for conjunctival redness on day 7). All eyes h ad cleared (all scores zero) by day 10. "The Maximum Mean Total Score of Glyphosate Technical is 40.3. Based on the classification system used the test substance is considered severely irritating to the eye. The classification was raised from moderately to severely because all three animals had scores greater than 10 on Day 7 of the study." (Merkel, 2005c).

In an eye irritation study with Glyphosate Technical (980.5 g/kg), 0.1 g was i nstilled in one eye of each of male and female New Zealand White rabbits. Because of the severity of the effects only 2 rabbit eyes were tested. The pH of a 1% solution is reported as 2.2.

In one rabbit there was corneal opacity, iritis and conjunctival effects through Day 4 with clearing by Day 7. In the other rabbit there was corneal opacity at 1, 24, 48 and 72 hours and at 7, 14

and 21 days. The eye was also positive for conjunctival irritation on Day 14 (Canabrava Frossard de Faria, 2008b).

In an eye irritation study with Glyphosate (97.76%), 0.1 g was instilled in the conjunctival sac of one eye of each of 6 New Zealand White rabbits (sex not reported). The eyes were not washed out until 24 hours after instillation of the test material.

Glyphosate produced corneal opacity and conjunctival irritation with blisteri ng in 6/'6 rabbits. One rabbit (which still had corneal opacity on Day 14) was found dead at 20 days after instillation; the death was considered to be unrelated to exposure to the test material. Three of the five surviving rabbits still had corneal opac ity on Day 21. Because the Glyphosate (97.76%) was severely irritating to the eye, it was assigned to EPA Toxicity Category I by this exposure route (Reagan and Laveglia, 1988c).

In an eye irritation study with Glyphosate Acid (95.6%), 100 mg was initial ly applied into the conjunctival sac of one female New Zealand White rabbit. Application resulted in moderate initial pain in this first rabbit so the subsequent 5 animals were pre-treated with a local anesthetic. "Between ¼ and ½ of the test substance was displaced from the eye of each animal immediately after dosing."

Corneal, iridial and conjunctival effects were seen in all rabbits for up to 4 days. Corneal opacity was present in 5/6 rabbits on day 4, but had cleared in all rabbits by day 7. All s cores were zero on Day 7 except for one rabbit which had grade 1 (not considered positive for irritation) conjunctival redness, which had cleared by Day 8. Glyphosate Acid (95.6%) was classified as a mild irritant (class 5 on a 1-8 scale) to the rabbit eye (Johnson, 1997).

In an eye irritation study with Glyphosate Technical Material (96.1%), 0.1 g was instilled into the conjunctival sac of the left eye of each of 3 male and 3 female New Zealand White rabbits. The pH of the test item is reported as 2.12.

There was no corneal opacity or iritis. All 3 rabbit eyes were positive for conjunctival irritation at 1 hour, and 2/3 were positive for these effects at 24 and 48 hours. None of the eyes was positive for conjunctival irritation at 72 hours, and all sc ores were zero by day 7. "…the test item did not induce significant or irreversible damage to the rabbit eye." (Arcelin, 2007d).

In an eye irritation study with Glyphosate Technical Material (96.3%; glycine mode of synthesis), 0.1 g was instilled into the conjunctival sac of the left eye of one male New Zealand White rabbit. The pH of the test item is reported as 1.99.

An Initial Pain Reaction of score of 3 was observed. Irritation effects were scored at 1 and 24 hours after instillation. "Conjunctiv al redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit at 1 and 24 hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema o f the eyelids, and a few black points on the conjunctiva and dry surface of the eye were noted at one hour after the treatment. Fluorescein staining was positive at the 24 hour observation. Based on the symptoms, no further animals were dosed and the stu dy was terminated after the 24 hour observation… Glyphosate Technical was classified as corrosive to the eye." (Tavaszi, 2011b).

In an eye irritation study with Glyphosate wet cake (85.5% purity), 0.1 cc (68.9 mg) was instilled into the lower conjunctival sac of the right eye of 6 New Zealand White rabbits. The eyes were not washed out until 24 hours after instillation.

All rabbits showed positive irritation effects (corneal opacity and/or grade 2 chemosis and/or redness and/or iritis) at 1-48 hours, and 2/6 showed positive irritation effects at 72 hours. None of the eyes was positive for irritation on day 7. "Glyphosate Wet Cake produced moderate to severe but reversible ocular irritation in all animals… Five had iritis and corneal opacities." (Blaszcak, 1988).

In an eye irritation study with MON 77945 (described as an amber liquid, pH = 4.59, containing 46.6% glyphosate acid) 0.1 mL was instilled into one eye of each of 6 rabbits.

There were no positive irritation effects (one eye scored 1 for conjunctival redness at 1 hour, all other scores were zero). "…Under conditions of this study, MON 77945 produced very mild, transient ocular irritation." (Blaszcak, 1998).

In an eye irritation study with MON 78623 (described as an amber liquid contain ing 57.8% potassium salt of glyphosate; 47.13% glyphosate acid acid equivalent) 0.1 mL was instilled into one eye of each of 3 rabbits. Two rabbits vocalized following instillation.

There was no corneal opacity. All eyes scored 1 for iritis, 2 for conj unctival redness and 2 for conjunctival swelling at 1 hour. At 24 hours one eye scored for 1 for iritis. All scores were zero at 48 hours. "Based on the EEC labeling criteria, MON 78623 is classified as a nonirritant to the ocular tissue of the rabbit…" (Bonnette, 2001).

In an eye irritation study with MON 0139 (described as an amber liquid, no information as to pH, active ingredient) 0.1 mL was instilled into one eye of each of 9 rabbits. Six eyes were unwashed; 3 were washed out with physiological saline ~20 seconds after instillation.

All irritation scores were zero. No signs of irritation were observed in any rabbit eye (Branch, 1981).

In an eye irritation study with MON 8750 (described as a white powder, 90.8% purity, which was ground with a mo rtar and pestle prior to dosing), 0.1 g was instilled into one eye of each of 6 rabbits.

There was no corneal opacity or iritis. At one hour there were positive (grade 2 redness and/or chemosis) conjunctival irritation effects in 5/6 rabbit eyes. At 24 and 48 hours some eyes scored 1 for conjunctival redness. At 72 hours all scores were zero (Busch, 1987a).

In an eye irritation study with MON 8722 (described as a white powder, 70.7% purity, which was ground with a mortar and pestle prior to dosing), 0.1 g [0.1 mL] was instilled into one eye of each of 6 rabbits.

There was no corneal opacity or iritis. At one hour there were positive (grade 2 redness and/or chemosis) conjunctival irritation effects in 5/6 rabbit eyes. At 24 hours one eye scored 1 for conjunctival redness (not considered a positive irritation effect). At 48 hours all scores were zero (Busch, 1987b).

In an eye irritation study with glyphosate technical (99%), 0.1 mL of a 25% w/v solution of test material in distilled water was instille d into the conjunctival sac of one eye of each of 9 rabbits. Six eyes were unwashed, while the other 3 were washed out for one minute with lukewarm water starting 20 seconds after instillation.

One unwashed eye and two washed eyes showed corneal opacity, with clearing by Day 4. All scores were zero by Day 7. In this study, the test material was moderately irritating to the eye (Heenehan, 1979).

In an eye irritation study with glyphosate (97.76%), 0.1 g of test material was instilled into the conjunctival sac of one eye of each of 6 rabbits.Corneal opacity and conjunctival irritation were noted in all rabbits at the 24, 48 and 72 hour and 7 day examinations.

One rabbit was found dead at 20 days; the death was considered unrelated to exposure. On day 21, 3/5 rabbits still showed corneal opacity. In this study, glyphosate (97.76%) was severely irritating to the eye (Reagan, 1988).

(f) Dermal sensitisation

Table 15 Results of studies of skin sensitisation with glyphosate

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

Guinea-pigs

In a dermal sensitization (Magnusson -Kligman Maximization Test with female Dunkin-Hartley guinea pigs) study with Glyphosate Technical (95.1%) intradermal induction treatments were with a 3% dilution of the test item in PEG 300 and in an emulsion of Freund's Complete Adjuvant/physiological saline. Epidermal induction (one week after the intradermal induction) was for 48 hours under occlusion with the test item at 50% in PEG 300. Two weeks later the 5 control and 10 test guinea pigs were challenged. Patches (3 cm x 3 cm) of filter paper were saturated with ~ 0.2

EPA-HQ-2018-002024_0004841

mL of the test item at the highest tested non-irritating concentration of 25% in PEG 300 (applied to the left flank) and ~0.2 mL PEG 300 alone (applied to the right flank), with 24-hour exposure. The application sites were scored at 24 and 48 hours after exposure ended.

All challenge irritation scores (for the 10 test and 5 control animals) were zero. A positive control assay with α-Hexylcinnamaldehyde gave appropriate results. Based on these findings, Glyphosate Technical does not have to be classified and labelled as a skin sensitizer (Talvioja, 2007c).

In a dermal sensitization (Magnusson-Kligman Maximization Test with female Dunkin-Hartley guinea pigs) study with Glyphosate TC (two analyses: 95.23% and 96.4%) intradermal induction treatments were with a 0.01% concentration of Glyphosate TC in *aqua ad iniectabilia*. The day before topical induction, the application site was treated with 0.5 mL sodium laurylsulfate 10% in vaseline. Topical induction (one week after the intradermal induction) was for 48 hours to 2 mL of a 50% concentration of Glyphosate TC in *aqua ad iniectabilia*. Challenge was two weeks after the intradermal induction. Filter paper containing 2 mL of the test material was applied to the left flank; filter paper containing 2 mL vehicle was applied to the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and 5 control guinea pigs) were zero. A positive control assay with Benzocaine gave appropriate results. Glyphosate TC was found to be not sensitizing to guinea pigs (Haferkorn, 2010b).

In a dermal sensitization (Magnusson-Kligman Maximization Test with female Hartley guinea pigs) study with Glyphosate TC (97.56%) intradermal injection treatments were with a 5% suspension of Glyphosate TC in paraffin oil. Six days later the treatment site was treated with 10% sodium lauryl sulfate in white petrolatum; the topical induction (the following day) was with 0.4 g of the test material preparation (25% test material in white petrolatum) on a 2 cm x 4 cm piece of filter paper with 48-hour exposure. The challenge application (two weeks after the topical induction) was with 25% test material in white petrolatum with 24-hour exposure, with scoring at 24 and 48 hours following the end of this exposure.

None of the 20 induced guinea pigs and none of the 10 negative control guinea pigs showed any signs of irritation at the application site following challenge. A positive control assay with DNCB (2,4-dinitrochlorobenzene) gave appropriate results. It was concluded that the test material had no dermal sensitization potential in the guinea pig maximization test (Hideo Ueda, 1995c).

In a dermal sensitization (Magnusson-Kligman Maximization Test with male Hartley guinea pigs) study with Glyphosate Technical (96.66%) intradermal injection treatments were with a 10% dilution of the test item in purified water and Freund's Complete Adjuvant. Seven days later the application site was treated with the test item at 50% in purified water (applied as a 2 cm x 4 cm filter paper containing ~0.3 mL), with 48-hour exposure. Two weeks later, the guinea pigs were treated with the test item at 15% in purified water (applied as a 3 cm x 3 cm filter paper containing ~0.2 mL), with 24-hour exposure, with scoring at 24 and 48 hours following the end of this exposure.

None of the 10 induced guinea pigs and none of the 5 control guinea pigs showed any signs of irritation at the application site following challenge. A positive control assay with α-Hexylcinnamaldehyde gave appropriate results. Based on the results of this study, no sensitization potential of the test item was observed in the guinea pig maximization test (Simon, 2009d).

In a dermal sensitization (Magnusson-Kligman Maximization Test with male Dunkin-Hartley guinea pigs) study with Glyphosate TC (two analyses: 97.52% and 98.8%) intradermal inductions were with a 0.5% concentration of Glyphosate TC in *aqua ad iniectabilia*. The day before topical induction, the application site was treated with 0.5 mL sodium laurylsulfate 10% in vaseline. Topical induction (one week after the intradermal induction) was for 48 hours to 2 mL of a 50% concentration of Glyphosate TC in *aqua ad iniectabilia*. Challenge was two weeks after the intradermal induction.

Filter paper containing 2 mL of the test material was applied to the left flank; filter paper containing 2 mL vehicle was applied to the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and 5 control guinea pigs) were zero. A positive control assay with Benzocaine gave appropriate results. Glyphosate TC was found to be not sensitizing to guinea pigs (Haferkorn, 2009d).

In a dermal sensitization (Magnusson-Kligman Maximization Test with male Dunkin-Hartley guinea pigs) study with Glyphosate TC (two analyses: 966 g/kg and 97.3%) intradermal induction were with a 0.5% concentration of Glyphosate TC in *aqua ad iniectabilia*. The day before topical induction, the application site was treated with 0.5 mL sodium laurylsulfate 10% in vaseline. Topical induction (one week after the intradermal induction) was for 48 hours to 2 mL of a 50% concentration of Glyphosate TC in *aqua ad iniectabilia*. Challenge was two weeks after the intradermal induction. Filter paper containing 2 mL of the test material was applied to the left flank; filter paper containing 2 mL vehicle was applied to the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and 5 control guinea pigs) were zero. A positive control assay with Benzocaine gave appropriate results. Glyphosate TC was found to be not sensitizing to guinea pigs (Haferkorn, 2010h).

In a dermal sensitization study with Glyphosate Tech (2 analyses: 96.40% and 96.71%), 15 male and 15 female short-haired Hartley albino guinea pigs were divided into 2 groups: Group I (5 males and 5 females) and Group II (10 males and 10 females). For each induction treatment, Group II animals were exposed by introducing an application of 400 mg test item moistened with 2 mL deionized water beneath a 4 ply 2.5 cm x 2.5 cm gauze pad, which was secured with non-irritating adhesive tape, which in turn was covered with a strip of clear polyethylene film. Exposures lasted for at least 6 hours and took place on days 1, 8 and 15. Group I animals were untreated during this period. After a two week rest period, all animals (Groups I and II) were challenged at a previously unexposed site with 400 mg test item moistened with 2 mL deionized water.

All challenge irritation scores (for the 20 induced and 10 control guinea pigs) were zero. A positive control assay with α-Hexylcinnamaldehyde gave appropriate results. Glyphosate Tech did not elicit a sensitizing reaction in guinea pigs (You, 2009e).

In a dermal sensitization study (Buehler method) with Glyphosate Acid Technical (97.23%), a group of 20 male and 20 female Hartley albino guinea pigs received once-a-week exposure to 0.4 g of a 70% w/w mixture of Glyphosate Acid Technical in distilled water. The mixture was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber, which was secured in place and wrapped with non-allergenic adhesive tape. After each 6-hour exposure, the chambers were removed and any residual test material was gently cleansed off. Twenty-seven days after the first induction dose, 0.4 g of a 70% w/w mixture of the test item in distilled water was applied to a naïve site on the right side of each guinea pig. These sites were evaluated and scored approximately 24 and 48 hours after the challenge application. A group of 10 controls was also similarly treated.

There were no positive irritation scores (defined as > 0.5). A positive control assay with α-Hexylcinnamaldehyde gave appropriate results. Based on the results, Glyphosate Technical is not considered to be a contact sensitizer (Merkel, 2005f).

In a dermal sensitization study (Buehler method) with Glyphosate Technical (980.5 g/kg), a group of 20 male Hartley guinea pigs received three once-a-week 6-hour exposures to 1.0 mL of a 50% w/v solution of test item in DMSO vehicle. The solution was applied in a cotton lint patch which covered approximately 6 cm^2 of the left flank. A group of 10 control guinea pigs was similarly treated with 1.0 mL DMSO. Two weeks after the last induction treatment, all (induced and control)

EPA-HQ-2018-002024_0004843

guinea pigs were exposed for 4 hours to 1.0 mL of a 50% w/v solution of test item in DMSO on the right flank.

One of the 20 induced guinea pigs had a score of 1 (positive response) at 24 and 48 hours following challenge. All of the other induced and control animals scored zero. It was concl uded that the epidermal application of Glyphosate Technical with DMSO as vehicle did not cause skin sensitization in guinea pigs according to the Buehler Test Method (Lima Dallago, 2008).

In a dermal sensitization (Magnusson -Kligman Maximization Test with Dunkin-Hartley guinea pigs) study with Glyphosate Technical (95.7%), a group of 20 female Dunkin-Hartley guinea pigs received induction treatments. Shortly before treatment on Day 0 the hair was removed from an area approximately 4 cm x 6 cm on the shoul der region of each animal with veterinary clippers. A row of 3 injections (0.1 mL each) was made on each side of the spine. The injections were: a) 1:1 Freund's Complete Adjuvant in isotonic sodium chloride; b) a 0.195% (v/v) formulation of the test mate rial in isotonic sodium chloride; c) a 0.195% (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. On Day 6 the scapular region was treated with 10% sodium lauryl sulphate (10% in petrole um jelly). On Day 7 the same area used for the intradermal injections was treated with a 60% w/w mixture of the test item in distilled water, with 48-hour occluded exposure. Challenge was approximately 2 weeks later. One site was treated with 60% w/w mixture of the test item in distilled water, a second site was treated with a 30% w/w mixture of the test item in distilled water. The sites were scored for irritation at 24 and 48 hours following exposure.

A group of 10 control guinea pigs was similarly t reated, except they were only exposed to vehicle during the induction period

All 18 induced guinea pigs (two had died during the course of the study) scored zero at 24 and 48 hours following challenge, as did all 10 controls. It is reported that the test material produced a 0% (0/18) sensitization rate and was classified as a non -sensitizer to guinea pig skin under the conditions of the test (Richeux, 2006).

In a dermal sensitization (Magnusson -Kligman Maximization Test with albino Crl (HA) BR guinea pig s) study with Glyphosate Acid (95.6%), a group of 20 female guinea pigs received induction treatments. The hair was clipped from an area ~5 cm x 5 cm on the scapular region of each animal. A row of 3 injections (0.05 to 0.1 mL each) was made on each side of the spine. The injections were: a) 1:1 Freund's Complete Adjuvant in deionized water; b) a 0.1% (w/v) preparation of the test material in deionized water; c) a 0.% (w/v) preparation of the test material in a 1:1 preparation of Freund's Complete Adjuva nt plus deionized water. On Day 6 the application site was clipped and 0.5 mL of a 10% preparation of sodium lauryl sulphate in paraffin wax was applied. On Day 7 the test area was treated with a topical application of gthe test substance (75% w/v) in deionized water. The preparation (0.2 -0.3 mL) was applied to a ~4 cm x 2 cm piece of filter paper which was held in place with surgical tape. The tape was covered by a strip of adhesive tape secured in place by self -adhesive PVC tape. This occlusive dress ing was kept in place for ~2 days. Ten control animals were treated the same as the test animals, except for being treated with deionized water in place of the test substance. Challenge (for both the induced animals and their controls) was at approximately 21 days. An area ~15 cm x 15 cm, on both flanks of the test and control animals was clipped free of hair. An occlusive dressing was prepared with two pieces of filter paper (~1 cm x 1.75 cm) stitched to a piece of rubber sheeting (~12 cm x 5 cm). A 7 5% w/v preparation of the test substance in deionized water (0.05 -0.1 mL) was applied to one piece of filter paper and a 30% w/v preparation in deionized water (0.05 -0.1 mL) was applied to the second piece. It was then covered with a strip of adhesive ban dage (~25-40 cm x 7.5 cm) which was secured by a self -adhesive PVC tape. Exposure was for ~24 hours. The sites were scored for irritation at 24 and 48 hours following the end of exposure.

Exposure to the 75% w/v preparation resulted in mild and scattere d redness (score of 1) in 3/20 induced and 1/10 control animals and 24 hours only, with all scores zero at 48 hours. Because it was

observed at similar incidences in both induced and control guinea pigs, and it occurred only at 24 hours, it was considered to be due to skin irritation. All sites exposed to the 30% w/v preparation scored zero at both 24 and 48 hours. A positive control assay with α -Hexylcinnamaldehyde demonstrated the sensitivity of the test system. It was concluded that Glyphosate Acid is not a skin sensitizer under the test conditions (Doyle, 1996d).

Dermal sensitization (LLNA)

Mouse

In a dermal sensitization (LLNA) study with glyphosate technical material (96.1% glyphosate acid), groups of 4 female CBA/Ca mice were used. Approximately 25 µL of a 10, 25 or 45% w/v preparation of the test item in dimethyl sulphoxide (DMSO) was applied to the dorsal surface of each ear. 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 ~250 µL of phosphate buffered saline (PBS) containing 20 µCi ^{3}H-methyl thymidine. The mice were sacrificed ~5 hours later. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in that group, were placed in a container of PBS.

Single cell suspensions were prepared by straining the lymph nodes from a single group through a 200-mesh stainless steel gauze. The cell suspensions were washed 3X by centrifugation with ~10 mL PBS. Approximately 3 mL of 5% w/v trichloroacetic acid (TCA) was added and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were resuspended in approximately 1 mL of TCA. The suspensions were transferred to scintillation vials and 10 mL of scintillant was added prior to ß-scintillation counting.

The following Disintegrations per minute (dpm) were obtained : 0% (DMSO alone): 3912; 10%: 2394 (Stimulus Index or SI of 0.61 relative to vehicle control); 25%: 3292 (SI: 0.84); 45%: 508 (SI: 1.04 SI). The following dpm were obtained from the positive control (α -Henxylcinnamaldehyde in 4 parts acetone and 1 part olive oil): 0% (vehicle alone): 5939; 5%: 10111 (SI: 1.70); 10%: 13747 (SI: 2.31); and 25%: 38015 (SI: 6.40, positive response >3). It was concluded that glyphosate technical material is considered not to be a skin sensitizer under the conditions of the test (Betts, 2007).

In a dermal sensitization (LLNA) study with glyphosate technical (96.3%), groups of 4 female CBA/J Rj mice were used. Each mouse was topically dosed on the dorsal surface of each ear with 25 µL of the appropriate formulation (10, 25 or 50% w/v preparation of the test item in propylene glycol (PG), PG alone, or 25% α -Hexylcinnamaldehyde in PG). 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 ~250 µL of phosphate buffered saline (PBS) containing 20 µCi ^{3}H-methyl thymidine (^{3}HTdR). The mice were sacrificed ~5 hours later. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in that group, were placed in a petri dish containing 1-2 mL PBS.

Single cell suspensions of pooled lymph node cells were prepared and collected in tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer. The cell strainer was washed with PBS. Pooled lymph node cells were pelleted in a centrifuge at ~190g for 10 minutes at 4°C. After centrifugation supernatants were discarded. Pellets were gently resuspended and 10 mL PBS was added to the tubes. The washing step was repeated twice. This was repeated for each group of pooled lymph nodes. After the final washing, suspensions were centrifuged and most of the supernatant was removed except for a small volume (<0.5 mL) above each pellet. Each pellet was resuspended in 3 mL of 5% trichloroacetic acid (TCA). After ~18 hour incubation with 5% TCA at 2-

8°C, precipitate was recovered by centrifugation at 190g for 10 minutes. Supernatants were removed and pellets were resuspended in 1 mL of 5% TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a scintillation vial with 10 mL of scintillation liquid and thoroughly mixed prior to ß-scintillation counting.

The following Disintegrations per minute (dpm) were obtained after subtraction of background: 0% (PG alone): 681; 10%: 794 (Stimulus Index or SI of 1.2 relative to vehicle control); 25%: 678 (SI: 1.0); 50%: 683 (SI: 1.0); positive control (α-Hexylcinnamaldehyde in PG): 25%: 8302 (12.2 SI, positive response >3). It was concluded that under the conditions of this assay Glyphosate Technical was shown to have no skin sensitization potential (non-sensitizer) in the Local Lymph Node Assay (Török-Bathó, 2011).

2.2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

In a 13-week oral toxicity study, groups of 15 male and 15 female CD-1 mice were fed diets containing glyphosate (purity, 98.7%) at dietary concentrations of 0, 5000, 10000, and 50000 ppm. The mean daily intake of glyphosate was 0, 944, 1870 and 9710 mg/kg bw per day for the males and 0, 1530, 2740 and 14800 mg/kg bw per day for the females.

No treatment related mortality, clinical signs of toxicity, organ weights, macroscopic and histopathological findings. Body weight gains of the 50000 ppm group males and females were about 24% and 18% lower, respectively than that of the control animals at study termination. Body weight gains of both sexes of the 5000 ppm and 10000 ppm group animals were comparable to those of the controls.

The NOAEL in the 13 week toxicity study in mice is 10,000 ppm; equal to 1870 mg/kg bw per day based on reduced body weights seen at 50,000 ppm; equal to 9710 mg/kg bw per day (Tierney and Rinehart, 1979).

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

No mortalities, clinical signs, haematological or biochemical findings and no organ weight changes were observed that could be attributed to treatment. Gross or histopathological examination did not reveal effects of glyphosate administration. Taking into account the limited range of clinical chemistry parameters evaluated,

The 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., 1991b).

In a 13-week oral toxicity study, groups of 10 male and 10 female B6C3F1 mice were fed diets containing glyphosate (purity, 99%) at concentration of 0, 3125, 6250, 12500, 25000 or 50000 ppm. The calculated mean intakes were equal to 507, 1065, 2273, 4776 and 10780 mg/kg bw per day for males and 753, 1411, 2707, 5846 and 11977 mg/kg bw per day for females. All tissues from

animals in the control group and in that receiving the highest dose were examined microscopically. Salivary glands were also examined in all groups receiving lower doses.

Reduced body weight gain was observed at 25000 and 50000 ppm in males and females. There were no differences in food consumption between control and treated mice. The only significant gross finding in the study was a "dark" salivary gland in a male at th e highest dose; no other gross abnormalities were observed at necropsy. Histological changes were observed only in the parotid salivary gland (Table 16). The cytoplasmic alterations consisted of a diffuse increase in the basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared to be enlarged with an appearance of reduced numbers of ducts. No histological changes were observed in the submandibular and sublingual glands.

Table 16. Incidence and severity[a] of cytoplasmic alteration of the parotid and submandibular salivary glands (combined) in mice given diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)					
	0	3125	6250	12500	25000	50000
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)

From Chan & Mahler (1992)
[a]Average severity score (in parentheses) was based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

The NOAEL in the 13-week toxicity study in mice is 3125 ppm (equal to 507 mg/kg bw per day) on the basis of 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 fed diets containing glyphosate (purity, 97.56%) at dietary concentrations of 0, 5000, 10000, and 50000 ppm. The mean daily intake of glyphosate was 0, 600, 1221 and 6295 mg/kg bw per day for the males and 0, 765, 1486 and 7435 mg/kg bw per day for the females.

There were no treatment-related effects in clinical signs, mortality, opthalmological findings, and haematology measurements. In the 50,000 ppm group, mean body weights of males were lower than those of the control from week 2 to the end of the treatm ent period. Mean body weight of males at week 13 was 91% of that of the control. Body weights of females were comparable to the control during the treatment period. Similarly, the food consumption was decreased slightly in males of the 50,000 ppm. In the 5 0,000 ppm group, food efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment

In the 50,000 ppm group, females showed a significant increase creatine phosphokinase (CPK) which is considered d ue to the treatment. Other s tatistically significant changes in clinical chemistry were observed in the high dose male and female mice, howerver, these changes were minor and not collaborated with histological findings, therefore the y were not considered as adverse. In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

In the 50,000 ppm group, males and females showed significant increases in both absolute and relative weights of the cecum. The absolute weights of the cecum of males and females were 238 % and 187 % of that of the respective control. For relative weight, the ratio of the value to the respective control was 263 % or 195 % in males or females, respectively (Table 17).

Table 17. Mean Absolute and Relative Cecum Weight

	Dietary Concentration (ppm)			
	0	5000	10000	50000
Males				
Absolute weight	624	609	718	1484 (238%)
S.D.	86	116	177	359
Relative weight#	1.45	1.38	1.61	3.82**
S.D.	0.19	0.26	0.33	1.15

Females				
Absolute weight	497	474	604	958** (187%)
S.D.	96	115	123	163
Relative weight#	1.43	1.37	1.67	2.79
S.D.	0.26	0.30	0.42	0.53**

#: (organ weight/body weight) x 100.
S .D.: Standard deviation.
**: Significantly different from the control at 1% level of probability.

In the 50,000 ppm group, males and females showed a significant increase in incidence of distention of the cecum (12/12 in males and 10/12 in females, in contrast to 0/12 in males and females of the control group).

In the 50,000 ppm group, males showed significant increases in incidence of cystitis of the urinary bladder (4/12 as compared to 0/12 in the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distention of the cecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the cecum.

The NOAEL in the 13-week toxicity study in mice is 10,000 ppm equal to 1221 mg/kg bw per day based on decreased in body weights in males, increased in absolute and relative to body cecum weight in both sexes, increased incidence of distention of the cecum in both sexes seen at 50000 ppm; equal to 6295 mg/kg bw per day (Kuwahara, 1995).

Rats

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

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

Because of frequent occurrence of soft stools and/or diarrhoea at all doses, no NOAEL could be derived from this 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; 1,000 or 2,500 mg/kg bw per day . At study termination, all animals were sacrificed and necropsied. The liver, heart, kidney, spleen and adrenals were processed and examined histopathologically for all animals in the control group and at a doses of 2500 mg/kg bw per day . Examination was subsequently extended to kidneys from all females in the groups receiving the lowest and intermediate doses.

Soft faeces were noted in three males from the group receiving the highest dose during weeks 3-4 of the study, but were not seen in any other group. No treatment related effects were observed on mortality, clinical signs of toxicity, body weights, food and water consumption, or haematology parameters. In males, equivocal increases in plasma alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were seen at the three higher doses. In females, plasma ALT activity was significantly increased at the highest dose, as was total bilirubin. In addition, increased plasma concentrations of phosphate were noted in males at the two higher doses. There were neither notable intergroup differences in organ weights nor gross pathological findings. However, an increase in the

incidence of very mild to slight nephrocalcinosis was observed by means of histopathology in female rats dosed at 250 mg/kg bw per day and greater (Table 18).

Table 18. Incidence And Severity Of Nephrocalcinosis In Female Rats Given Diets Containing Glyphosate For 4 Weeks

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

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

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

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 a concentration of 0; 1,000; 5,000 or 20,000 ppm. The calculated mean intakes were equal to 63, 317 and 1,267 mg/kg bw per day for males and 84, 404 and 1,623 mg/kg bw per day for females. Clinical signs, body weight, food consumption, haematology and clinical chemistry parameters were monitored routinely. Gross examinations were performed for all groups, and kidneys, liver, and testes were weighed. A standard range of tissues from animals in the control group and at the highest dose was examined microscopically, as well as kidneys, livers, and lungs from animals at the lowest and intermediate doses.

No treatment-related effects were observed at up to the highest dose. However, parotid salivary glands were not included in the histopathological examination.

The NOAEL in the 90-day dietary toxicity study in rats was 20,000 ppm (equal to 1,267 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 a concentration that was adjusted weekly to give doses of 0, 30, 300 or 1,000 mg/kg bw per day. All tissues from animals in the control group and at the highest dose, in addition to kidneys, liver, lungs and parotid salivary glands of all the other test animals, underwent a full histopathological examination.

There were no mortalities, clinical signs, body weights, food and water consumption, haematological parameters, ophthalmoscopy, organ weights and macroscopic findings. Females at the highest dose showed slight but statistically significant increases in concentrations of glucose (11%), total protein (9%), albumin (9%) and creatinine (8%) compared with those in the control group. Urine analysis revealed a reduction in pH in males at the highest dose.

In contrast to 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 among dose groups and sexes and did not follow a dose–response relationship, and is therefore clearly not treatment-related. Thus, the previous finding was not confirmed.

An increase in the incidence of cellular alterations (deep basophilic staining and enlargement of cytoplasm) was noted in the parotid salivary glands of both sexes in all treated groups. In addition, the severity (graded as very mild, mild, moderate, severe, and very severe) of these findings showed a dose-related increase, but reached statistical significance in males at the highest dose only (Table 19).

Table 19. Incidence And Severity Of Cytoplasmic Alteration Of The Parotid Salivary Gland In Rats Given Diets Containing Glyphosate For 13 Weeks

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

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

In conclusion, rats treated with glyphosate for 13 weeks showed dose-related histopathological changes in the parotid salivary gland. However, at the lower doses of 30 and 300 mg/kg bw per day , these changes were only minimal with respect to severity and incidence and are considered to be of equivocal toxicological significance.
The NOAEL in this 90-day toxicity study in rats is 300 mg/kg bw per day on the basis of the more pronounced severity of cellular alterations in the parotid salivary gland at 1,000 mg/kg bw per day (Perry et al., 1991a).

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 a concentration of 0, 3,125; 6,250; 12,500; 25,000 or 50,000 ppm for approximately. Ten additional animals of each sex were included at each dietary concentration for evaluation of haematologic and clinical pathology parameters. The calculated mean intakes were equal to 205, 410, 811; 1,678 and 3,393 mg/kg bw per day for males and 213, 421, 844; 1,690 and 3,393 mg/kg bw per day for females. All tissues from animals in the control group and at the highest dose were examined microscopically. Salivary glands were also examined for animals at all lower doses.

Diarrhoea was observed in males at the highest dose and in females for the first 50 days of the study. Weight gain was reduced in males at 50,000 and 25,000 ppm, and the final mean body weight was approximately 18% and 6% less than that of controls, respectively. Small increases in several erythrocyte parameters were noted in males at doses of 12,500 ppm and greater. These changes were unremarkable and generally consistent with a mild dehydration. Plasma ALP and ALT activities were slightly increased in males at 6,250 ppm and greater and in females at 12,500 ppm and greater. In the absence of histopathological findings in the liver, these increases are considered to be of no toxicological significance.

No treatment-related gross abnormalities or organ weight changes were observed at necropsy. Histopathological changes were observed only in the parotid and submandibular glands of male and female rats. The study authors combined the findings for these two glands (Table 20). 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 3,125 ppm and the highest dose of 50,000ppm, the incidence response curve appears to be relatively flat and the degree of change is slight, progressing from only minimal to moderate.

Table 20. Incidence And Severity[a] Of Cytoplasmic Alteration Of The Parotid And Submandibular Salivary Glands (Combined) In Rats Given Diets Containing Glyphosate For 13 Weeks

	Dietary concentration (ppm)					
	0	3125	6250	12500	25000	50000
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)

From Chan & Mahler (1992)
[a]Average severity score (in parentheses) was based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

In conclusion, the administration of glyphosate to rats for 13 weeks produced do se related histopathological changes in the parotid and submandibular salivary glands. However, at the lower doses of 3,125 and 6,250 ppm, these changes were only minimal with respect to severity and are considered to be of equivocal toxicological significance.

The NOAEL in the 13-week dietary toxicity study in rats is 6,250 ppm (equal to 410 mg/kg bw per day) on the basis of the more pronounced severity of cellular alterations in the salivary glands at 12,500 ppm and greater (Chan & Mahler, 1992).

In a 90 day feeding study, groups of 10 Sprague Dawley rats per sex received daily dietary doses of 0; 2,000; 6,000 and 20,000 ppm (equal 0, 125.2, 371.9, and 1262.1 mg/kg bw per day for males and 0, 156.3, 481.2 and 1686.5 mg/kg bw per day for females) techni cal glyphosate (purity 97.5%) in the diet. Blood was collected pre-treatment and at termination for measurement of selected haematological and clinical chemistry parameters. At necropsy, selected organs were weighed. Histopathological examination was conducted on all tissues taken at necropsy.

Diets were homogeneously distributed and were stable for at least 10 days. Analytical concentrations were within 10% of the nominal concentrations. No treatment related effects was observed on mortality, body weights, body weight gains, food consumption, urinalysis, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights and macroscopic and microscopic examinations. The only obvious treatment related clinical observations was diarrhoe a seen in 10/10 males and 9/10 females in the 20,000 ppm treatment group.

The NOAEL in the 90-day toxicity study in rats is 6,000 ppm equal to 371.9 mg/kg bw per day; the highest dose tested (Parker 1993).

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 a concentration of 0; 1,000; 5,000 or 20,000 ppm for 90 days. The calculated mean intakes were equal to 81, 414 and 1,693 mg/kg bw per day for males and 90, 447 and 1,821 mg/kg bw per day for females. Achieved concentrations, homogeneity and stability were satisfactory throughout the study.

No mortality eas observed in the study. A low incidence of diarrhoea and light colored faeces were seen in both sexe s 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 when compared with controls. There was some evidence for a reduction in platelet coun t in males and females fed diets containing glyphosate at 5,000 or 20,000 ppm. Also, a marginal dose -related increase in prothrombin time was observed in males at all doses. The differences, however, were small and considered not to be of h aematological significance. Plasma ALP and ALT activities were increased in both sexes at 20,000 ppm and, to a lesser extent, in males at 5,000 ppm. In addition, plasma AST activity was increased in females at the highest dose at this early time -point, but not at study te rmination. The changes in clinical chemistry parameters wer e small in magnitude, often lacking clear dose response, therefore they were not considered as biologicall y relevant. There were no treatment-related effects on urine biochemistry and organ weights.

The only notable histopathological finding was a uterine leiomyosarcoma in one female at 5000ppm. Although rare, the finding of such a tumor in an animal receiving the intermediate dose was considered to be incidental to treatment.

The NOAEL in the 90 -day toxicity study in rats is 5,000 ppm, equal to 414 mg/kg bw per day, on the basis of reduced growth in males (Botham, 1996).

In a 90 day feeding study, groups of 10 Sprague Dawley rats per sex received daily dietary doses of 0; 1,000; 10,000 and 50,000 ppm (equal to 0, 79, 730 and 3,706 mg/kg bw per day for male and 0, 90, 844, 4188 mg/kg bw per day for female) glyphosate (purity 95.3 %) in the diet.

There were no deaths during the study. Animals of both sexes treated with 50,000 ppm showed soft faeces and diarrhoea from Day 4 which continued throughout the study period.

Animals of both sexes treated with 50,000 ppm showed a reduction in bodyweight gain over the first four weeks of treatment when compared with controls (Table 21). Body weight development was unaffected by treatment with the test material at the remaining dose levels.

Table 21. Group mean weekly bodyweights and standard deviations (sd)

Dietary concentration		**Bodyweight (g) at Day**													
(ppm)		0	7	14	21	28	35	42	49	56	63	70	77	84	90
		Males													
0	mean	206	269	315	354	382	411	444	457	488	508	523	537	536	551
	sd	8	12	17	24	33	38	45	44	49	52	55	58	56	58
1000	mean	199	260	309	350	377	400	427	446	470	485	497	513	516	528
	sd	11	14	19	21	24	26	30	31	32	32	35	37	36	37
10000	mean	200	257	303	338	364	393	414	429	454	470	483	494	495	506
	sd	12	12	15	21	25	30	35	35	38	38	38	40	39	43
50000	mean	198	215	247	268	283	306	329	335	356	369	382	394	395	408
	sd	8	8	15	21	26	31	33	38	41	43	43	44	42	44
		Females													
0	mean	173	197	214	232	243	256	269	276	284	291	295	306	304	307
	sd	9	11	12	15	16	18	20	19	20	21	24	25	25	27
1000	mean	173	199	218	238	249	261	272	280	286	292	300	308	304	313
	sd	10	13	14	16	16	17	18	19	18	18	19	21	20	20
10000	mean	166	184	201	217	226	237	246	256	262	267	272	277	276	282
	sd	14	18	21	25	24	26	27	27	27	27	27	29	28	29
50000	mean	173	183	197	214	219	231	240	246	251	260	265	271	267	273
	Sd	11	12	14	15	14	18	21	21	20	23	23	26	22	25

Animals of both sexes treated with 50,000 ppm showed a reduction in both dietary intake and food efficiency over the first four weeks of treatment when compared with controls . There were no treatment-related effects on water consumption, measured ocular parameters or h aematological parameters for either sex noted during the study.

Animals of both sexes treated with 50,000 or 10,000 ppm showed a statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase (AP) when compared with controls. A statistically significant increase in inorganic phosphorus and reduc tion in plasma creatinine were also evident among animals of both sexes treated with 50,000 ppm while females at this dose level showed statistically significant reductions in total plasma protein and albumin in comparison with controls. There were no furt her treatment-related effects. The changes in clinical chemistry parameters were minor in magnitude and lacking the dose response. Therefore, they were not considered as adverse.

Animals of both sexes treated with 50,000 ppm showed statistically significa nt increases in both relative liver and kidney weight when compared with controls (Table 22).

Table 22. Group mean organ weights and standard variations (sd)

Dietary concentration (ppm)		Relative organ weight (%)			
		Liver		Kidney	
		Male	Female	Male	Female
0	mean	2.9749	2.9734	0.5861	0.6516
	sd	0.2629	0.1558	0.0575	0.0523
1000	mean	2.8868	2.9093	0.5901	0.6257
	sd	0.2552	0.2146	0.0804	0.0375
10000	mean	2.8853	2.9801	0.6070	0.6454
	sd	0.3758	0.1556	0.0552	0.0532
50000	mean	3.2433*	3.1989*	0.6963***	0.7180*
	sd	0.2452	0.2098	0.0436	0.0707

* - significantly different from control group ($p < 0.05$)
*** - significantly different from control group ($p < 0.001$)

Macroscopic abnormalities were detected in the 50,000 ppm dose group with all animals showing enlarged and fluid-filled cecums while one female treated with 50000 ppm showed gaseous distension of the stomach at terminal kill. There were no treatment-related macroscopic abnormalities detected at 10,000 or 1,000 ppm (Table 23).

Treatment-related changes were observed in the cecum. Atrophy, characterized by flattening of the intestinal mucosa, was observed for five rats of both sexes dosed at 50,000 ppm ($p < 0.05$ for male rats) and for one male and two female rats receiving 10, 000 ppm of the test material. The etiology of this change is uncertain and may represent no more than a stretch atrophy of the mucosa resulting from cecal distension. There were no further treatment-related changes.

Table 23. Incidence of Cecum Macroscopic and Microscopic Findings

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	1000	10000	50000	0	1000	10000	50000
Enlarged and Fluid Filled	0	0	0	10	0	0	0	10
Cecum Mucosal Atrophy	0	0	1	5	2	0	2	5

The NOAEL in the 90-day toxicity study in rats is 10,000 ppm; equal 730 mg/kg bw per day based on histological findings in the cecum, soft faeces, diarrhoea, decreased in body weight and food consumption at 50,000 ppm equal to 3706 mg/kg bw per day (Coles et al., 1996).

In a 13-week feeding study, groups of 12 Sprague Dawley rats per sex received daily dietary doses of 0; 3,000; 10,000 and 30,000 ppm (equ al to 0, 168.4, 569, and 1,735 mg/kg bw per day for male and 0, 195.2, 637 and 1892 mg/kg bw per day respectively) glyphosate (purity 95.3 %) in the diet

There were no treatment related mortalities or clinical signs of toxicity. In the 30,000 ppm group, body weights of males and females were slightly lower (about 5-10 % decrease in males and 5 % in females) than those in the control throughout the treatment period. The overall food consumption by males and females was comparable to the control . There were no treatment related ocular effects and haematological and clinical chemistry parameters. In the 30,000 ppm group, urine pH in males and females was si gnificantly lower than that in the control. Urine protein showed a significant decrease in males and a decreasing trend in females. In addition, females showed a significantly

higher urine volume than that of the control, but males showed a decreasing tren d in urine volume as compared with the control. In the 10,000 ppm group, urine, pH and protein in males were lower than those in the control. In females, no statistically significant change was observed in any parameter. In the 3,000 ppm group, no statistically significant changes was observed in either sex.

In the 30,000 ppm group, both sexes showed significant increases in absolute and relative weights of the cecum (containing contents). In addition, females in this group also showed significant increases in relative weights of the brain and liver. In the 10,000 ppm group, the absolute and relative weight of the cecum showed a statistically significant increase in males and increasing trend in females. In the 3,000 ppm group, there were no abnormalities a ttributable to the treatment in either sex (Table 24).

Table 24. Mean Absolute and Relative Cecum Weight

	Dietary Concentration (ppm)			
	0	3000	10000	30000
Males				
Absolute weight	2823	3187	3383	5854**
S.D.	794	609	1081(11)	2053
Relative weight#	0.55	0.62	0.64	1.22**
S.D.	0.16	0.13	0.20(11)	0.41
Females				
Absolute weight	2367	2586	3546*	5268**
S.D.	582	462	959	1189
Relative weight#	0.79	0.84	1.22*	1.92**
S.D.	0.17	0.17	0.32	0.41

#: (organ weight/body weight) x 100.
S .D.: Standard deviation.
**: Significantly different from the control at 1% level of probability.

In the 30,000 ppm group, distention of the cecum was observed in 9/12 males and 7/12 females with statistical significance. There were no other macroscopic abnormalities attributable to the treatment. In the 10,000 ppm group, 3/12 males showed distention of the cecum, but there were no macroscopic abnormalities in females. In the 3,000 ppm group, there were no macroscopic abnormalities attributable to the treatment in either sex (Table 25).

Table 25. Incidence of Cecum Macroscopic and Microscopic Findings

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	3000	10000	30000	0	3000	10000	30000
Distended with contents	0	0	3	9**	0	0	0	7**
Cecum Mucosal Atrophy	0	0	1	5	2	0	2	5

N = 12
**Significantly different from the control at 1% level of probability.

Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment -related changes were not observed. One male in the 10,000 ppm group and one female in the 30,000 ppm group showed renal lesion (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). It is generally regarded that these lesions were caused by genetic disorder and were not considered to be treatment-related.

The NOAEL in this 90 day toxicity study in rats is 3,000 ppm (equal to 168.4 mg/kg bw per day) based on increased in cecum weight at 10,000 ppm and above (Kinoshita, 1995).

Dogs

In a 7-day oral toxicity study, one male and one female beagle dogs were fed gelatin capsules containing glyphosate (purity, 99.5%) at increasing doses of 100, 300 or 1,000 mg/kg bw per day. A second group of one male and one female dog received gelatin capsules containing glyphosate at a dose of 1,000 mg/kg bw per day for 14 consecutive days.

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

Glyphosate technical (purity 94.61 %) was offered on a continuous basis in the basal diet to groups of 4 males and 4 females Beagle dogs for a minimum of 90 days. Dietary concentrations were 0; 1,600; 8,000 and 40,000 ppm (equal to 0, 39.7, 198, and 1015 mg/kg bw per day for males and 0, 39.8, 201 and 1014 mg/kg bw per day.

There was no treatment related effects on mortality, clinical signs, changes in body weight, food consumption, test substance intake, ocular changes or macroscopic findings.

Statistically significant changes in haematology parameters were observed in the treated groups. There were statistically significant differences in some haematological parameters in the treated groups of both sexes; however, no dose dependency was conceived in the changes. There were statistically significant differences in some clinical chemistry parameters in the treated group of both sexes; however, no dose dependency was conceived in the changes. In the 40,000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis. There were no significant changes in urinalysis in males and females treated at 16,000 ppm or less.

Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1,600 ppm group, the change was considered to be incidental due to the lack of dose-dependency. There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40,000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.

The NOAEL in this 90 day toxicity study in dogs 40,000 ppm; equal to 1015 mg/kg bw per day, the highest dose tested (Yoshida, 1996).

Glyphosate acid (purity 99.1%) was administered at doses of 0; 2,000; 10,000 or 50,000 ppm (equal to M/F: 68/68, 323/334, 1680/1750 mg/kg bw per day) via the diet for 90 days to one control and three treatment groups each containing 4 male and 4 female Beagle dogs.

There were no mortality and treatment related clinical signs of toxicity. Body weight gain of males given 50,000 ppm glyphosate acid showed a slight depression throughout the study, but the differences were not statistically significant. Females given 50,000 ppm glyphosate acid showed slightly reduced bodyweight gains throughout the study and these were occasionally statistically significantly different from the controls. There were no treatment-related ophthalmological and haematological findings. There were statistically significant changes in clinical chemistry parameters but they were small in magnitude of change, therefore, they were not considered as biologically relevant. There were no differences in urine clinical chemistry parameters, nor in urinary sediment examinations, which were considered to be related to treatment.

Kidney weights of males given 10,000 or 50,000 ppm glyphosate were slightly increased above control values, but the increase was not proportional to dose. There was also a small increase in liver weight at these dose levels, but in male dogs only

No macroscopic or microscopic findings were observed attributable to the administration of glyphosate acid.

The NOAEL was 10,000 ppm; equal to 323 mg/kg bw per day based on decreased in body weight gains in female dogs at 50,000 ppm; equal to 1750 mg/kg bw per day (Hodge, 1996).

In a 90 day feeding study, groups of four Beagle dogs per sex received daily doses of 0, 200; 2,000 and 10,000 ppm glyphosate technical (purity >95%) in the diet (corresponding to 5.3, 53.5 and 252.6 mg/kg bw per day).

All animals survived until s cheduled necropsy. No clinical signs of toxicity were observed. There were no treatment related effects observe in body weights, urinalysis, organ weights, gross pathology or histopathology.

A significant increase in clotting time and GGT-activity was observed in both sexes at the 45-day interim bleed; however, in the absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to rather reflect a systemic error during determination than a real effect of the test item. Total bilirubin was higher; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

The NOAEL in this 90-day toxicity study in dogs was 10,000 ppm; equal to 252.6 mg/kg bw per day; HDT (Prakash, 1999).

In a 13-week oral toxicity study, groups of four Beagle dogs per sex received daily doses of 0, 30, 300 and 1,000 mg/kg bw per day glyphosate (purity 95.7%) by capsule application.

Two unscheduled sacrifices (one male and one female) were necessary in animals given 1,000 mg/kg bw per day: One male was sacrificed on day 61 on humane grounds. Vomiting was seen once in week 7 (before dosing) and liquid faeces were noted on many occasions in weeks 8 and 9. One female was s acrificed on day 72 for humane reasons. This animal showed liquid or soft faeces on many occasions from week 4 and dehydration from week 9. Vomiting was observed once in week 10.

No treatment-related clinical signs were noted in control animals or those given 30 or 300 mg/kg bw per day . The following treatment -related clinical signs were reported in animals given 1,000 mg/kg bw per day (excluding those killed in extremis, which are discussed separately): liquid or soft faeces on several occasions in all a nimals, vomiting in 2/3 females on one occasion within 30 minutes or 3 to 5 hours after treatment, thin appearance in 1/3 males and all females, dehydration in 1/3 males and 2/3 females, pallor of ears and mouth in 1/3 females.

No relevant differences in the mean body weight gain were noted between controls and animals given 30 or 300 mg/kg bw per day during the treatment period. At the end of the treatment period, only a slight mean body weight gain in males (+4 % vs. +31 % in controls) and a mean body weight loss in females (-7 % vs. +14 % in controls) when compared to their body weight on day 1. This effect on body weight was considered treatment -related. Reduced food consumption, varying from 25 to 75 % of the amount given, was observed on many occasions in animals given 1,000 mg/kg bw per day. There were no ophthalmological findings at the end of the treatment period. There were no treatment related effects on haematological and clinical chemistry parameters between trated gropus compared to contols. When compared to both pre -dose and control values, urine analysis resulted in the following findings were noted at 1000 mg/kg bw per day in Week 11: decrease in mean specific gravity in 1/3 males and 3/3 females . Absolute and relative weight of the prostate was significantly reduced by 68 and 56%, respectively at 1000 mg/kg bw per day . There were no other treatment related effects on organ weights. All the macroscopic changes noted in surviving animals at termination were considered to be normal variations, except for changes in the uterus (reduced in size) for females given 1,000 mg/kg bw per day. No test-substance related histopathological changes were observed in animals of both sexes at and below 300 mg/kg bw per day . Treatment-related changes observed i n surviving animals given 1,000 mg/kg bw per day consisted of increased number of adipocytes in the sternum of 2/3 males and 3/3 females, prostate atrophy in 2/3 males and uterine atrophy in 2/3 females.

The NOAEL in this 90-day toxicity study in dogs is 300 mg/kg bw per day based on mortality and decreased in body weight gains at 1000 mg/kg bw per day (Gaou, 2007). This study results demonstrate much pronounced toxic effects which is rather different from what was seen in other dog studies or other species.

In an oral toxicity, groups of six male and six female beagle dogs were fed gelatin capsules containing glyphosate (purity, 96.13%) at a dose of 0, 20, 100 or 500 mg/kg bw per day once daily for 52 weeks.

All dogs survived until study termination. There were no treatment related effects on body weights, food consumption, clinical signs of toxicity, ocular abnormalities, haematological parameters, urinary parameters, organ weights, macroscopic and histological findings.

The NOAEL in this 1-year toxicity study in dogs is 500 mg/kg bw per day, the highest dose tested (Reyna and Ruecker, 1985).

In an oral toxicity, groups of four male and four female beagle dogs were fed gelatin capsules containing glyphosate (purity, 98.6-99.5%) at a dose of 0, 30, 300 or 1,000 mg/kg bw per day once daily for 52 weeks.

There were no mortalities throughout the test period. Changes in faecal consistency (soft/loose/liquid) were recorded frequently for animals in the group receiving glyphosate at a dose at 1,000 mg/kg bw per day. This finding was observed 4-6 hours after dosing and was also recorded on isolated occasions for a few animals at 300 mg/kg bw per day. It was considered to be related to the administration of glyphosate. Food consumption was maximal or near maximal for all test groups. Mean body weight gain showed a non-statistically significant reduction in males at all doses (approximately 83, 75 or 75% of that of the control group for the groups receiving the lowest, intermediate, and highest dose, respectively) and in females at the highest dose (81% of that of the control group). Ophthalmoscopy and laboratory examinations revealed no treatment-related abnormalities. Plasma concentrations of glyphosate suggested that absorption was dose-related and remained constant throughout the duration of the study. Mean values detected were 0.36, 1.82 and 6.08 µg/ml for the groups receiving the lowest, intermediate and highest doses, respectively. At necropsy, no abnormal gross findings and no significant intergroup organ weight differences attributable to treatment with glyphosate were noted. In males, absolute and relative weights of the liver were slightly increased (4%, 8% and 10% above that of the control group, and 10%, 17% and 19% above that of the control group for the groups receiving the lowest, intermediate and highest doses, respectively), but the differences did not achieve statistical significance. There were no significant histopathological findings at any dose. The faecal inconsistencies seen a few hours after dosing were most likely to be related to high local concentrations of glyphosate in the gastrointestinal tract that were attributable to the administration of the test substance in capsules.

The NOAEL in this 52-week study in dogs was 30 mg/kg bw per day on the basis of the changes in fecal consistency and the reduced body weight gain in males at 300 mg/kg bw per day and greater (Goburdhun, 1990).

In an oral toxicity study, groups of four male and four female beagle dogs were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0; 3,000; 15,000 or 30,000 ppm (equal to 0, 91, 440 and 907 mg/kg bw per day for males and o, 92, 448 and 926 mg/kg bw per day for females) for 1-year. Selected organs were weighed and specified tissues taken from all groups for histopathological examination.

There were no mortalities during the study. There was no effect on food consumption; only three dogs left small amounts of food intermittently during the study. Body weight was slightly reduced in females at 30,000 ppm, with a maximum reduction of 11% (compared with that of controls) in week 51. These dogs showed a gradual reduction in growth rate, compared with that of controls, which was consistently significant from week 23 onwards. A similar change in body-weight gain in females receiving glyphosate at the lowest dose of 3,000 ppm, although occasionally reaching statistical significance, was not regarded as treatment-related since a dose response relationship was lacking. There was no effect on body weight in males at any dose tested. There were no toxicologically significant effects on any of the haematological and clinical chemistry parameters

measured. There were no treatment -related effects in any of the clinical chemical parameters measured in urine. No adverse effects of glyphosate were seen at examination post mortem and there were no treatment -related effects on organ weights. No histopathological changes attributable to administration of glyphosate were found.

The NOAEL in this 1-tear toxicity study in dogs is 15,000 ppm (equal to 448 mg/kg bw per day) on the basis of a reduction in body weight at 30,000ppm in female dogs (Brammer, 1996).

In an oral toxicity study, groups of 4 males and 4 females Beagle dogs received glyphosate technical (purity 94.6 1%) via the diet at a level of 0; 1,600; 8,000 or 50,000 ppm (equ al to M/F: 34.1/37.1, 182/184; 1,203/1,259 mg/kg bw per day) for a period of 12 months. A detailed histo-pathological examination was performed on all sampled tissues of all dogs, except for femur, larynx, oviducts, tongue, ureter and vagina.

There were no deaths in any dose groups of either sex. There were no treatment related effects observed during ocular examination, in urine analysis , organ weights and macroscopic and histopathological findings. In the 50,000 ppm group, loose stool was observed in 3 of 4 males and 4 of 4 females. The animals in the 8,000 and 1,600 ppm groups did not show the clinical sign at all. The mean body weights in the 50,000 ppm group at termination of treatment were reduced by 6% in males and 11% in females when compared to the controls. However, statistically significant differences in mean body weights were not observed. Food consumption was not affected by the treatment.

Male groups showed no significant changes in any haematological parameters. Females in the 50,000 ppm group showed significantly decreased values of haematocrit (Ht), haemoglobin concentration (Hb), and erythrocyte count (RBC) , however, these changes were minor in magnitude, often lacking the dose response and therefore they were not considered as biologically relevant.

Statistically significant changes in blood biochemistry were observed as follows. Females in the 50,000 ppm group showed a significant changes in clinical chemistry parameters. These changes were within biological variability and therefore they were not considered as adverse.

The NOAEL in this 12 months toxicitt study in dogs is is 8,000 ppm (equ al to 182 and 184 mg/kg bw per day based on loose stools in both sexes and decr eased in body weights in females (Nakashima, 1997).

In a 12-month oral toxicity study, groups of four beagle dogs per sex received daily doses of 0, 30, 100 and 300 mg/kg bw per day glyphosate technical (purity 97.5%) in gelatin capsules. Dose formulations were prepared weekly by adding the required amount to the capsules.

During the administration period, no deaths occurred in any group. In the 300 mg/kg bw per day group, all males and females showed soft stool, diarrhoea, mucous faeces or watery diarro hea. They also showed vomiting, bloody stool or coloration of the faeces by the test article in rare cases. In the 100 mg/kg bw per day group, changes similar to those observed in the 300 mg/kg bw per day group were observed at lower frequencies, but one m ale in this group that showed bloody faeces continually from Day 346 of administration onward showed ulcer by intussusception in histopathological examination. It was thought that the change observed in the 30 mg/kg bw per day group was comparable to that observed in untreated animals based on the incidence of its occurrence. A significant decrease in body weight compared to that of the control group was recorded from Week 24 of administration in females in the 300 mg/kg bw per day group and from Week 27 of administration in females in the 30 mg/kg bw per day group largely continually until the end of the administration period. There were no treatment related effects in females at 100 mg/kg per day. There were no changes that are thought to be caused by admi nistration of the test article in food consumption, urinalysis, haematological examination, blood biochemistry examination, ophthalmo-scopy, organ weight, necropsy or histopathological examination.

In conclusion, the NOAEL was 30 mg/kg bw per day based on changes in faeces observed in male and female dogs and reduced body weights in females at the LOAEL of 100 mg/kg bw per day group (Teramoto, 1998).

In a 1-year oral toxicity study groups of four beagle dogs per sex received daily doses of 0, 30, 125 and 500 mg/kg bw per day glyphosate technical (purity 95.7%) in gelatin capsules for 52 consecutive weeks.

No mortalities or premature sacrifices occurred during the treatment period. There were no treatment-related effects in clinical signs, body weight, food consumptions, haematology and clinical chemistry parameters, ophthalmoscopic findings, organ weights, macroscopic or microscopic findings.

The NOAEL in this 1-year toxicity study in dogs is 500 mg/kg bw per day ; HDT (Haag, 2008).

(b) Dermal application

Rats

In a 21-day dermal toxicity study, groups of five male and five female Alpk:APfSD rats were exposed to glyphosate (purity 95.6%) at 0, 250, 500 or 1000 mg/kg bw per day. The test material was moistened with deionized water and the paste was applied to previously clipped back area. The paste was applied in gauze patch and covered with occlusive dressing for six hours. The application site was rinsed after 6 hours of exposure. A total of 15 six hour applications were made during a period of 21 days.

No compound related effects were noted on mortality, body weights, body weight gains, food consumption, haematology, clinical chemistry parameters, macroscopic findings, organ weights, and histopathological findings at any dose levels.

Based on the results of the 21-day dermal toxicity study in rats, the systemic toxicity NOAEL was 1000 mg/kg bw per day, the highest dose tested (Pinto, 1996).

Rabbits

In a 21-day dermal toxicity study, groups of ten male and ten female New Zealand White rabbits were exposed to glyphosate (purity not reported) at 0, 100, 1000 or 5000 mg/kg bw per day. The test material was moistened with physiological saline and applied onto the skin covered with a gauze patch secured with a tape. The material was applied on intact skin (5/sex/dose) and abraded skin (5/sex/dose) for 6 hours/day, 5 days/week, for 3 weeks. The control group physiological saline only. The study was conducted in accordance with GLP.

There were no deaths and no clear effects on clinical condition. No dermal irritation was observed in the control, 100 and 1000 mg/kg bw per day dosed group. Slight irritation was noted in both intact and abraded skin at 5000 mg/kg bw per day. No treatment related effects were observed on body weights, body weight gains, food consumption, haematology and clinical chemistry parameters at any dose levels tested. At the terminal sacrifice, no compound related macroscopic lesions were observed on the skin at the application site or in any other tissues or organs in rabbits from the test groups. No compound-related organ weight variations occurred in any of the test groups in this study. No compound related histopathological findings were noted.

Based on the results of the 21-day dermal toxicity study in rabbits, the systemic toxicity NOAEL was 5000 mg/kg bw per day, the highest dose tested (Johnson, 1982).

In a 28-day dermal toxicity study, groups of five male and five female New Zealand White rabbits were exposed to glyphosate (purity 99.6%) at 0, 500, 1000 or 2000 mg/kg bw per day. The test material was homogenized in water. The test substance was placed on a gauze pad and then the compound-coated side of the pad applied to the clipped area of the rabbit. The pad was covered with a sheet of polyethylene material that was secured by tape. The test material was applied to approximately 10% of the body surface area.

No compound related effects were noted on mortality, body weights, body weight gains, food consumption, haematology, clinical chemistry parameters, macroscopic findings, organ weights, and

EPA-HQ-2018-002024_0004859

histopathological findings at any dose levels. Very slight erythema was observed in 2000 mg/kg bw per day dose group.

Based on the results of the 28-day dermal toxicity study in rabbits, the systemic toxicity NOAEL was 2000 mg/kg bw per day, the highest dose tested (Tornai, 1994).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Study 1:

In a carcinogenicity study, glyphosate (purity 99.7%) was administered to groups of 50 male and 50 female CD-1 mice/sex/dose in the diet at dose levels of 0, 1000, 5000, or 30,000 ppm (equal to 0, 157, 814, 4841 mg/kg bw per day for males and 0, 190, 955, and 5874 mg/kg bw per day for females) for 24 months. Cage-side and detailed clinical observations were done. Body weight and food intake were monitored throughout the study. Water consumption was measured during months 12 and 24. Erythrocyte, as well as total white cell counts and differentials, were done at months 12, 18, and 24. Tissues and organs were collected from all mice whether dying during the study or at terminal sacrifice. Microscopic analyses were done on all collected tissues. This study was conducted prior to the establishment of GLP requirements.

Analysis of treated diets demonstrated that glyphosate could be homogeneously mixed with rodent diet and that it remained stable in the diet for the one week feeding period used in this study. Glyphosate test concentrations averaged approximately 95% of the target concentrations throughout the study. There were no physical or behavioural signs of toxicity, which were considered to be related to glyphosate administration. The incidences of yellow staining of the anogenital area, scabbing on the ears, alopecia, excessive lacrimation, displacement of the pupils, and ocular opacities were observed in all groups of male and female mice, none were dose-related and all occurred at low incidences. Mortality was not affected by the treatment. Body weights for both males and females of the high-dose group were consistently less than controls throughout the study. Although the decreases were slight (1% to 11%), several were statistically significant. Other statistically significant decreases were also noted in the mid- and low-dose animals; however, these changes were sporadic and did not reflect a recognizable dose-response relationship. Although sporadic statistically significant effects were noted for food 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 RBC or WBC counts, HGB, HCT, or platelet counts. There were no changes observed in the absolute or relative organ weights that were considered to be due to glyphosate administration. 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 a specific organ effect. 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 26) and chronic interstitial nephritis in males, and for proximal tubule epithelial basophilia and hypertrophy in females. Statistically significant increases in the incidence of lesions in treatment groups vs. control were observed for centrilobular hepatocyte necrosis in high-dose males and proximal tubule epithelial basophilia and hypertrophy in high-dose females. Regarding the kidney findings, while the incidences and/or dose response trends of these individual microscopic kidney lesions were found to be statistically significant, they are considered to be part of a spectrum of lesions, which, as a whole, constitute spontaneous renal disease.

Table 26. Incidences of hepatocellular lesions in all mice

	Sex	Concentration in the diet (ppm)			
		0	1000	5000	30000
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[a]	2/50	2/50	10/50[a,b]

[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-Square test

Neoplastic outcomes were of the type commonly encountered 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 were seen in all treatment groups (Table 27). Lymphoreticular tumours were more frequently observed in female mice, but the incidences were low and did not approach statistical significance. With the possible exception of kidney tumours (renal tubular adenomas) in males, all tumour types were considered spurious and unrelated to treatment. The renal tumour incidence as provided in the study report is sh own in Table 39. At the request of the US Environmental Protection Agency (USEPA), the Pathology Work Group (PWG) examined all sections of the kidneys from this study as well as additional renal sections. The PWG evaluation included a renal tubule adenom a in one control male mouse that was identified during a re-evaluation of the original renal section. This control animal tumor was not included in the original study report, but was considered subsequently in the PWG statistical analysis. The PWG noted t hat 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 purposes of evaluation of statisti cal analysis. Statistical analyses performed by PWG are presented in Table 28. The PWG concluded that these lesions are not compound-related based on the following considerations: 1) renal tubular cell tumours are spontaneous lesions for which there is a p aucity 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) compound -related nephrotoxic lesions, including pre -neoplastic changes, were not present in male mice in this study. Additionally, there was no increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/rege neration, hyperplasia or hypertrophy). Although the incidence of tubular adenomas exceeded the historical control range (0 -3.3%) for the testing laboratory, the increase at the high dose was not statistically significant compared to the concurrent controls.

Table 27. Incidence of neoplasia in male and female mice treated with glyphosate for 24 months

Organ / Effect	Dose (ppm)			
	0	1000	5000	30,000
Males				
Lung				
Bronchiolar alveolar adenoma	5/48	9/50	9/50	9/50
Bronchiolar alveolar adenocarcinoma	4/48	3/50	2/50	1/50
Lymphoblastic lymphosarcoma with leukemic manifestations	1/48	4/50	3/50	1/50
Liver				
Hepatocellular adenocarcinoma	5/49	4/50	6/50	4/50
Hepatocellular carcinoma	0/49	0/50	0/50	2/50
Lymph node (mediastinal)				
Lymphoblastic lymphosarcoma with leukemic manifestations	1/45	2/49	1/41	2/49
Kidney				
Renal tubular adenoma	0/49	0/49	1/50	3/50
Lymphoblastic lymphosarcoma with leukemic manifestations	1/49	3/49	2/50	2/50
Females				
Lung				
Bronchiolar alveolar adenoma	10/49	9/50	10/49	1/50
Bronchiolar alveolar adenocarcinoma	1/49	3/50	4/49	4/50
Liver				
Hepatocellular adenocarcinoma	1/49	2/50	1/49	0/49
Composite lymphosarcoma	2/49	1/50	0/49	4/49

EPA-HQ-2018-002024_0004861

Table 28. Incidence of renal tumours in male mice as reported by the PWG using Cochran-Armitage Trend & Fisher's Exact Test

	Dose (ppm in diet)			
Tumor Type	0	1000	5000	30000
Adenomas (%) P =	1/49 (2) 0.4422	0/49 (0) 1.0000	0/50 (0) 1.00000	1/45 (2) 0.7576
Carcinomas (%) P =	0/49 (0) 0.0635	0/49 (0) 1.0000	1/50 (2) 0.5051	2/50 (4) 0.2525
Combined (%) P =	1/49 (2) 0.0648	0/49 (0) 1.0000	1/50 (2) 0.7576	3/50 (6) 0.3163

In conclusion, the NOAEL for the systemic toxicity in the carcinogenicity study in mice was 5000 ppm (equal to 814 mg/kg bw per day for males and 955 mg/kg bw per day for females) based on 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 and Hogan, 1983).

Study 2:

In a carcinogenicity study, trimethylsulfonium carboxymethyl aminomethylphosphonate (Company code SC-0224; trimesium salt of glyphosate; purity 56.17% active ingredient [glyphosate]) was administered to groups of 80 Crl:CD-1 (ICR) BR mice/sex/dose in the diet at doses of 100, 1000, or 8000 ppm for 22 months. The mean compound intake for male mice were 11.7, 118 and 991 mg/kg bw per day for th e 100, 1000 or 8000 ppm groups, respectively. The mean compound intake for female mice were 16.0, 159 and 1341 mg/kg bw per day for the 100, 1000 or 8000 ppm groups, respectively.One control group of 60 male and female mice were fed on basal diet only. Add itional control group of 80 male and female mice were fed on basal diet plus 1% propylene glycol vehicle. There were interim sacrifices of variable numbers of mice at 6, 12 and 18 months. The number of mice scheduled for full 22 month study duration was 50 sex/dose. Blood samples were drawn from fasted mice 10/sex/dose at 6, 12, 18 and 22 months for haematology and clinical chemistry measurements. Brain cholinesterase was measure from the left and right brain of 5 mice/sex/dose at 6, 12, 18 and 22 months. Urinalysis were performed from 10 fasted mice/sex/dose at 6, 12, 18 and 22 months. Ophthalmoscopic examination of mice were conducted at 6, 12, 18 and 22 months. Macroscopic examination was conducted on all animals. Histopathological examination was conducted on selected tissues from all animals. Selected organs were weighed.

The mean body weight values of the 8000 ppm male mice were decreased by 3 to 11% during most of the study. Similarly, the mean body weight values of the 8000 ppm female mice were decreased by 4 to 17% during most of the study. The food consumption was also slightly decreased in male and female mice at 8000 ppm. Survival of male mice was not affected by the treatment and the survival in female mice was apparently increased. There were no compound related effects on clinical signs, urinalysis, haematology and clinical chemistry parameters, opthalmoscopic examinations at 6, 12, 18 or 22 months. There were no compound related effects on organ weights (absolute or relative to body weight) and palpable masses. Analysis of the brain, red blood cells and serum cholinesterase activity did not reveal any toxicologically significant differences. In female mice, the non -neoplastic lesion which was considered compound -related was the increased incidenc e of epithelial hyperplasia of the duodenum at 8000 ppm. The percent response of hyperplasia in female was 10, 13, 16, 15 and 24% at 0, 100, 1000 and 8000 ppm, respectively. There was a compound related increased incidence

of white matter degeneration of t he lumbar region of the spinal cord in male mice at 8000 ppm. The percent response for increased white masses in male mice were 2, 3, 4, 4 and 8 at 0, 100, 1000 and 8000 ppm, respectively. There were no compound related neoplastic lesions in male and female mice. Additionally, there was no decrease in latency.

The systemic toxicity NOAEL in mice is 1000 ppm (equal to 118 mg/kg bw per day) based on decreased in body weight and food consumption in both sexes, increased incidence of white matter degeneration in the lambar region of the spinal cord in male mice and epithelial hyperplasia of the duodenum in female mice at 8000 ppm (equal to 991 mg/kg bw per day. There were no compound related neoplastic lesions in male and female mice (Pavkov and Turnier, 1987).

Study 3:

Bhide, M.B. (1988): Carcinogenicity and chronic toxicity study of glyphosate (technical) of Excel Industries Ltd. From Referenced in: Draft Assessment Report on Glyphosate, Annex B B -5: Toxicology and metabolism, 1998. Verbatim from Draft As sessment Report on Glyphosate, Annex B B-5: Toxicology and metabolism, 1998: The DAR assessment concluded that the study is unacceptable for a reliable assessment of carcinogenicity since the number of animals used was too small. In addition, the highest dose level of 300 ppm is considered too low. However, the study can be considered to provide supplementary information with regard to chronic toxicity. **Summarized here for JMPR DISCUSSION-**

Groups of 25 male and 25 female Balb/c inbred albino mice (sourc e not specified; 5 to 8 weeks old at the start of treatment) per dose were administered glyphosate technical (batch and purity not given; manufacturer: Excel Industries Ltd., Bombay, India) for 80 weeks at dietary levels of 0, 75, 150 and 300 ppm. The actual mean daily compound intake was not calculated.

Survival was not affected by treatment and overt clinical signs of toxicity did not occur. There was a trend of decreased body weight in high dose male animals towards the end of the treatment period. In fe males, a similar trend was obvious from the beginning of the study up to week 21 at the highest and the mid dose level. During the last 20 weeks of the administration period, mean body weight was reduced again but only in the female group receiving 300 ppm . Food consumption was markedly diminished in high dose males from week 9 onwards and in high dose females from week 6. Haematology and clinical chemistry did not reveal treatment -related changes neither after 9 nor after 18 months. Mean organ weights were not affected. Gross and histopathological examination did not provide evidence of lesions that could be attributed to glyphosate administration. The incidence of neoplasia was not increased. The total number of tumours was considerably low in all groups.

The NOAEL for chronic toxicity was 150 ppm based on the impact of treatment on body weight and food consumption. When the usual conversion factor of 10 is applied, this value would correspond to a daily intake of 15 mg/kg bw. A NOEL could not be established since a weak effect on body weight also in mid dose females cannot be completely excluded. In contrast, the study author concluded that toxicological effects did not occur up to the highest dietary level of 300 ppm although the reduction in body weight a nd food consumption was mentioned in the study report. It should be noticed that body weight and food intake were not affected at much higher doses in the other available long-term studies in mice. Thus, it is not likely that these effects were actually re lated to treatment (Bhide, 1988).

Study 4 :

Vereczkey, L. and Csanyi, E. (1992/ revised version): 18 -month carcinogenicity study of glyphosate in mice. Referenced in: Draft Assessment Report on Glyphosate, Annex B B -5: Toxicology and metabolism, 1998. The DAR assessment concluded that no conclusion available due to low quality of the study report. The study is unacceptable for a reliable assessment of carcinogenicity since the number of animals surviving up to scheduled termination and subjected to pathological examination was too small. In addition, the highest dose of 300 ppm is apparently not sufficient for evaluation of carcinogenicity since no evidence of toxicity was obtained at that dose level. However, the study can be considered to provide suppl ementary information with regard to

chronic toxicity. The study was not conducted in accordance with GLP. **Summarized here for JMPR DISCUSSION-**

Glyphosate (purity not indicated since the respective supplement was not submitted to the Rapporteur; manufacturer not given) was administered to groups of 50 male and female CFLP/LATI mice (bred in a facility in Godollo, Hungary; 26 - 30 days old at study initiation) per dose at dietary levels of 0, 100 and 300 ppm. The actual daily intake was not calculated. The a dministration period was 18 months.

There was a considerably high mortality rate in all study groups. Thus, only 11, 14, and 23 male animals and 14, 16 and 14 females survived up to scheduled termination in the control, low and high dose groups and were a vailable for pathological examination. Because clinical signs of toxicity were lacking and since mortality did not increase with dose, a treatment -related impact on survival is not likely. Body weight and food consumption were not affected. Gross and histo pathological examination did not reveal treatment -related changes. The overall tumour rate was rather high in all study groups including the controls. However, no significant difference in tumour incidence was observed between the groups.

There was no clear evidence of adverse effects of glyphosate administration up to the highest tested dose of 300 ppm (about 30 mg/kg bw per day) which is considered the NOEL in this study. However, the scientific value of this experiment is rather limited (Vereczkey and Cs anyi 1982, revised 1992).

Study 5:

In a carcinogenicity study, glyphosate (purity 97.5 – 100.2%) was administered to groups of 50 CD-1 mice/sex/dose in the diet at doses of 0, 100, 300, or 1000 mg/kg bw per day for 104 weeks. The dietary concentrations were adjusted weekly for the first 13 weeks and every 4 weeks thereafter. No interim sacrifices were done. Mortality, body weight, body weight gain, and food consumption were monitored throughout the study. WBC differential counts were done during Weeks 5 2, 77, and 102 of the study. Following premature deaths or at scheduled sacrifice, organ weights were measured and tissues collected for microscopic analyses.

The analytical data indicated that the mixing procedure was adequate and that the variance betwe en nominal and actual dosage to the animals was acceptable. There were no unscheduled deaths during the course of the study that were attributable to the administration of glyphosate. No treatment-related clinical signs of toxicity were observed. There wer e no biologically relevant or toxicologically significant effects over the 104 -week study on body weight or body weight gain of male and female CD-1 mice. Although statistically significant effects were noted, none were due to treatment with the test mater ial and were typically higher than in corresponding control mice. No treatment -related effects were noted on food or water consumption. Ophthalmoscopic examinations, urinalysis and clinical chemistry parameters were not evaluated. There were no remarkable intergroup differences in differential blood counts in either sex at any of the time -points tested. The absolute and relative to body thymus weights of male mice in the 300 and 1000 mg/kg bw per day groups were statistically significantly increased. The in crease in thymus weights were slight in magnitude and lack of a dose response. No histologically correlates were found microscopically. No increase in absolute or relative to body thymus weights were found in female mice. The incidence of lung masses was s lightly increased in high -dose male mice (control 10/50, low -dose 13/50, mid -dose 12/50 and high -dose 18/50), however, histopathology failed to reveal adverse lung findings. No increase in lung masses was found in female mice. The occurrence of mineral deposits in the brain was significantly increased in males at the highest dose when compared with the control group (13/50 compared with 4/49). It should be noted that this is a common finding in mice of this age and strain.

There were no statisti cally significant increases in the incidence of any tumours, both benign and malignant in either sex when compared to the control. However, the number of animals with multiple tumour types was slightly increased in the high -dose group of both sexes (males 16/50 and females 11/50) compared to the control (males 11/50 and 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 and females 43) compared to the control (males 49 and 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

in the controls. Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue was evident in 2/50 low and high-dose males and 3 low- and intermediate- and 1/50 high-dose females when compared to the respective controls (0/50). Due to a lack of dose relationship, and the lack of statistical significance and the incidences in this study falling within the background ranges, these changes are not considered to be due to administration of glyphosate Other tumours seen were considered to be typical for mice of this age and strain.

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

Study 6:

In a carcinogenicity study, glyphosate (HR -001, purity 97.56 and 94.61%; two lots) was administered to groups of 50 male and 50 female Specific -Pathogen-Free (SPF) ICR (Crj: CD -1) mice/dose in the diet at dose levels of 0, 1600, 8000 or 40000 ppm (equal to 0, 165, 838.1, or 4348 mg/kg bw per day for males and 0, 153.2, 786.8, or 4 116 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 food consumption was measured. At week 21, urinalysis was carried out on 20 males from all groups. Differenti al leukocyte counts were determined on the blood smears from 10 males and 10 females of all groups at week 52 and after 78 weeks of treatment and also animals killed in extremis during the treatment as possible. At final necropsy after 78 weeks of treatment, organ weight analysis was conducted on 10 males and 10 females which were served to the determination of differential leukocyte counts. All animals of both sexes were subjected to necropsy and histopathological examinations.

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 6 and weeks 9-24. No treatment-related changes were seen in males. At 40000 ppm, the incidence of pale-colored skin increased in males. In addition, loose stool was observed in all cages beginning at week 21 in males and at week 20 in females. Retarded growth was persistently observed during the treatment period showing statistically si gnificant 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 food consumption and food efficiency. At necropsy, the increased incidences of distention of the cecum were noted in males and females at terminal kill and in all animals examined, which were consistent to increases in absolute and relative weights of the cecum. However, no abnormalities were recorded in the cecum histopathologically. In males, a significant increase was noted for the overall incidence of anal prolapse which was correspondent to erosion/ulcer of the anus histopathologically.

Histopathological examinations failed to show increases in incidence of any types of neoplastic lesions in all treatment groups of both sexes..

Based on these results, the no observable adverse effect level NOAEL is 1600 ppm (153.2 mg/kg bw per day) and the lowest observable adverse effect level (LOAEL) is 8000 ppm (838.1 mg/kg bw per day) for females based upon retarded growth with statistically significant decreases in weight at 6 and weeks 9-24. For males the NOAEL was 8000 (838.1 mg/kg bw per day) and the LOAEL was 40000 ppm (4116 mg/kg bw per day) based on a significant increase was noted for the over all incidence of anal prolapse which was correspondent to erosion/ulcer of the anus histopathologically (Sugimoto, 1997).

Study 7:

In a carcinogenicity study, glyphosate (purity 97.5%) was administered to groups of 50 male and 50 female Crj:CD-1 mice/dose in the diet at dose levels of 0, 500, 5000 and 50000 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 done. Body weight and food intake were monitored

throughout the study. Differential white cell counts were performed at week 52 and full haematological parameters evaluation at the end of t he treatment. Gross pathological examinations was conducted at sacrifice and on mice died pre -maturely and moribund. Selected organs (Brain, liver, (right and left) kidneys, (right and left) adrenal glands, and (right and left) testes) were weighed. Histopathological examination was performed on all sampled tissues from control and high dosed animals and on animals that died or killed in extremis.

Prepared diets were stable at room temperature for 4 months and the test compound was homogeneously distribut ed in the diet. Analysis of the prepared diet indicated that the measured concentrations were ranged from 80 to 110% of the nominal concentrations. In the 50,000-ppm group, loose stool was found throughout the treatment period in all males and females, of which some animals showed improvement as treatment was continued. In the same group, 9 males and 8 females had treatment-related anus prolapse at Week 10 or later. Other clinical signs and their incidences were similar in the 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 the mid and low dose males and females of all dose group was not affected by the treatment. In th e 50,000 ppm group, compared with the control group, body weight gain significantly decreased or appeared to decrease throughout the treatment period in males and at Week 24 or later in females. No effects of treatment was observed in treated males and fem ales in the mid and low dose at any time interval compared to controls. In the males and females from the 50,000 -ppm group, compared with the control group, food consumption decreased and the change was considered to be related to the test substance. No tr eatment related changes were observed in haematological parameters. In the females from the 50,000 -ppm group, compared with the control group, the relative weights of right and left kidneys significantly increased. These changes were considered to be relat ed to the test substance, 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 adr enal gland, and right and left testes in males, and a decrease in the absolute weight of brain in females were noticed in the 50,000 -ppm group. All these changes were considered to be unrelated to the test substance, because they were accompanied by decrea sed body weight. Macroscopic examination revealed luminal dilatation of the large intestine, which may be associated with loose stool, in most of the terminally sacrificed males and females from the 50,000 -ppm group. In the 50,000-ppm group, treatment-related non-neoplastic lesions were found in the kidney in males and the rectum in males and females. The renal findings included significant increases in tubular epithelial cell hypertrophy, tubular dilatation, degeneration / necrosis and an increasing tenden cy in basophilic tubules (based on data from all animals). The rectal findings included significant increases in anus prolapse-associated erosion and luminal dilatation (Table 29).

Table 29 Incidence of non-neoplstic lesions in mice treated with glyphosate for 78 weeks

	Male				Female			
Dose in ppm	0	500	5000	50000	0	500	5000	50000
Kidney								
No. of animals examined	50	50	50	50	50	50	50	50
Tubular Dilation	4	7	4	20**	8	12	5	8
Tubular epithelial cell hypertrophy	13	10	13	25*	13	17	14	13
Basophilic tubules	21	16	17	28	14	14	10	13
Tubular degeneration/necrosis	9	6	5	15	5	8	8	7
Rectum								
No. of animals examined	48	12	7	46	44	11	10	44
Luminal dilation	0	0	0	6*	0	0	0	6*
Erosion	0	0	0	3	0	0	0	6*

[a] The data represents in all animals at week 78
* $p<0.05$, ** $p<0.01$ (Fisher's exact probability test)

The observed neoplastic lesions of the kidney included renal cell adenoma in 3 males and renal cell carcinoma in 1 male in the 50,000 -ppm group, and renal cell adenoma in 1 male in the 5,000 -ppm group, with no renal tumo r formation in 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 Pesticide Expert Panel, Food Safety Commission of Japan requested additional information on historical control data and association with the non-neoplastic renal findings. The hematoxylin-eosin-stained kidney sections prepared in the original study were found to have faded and unevaluable; therefore, the paraffin-embedded blocks of 50 males in each group which had been stored for each observation period were sectioned and stained by hematoxylin and eosin for microscopic re-examination. The data from the re-examination and the original data are shown in Table 30

Table 30. Number and Incidence of Males with Renal Tumor by Dose

Dose (ppm)	Findings	Original study	Re-examination	Incidence
50,000	Renal cell adenoma	3	1	1/50 (2%)
50,000	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%)

The incidence of renal tumours in each treatment group did not statistically significantly differ from that in the control group, both in the original study report and re-examination (Fisher's exact probability test, P>0.05). The historical control data were not available. As described in the reexamination document, historical control values in the literature for renal cell carcinoma were 1/725 (0.13%) in males and 0/725 (0%) in females. The historical control values for renal cell adenoma were 3/564 (0.53%) in males and 0/564 (0%) in females (Baldrick and Reeve, 2007 and Chandra and Frith (1994). The re-examination report also provides reference data which were 0/55, 0/55, 1/55, 0/55, 0/55 (0%-1.8%) in males and 0/55, 0/55, 0/55, 0/55, 0/55 (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 tumors.

Baldrick P and Reeve L. Carcinogenicity Evaluation: Comparison of tumour data from dual control groups in CD-1 mouse. Toxicologic Pathology 2007; 35: 562-569.
Chandra M and Frith CH. Spontaneous renal lesions in CD-1 and B6C3F1 mice. Exp Toxic Pathol 1994; 46:189-198.

It is concluded that the renal cell tumours observed in this study are not considered to be treatment-related because (1) The incidence of renal tumors in the males from the 50,000-ppm group did not significantly differ from that in the control group; (2) It is uncertain that the non-neoplastic lesions found in males were associated with tumors, because these lesions developed even in the absence of tumors (see the EPA's evaluation mentioned above); (3) No females had a neoplastic lesion or non-neoplastic lesion, and (4) The highest dose (50,000 ppm) used in this study far exceeded the limit dose for mice (7,000 ppm) specified by the OECD and EPA.

In conclusion, the NOAEL was 5,000 ppm; equal to 685 mg/kg bw per day based on loose stool, decreased body weight gain, decreased food consumption, and the increased incidences of rectal and renal non-neoplastic lesions were observed in the males and females at the LOAEL 50,000 ppm; equal to 7470 mg/kg bw per day; the highest dose tested. Glyphosate was not carcinogenic in CD-1 mice (Takahashi, 1999a).

Study 8:

In a carcinogenicity study, glyphosate (purity >95%) was administered to groups of 50 HsdOla:MF1 Swiss Albino mice/sex/dose in the diet at doses of 0, 100, 1000, or 10000 ppm (equal to 0, 14.5, 149.7 or 1453 mg/kg bw per day for males and 0, 15.0, 151.2, or 1466.8 mg/kg bw per day for females) for 18 months. The stability, homogeneity and dietary concentrations were measured periodically. A detailed veterinary examination of all mice was done before and after grouping and monthly thereafter. A check for clinical signs of toxicity, appearance, behaviour, and neurological

changes and mortality was made once daily on all mice. Ophthalmological examinations were performed on all mice prior to start of treatment at 6, 12 and 18 month of the study. Mortality, body weight, body weight gain, and food consumption were monitored throughout the study. WBC differential counts were done at 9 months and at terminal sacrificed from all surviving animals and from mice killed in extremis. All animals that died or were killed in extremis during the conduct of the study, were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy. All surviving mice were sacrificed at scheduled termination. A gross pathological examination was performed on all mice. Adrenals, kidneys, liver and gall bladder, ovaries and testes from 10 mice/sex/dose were weighed and selected tissues were examined histopathologically from control and high dose animals and animals that died or were killed in extremis.

All prepared diets were stable for 30 days, homogenously distributed and achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 10% of the nominal concentration for all diet samples. There was no of effect of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, ophthalmoscopic examination, and organ weights (absolute and relative to body). There were no significant treatment-related changes in the white blood cell counts for either sex at both 9 and 18 month. Slightly higher neutrophil counts and slightly lower lymphocyte counts in high dose males at 9 month were within the historical control ranges. The slightly higher eosinophil counts, higher neutrophil and monocyte counts, as well as slightly lower lymphocyte counts observed in high dose females at 18 month were comparable with historical control values and therefore considered incidental.

In mice found dead or sacrificed moribund, cystic glands of the stomach were significantly increased in high dose males and for both sexes combined. However, the incidence of these findings was similar to historical control data and did not show a dose dependency. Therefore, these finding was considered incidental. Increased haematopoiesis was seen in the bone (femur) of high dose males, mid- and high-dose animals combined sex. Cell debris in tubules of epididymides was increased in mid dose males and the incidence of sub-capsular cell hyperplasia was increased in adrenals of low dose males. In addition, the incidence of kidney nephropathy in mid-dose females, as well as the incidence of lymphocyte in filtration of epididymides in mid dose males was significantly decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental. Furthermore, it is necessary to consider the frequency of this finding in animals surviving to scheduled termination. At terminal sacrificed, cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose-response. Degenerative heart changes were higher in high-dose males and females, and significantly higher when sexes were combined. However, the percentage incidences were similar or slightly higher than in the historical controls and severity did not increased dose hence considered incidental. In mandibular lymph nodes lymphoid hyperplasia was significantly increase in low and mid dose males and combined sex, whereas the incidence was significantly lower in high dose females. In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level in combined sex. In spleen extramedullary haematopoiesis was significantly increased in females and combined sex at the low dose level. In the absence of any dose-relation these findings, as well as several not statistically significant changes considered incidental.

The number of malignant lymphoma (Table 43) was slightly elevated in the high dose group compared to control. This tumour of the hemolymphoreticular system is one of the most common tumours of mice accounting for the highest percentage of spontaneous tumours in this species. Therefore, the observed tumours incidence is considered incidental and not treatment-related.

An increase in malignant lymphoma was noted in both the male and female groups receiving the highest dose. The incidence was statistically significantly elevated as compared to the actual control groups in this study, was above the mean values of the (relatively small) historical control and, for males, outside the historical control range. Even though malignant lymphoma is a common tumour in mice (accounting for 54.6% of all tumours in this study), it cannot be completely excluded that the higher incidence in the top dose groups were somehow related to treatment. However, as noted in Greim et al. 2015 there is concern that there was a viral infection within the colony of mice used in this study, which confounds the interpretation of the lymphoma findings.

Table 31 Incidence of malignant lymphoma in glyphosate treated mice and comparison with the historical control

			Dietary concentration of glyphosate (ppm)							
			Males				Females			
	♂	♀	0	100	1000	10000	0	100	1000	10000
Dead & moribund										
Number examined	75	77	22	20	22	27	16	16	20	20
HC Number affected	20	49	9	12	13	13	9	10	13	12
HC Percentage affected	26.7	63.6	41.0	60.0*	59.0*	48.0	56.0	63.0	65.0	60.0
HC Mean %	26	61.8	--	--	--	--	--	--	--	--
HC Range %	0-44	0-100	--	--	--	--	--	--	--	--
Terminal sacrifice										
Number examined	175	173	28	30	28	23	34	34	30	30
Number affected	26	50	1	3	3	6*	9	10	6	13
Percentage affected	14.9	28.9	3.6	10.0	10.7	26.1*	26.5	29.4	20.0	43.3*
HC Mean %	14.9	28.8	--	--	--	--	--	--	--	--
HC Range %	8-24	20-43	--	--	--	--	--	--	--	--
All fates										
Number examined	250	250	50	50	50	50	50	50	50	50
Number affected	46	99	10	15	16	19*	18	20	19	25
Percentage affected	18.4	39.6	20.0	30.0	32.0	38.0*	36.0	40.0	38.0	50.0*
HC Mean %	18.4	41.6	--	--	--	--	--	--	--	--
HC Range %	6-30	14-58	--	--	--	--	--	--	--	--

* significantly increased; -- not examined/determined; HC= historical control data as provided in the study report

In conclusion, the systemic toxicity NOAEL in this 18 months carcinogenicity study in mice is 1000 ppm; equal to 149.7 mg/kg bw per day based on increased incidence of malignant lymphomas in males compared to controls seen at the high dose of 10 ,000 ppm; equal to 1453 mg/kg bw per day. Glyphosate was not carcinogenic in mice at doses up to 10,000 ppm; the highest dose tested (Kumar, 2001).

Study 9:

In a carcinogenicity study, glyphosate (purity 95.7%) was administered to groups of 51 male and 51 female CD -1 mice/dose in the diet at dose levels 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. Additional 12 mice per sex, designate d for veterinary controls, were housed and maintained alongside treated animals. Ten animals per sex from each group were set aside for an interim kill (toxicity assessment), which was carried out on the survivors after 39 weeks of dosing. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were done. Body weight and food intake were monitored throughout the study. Water consumption was observed daily. Blood smear samples were collec ted after 12 months and at termination from all animals, and from mice that were killed in extremis. Differential white cell counts were performed on all control and high -dose animals and on the animals killed in extremis. Gross pathological examinations was conducted at sacrifice and on mice died pre -maturely and moribund. Selected organs were weighed from 10 mice/sex/dose. Histopathological examination was performed on all sampled tissues from control and high dosed animals and on animals that died or killed in extremis.

Analyses for homogeneity and stability indicated that the dose preparations were homogeneous and stable for at least six weeks. Analyses for achieved concentration demonstrated that the mean prepared dietary admixture concentrations were within ± 5 % of the nominal concentration for all but exception of one low dose sample, which was + 10 % of the nominal concentration. There were no treatment related effects on the number of mortalities observed and no significant differences

EPA-HQ-2018-002024_0004869

in the rate of death during the course of the study were observed. There were no significant treatment related clinical observations reported during the course of the study. There were no treatment related effects on body weights, body weight gains, food consumption and water consumption during the study. There were no significant differences in proportion of white cell populations of either sex when observed at both 12 and 18 months. There were no trends in the proportion of palpable masses observed during the study period. There were no treatment-related macroscopic findings observed for any mice sacrificed at termination or mice that died or were killed in extremis during the study period. There were no treatment-related findings observed in organ weights or relative organ weights. There appears to be a dose-related increase in lymphomas in the male mice (but not female mice). This increase is attributed by the authors to an unusually low incidence in the controls (and presumably low dose mice). The historical control value lymphomas in CD-1 mice from the Charles River is 4.5%. The observed increase appears to be well within the historical range so the increase would not appear to be biologically significant. Therefore, it is concluded that were no treatment-related histopathological findings observed in any dose group of either sex.

In conclusion, the NOAEL for carcinogenicity and systemic toxicity in mice is 5000 ppm (equal to 810 and 1081.2 mg/kg bw per day in male and female mice, respectively); the highest dose tested (Wood et al. 2009a).

Rats

Study 1:

In a combined chronic toxicity and carcinogenicity study, groups of Sprague-Dawley rats (50/sex/dose) were fed diets containing glyphosate (purity 98.7%) at concentrations of 0, 30, 100 or 300 ppm for the first week. These concentrations were adjusted during the course of the study so that actual doses of 0, 3.05, 10.30, and 31.49 mg/kg bw per day in males and 0, 3.37, 11.22, and 34.02 mg/kg bw per day in females were maintained for approximately 26 months. Diets were analyzed for stability, homogeneity and dietary concentrations periodically. All rats were observed twice daily for mortality and toxic signs. Body weights and food consumption were determined at pre-test, weekly for 14 weeks and bi-weekly thereafter. Water consumption was determined for 10 rats/sex/group for two separate three-day periods at 18 and 24 months. Blood and urine samples were collected at months 4, 8, 12, 18 and 24 from 10 rats/sex/group. Selected haematological and clinical chemistry parameters were evaluated. Complete necropsies were performed on all rats that died or were sacrificed during or at the end of the study. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, testes/ovaries, pituitary, spleen and thyroid. The tissues were preserved for histopathology.

This study was conducted prior to the establishment of GLP requirements. The prepared diets were stable for one week and homogeneously distributed. There was no significant difference between the control and treated groups of both sexes with regard to the survival rate during the course of this study. Survival was approximately 80-90% through Month 20 of the study for all groups. No clinical observations attributable to substance administration were reported in any of the treated groups. Although statistically significant differences in mean food consumption were occasionally noted in the treated groups relative to the controls, these differences occurred sporadically and there was no dose-effect relationship. Water consumption of the treated groups was similar to that of the controls at the 18 and 24-month intervals. During the intermediate months of this study, the mean body weights of the treated animals were slightly lower than the controls. The maximum body weight reductions for males ranged from 6% in the high-dose group to 2-3% in the low-dose group. For females these differences were statistically significant only during months 20 and 21 and they were not dose-related. From month 24 until study termination the mean body weights of all treated groups were comparable to the controls. Haematology, blood biochemistry and urinalysis parameters deviated occasionally and some of them were statistically significantly different from controls. These differences were not dose-related and not consistent over time or between sexes. No statistically significant differences were noted in the terminal absolute and relative organ weights of the treated groups when compared to the controls. The few inter-group differences were neither dose-related nor consistent. Gross observations at necropsy were similar in incidence between treatment groups and

controls of both sexes. These lesions consisted primarily of inflammatory and structural changes that are commonl y found in rats of this strain in lifetime studies. The incidence and severity of the microscopic lesions were similar between the treatment groups and controls of both sexes. The most frequently observed changes occurred in the lungs and the kidneys, an d were associated with chronic respiratory disease and chronic progressive nephropathy. Both lesions are a common age -related disease in this strain of rats.

A variety of neoplasms were found in both control and treated animals. The most common tumours were found in the pituitary of both sexes and in the mammary glands of the females. The incidence of all tumour -bearing animals in the treated groups and the controls were similar and did not exhibit any dose-effect relationship.

Table 32 A summary of interstitial cell tumours finding in testes of rats after 26 -months dietary exposure to glyphosate and historical control data

Group	Controls (0)	3.05 mg/kg bw per day	10.3 mg/kg bw per day	31.49 mg/kg bw per day		
Interstitial Cell Tumours of the Testes						
Terminal sacrifice	0/15 (0%)	2/26 (7.7%)	1/16 (6.3%)	4/26 (15.4%)		
All animals	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50 (12%)		
Recent Historical Control Data						
Study no.	1	2	3	4	5	6
Terminal Sacrifice	4/65 (6.2%)	3/11 (27.3%)	3/26 (11.5%)	3/24 (12.5%)	3/40 (7.5%)	6/60 (10%)[a]
All animals	4/116 (3.4%)	5/75 (6.6%)	4/113 (3.5%)	6/113 (5.3%)	5/118 (4.2%)	

Combined historical control data for 5 studies are 16/166 (terminal sacrifice) and 24/535 (al1 animals).
[a] Prejean, J.D., et al. (1973). Spontaneous tumors in Sprague-Dawley rats and Swiss mice. Cancer Res. 33(11):2768-73.
*number of animals affected / total number of animals examined
( ): Percentage

The incidence of interstitial cell tumours in the testes was increased in the treated animals when compared to the controls (1 2% at the highest dose at terminal sacrifice). The increased incidence of interstitial tumours in males rats were not considered as treatment -related based on the following weight of evidence considerations: 1) lack of monotonic dose -response; 2) absence of pre -neoplastic lesions (i.e., interstitial cell hyperplasia); 3) the incidences were within the normal biological variation seen for this tumour type in this strain of rats; 4) the incidences in the concurrent controls (0%) was not representative of the normal background incidences noted in the historical control animals and 5) no interstitial cell tumours were seen when tested at much higher doses in the same strain of rats in an another study of glyphosate (Stout and Ruecker, 1990).

In conclusion, the NOAEL for systemic toxicity in rats after 26 months dietary exposure to glyphosate was 31.5 mg/kg bw per day, the highest dose tested. It was concluded that the glyphosate was not carcinogenic in rats (Lankas, 1981).

STUDY 2

In a 24 -month combined chronic toxicity and carcinogenicity study groups of Sprague -Dawley rats received daily dietary doses of 0 (Group 0: basal diet, no vehicle; 60 rats/sex), 0 (Group 1: basal diet plus vehicle; 80 rats/sex), 100 (Group 2: 80 rats/sex), 500 (Gro up 3: 80 rats/sex), and 1000 (Group 4: 90 rats/sex) ppm of active ingredient (0, 178, 890 and 1779 ppm technical trimesium salt of glyphosate (Trimethylsulfonium carboxymethyl -aminomethylphosphonate , company code SC-0224; sulfosate, glyphosate trimethyl s ulfonium salt). Average dose levels for the 2 -year treatment period, based on the nominal concentrations of active ingredient, were 4.2, 21.2 and 41.8 mg/kg bw per day for males and 5.4, 27.0, and 55.7 mg/kg bw per day for females.

EPA-HQ-2018-002024_0004871

Interim sacrifices were as follows: Group 0: 10 rats/sex at 12 months; Groups 1, 2 and 3: 10 rats/sex at 6, 12 and 18 months; Group 4: 10 rats/sex at 6 and 18 months; 20 rats/sex at 12 months. All surviving rats in all groups were sacrificed at 24 months.
The only indication of toxicity was a significant reduction in growth in both sexes in Group 4 (1000 ppm). The test compound at the doses tested did not cause treatment or dose-related effects involving survival, histopathological changes, or any indications of carcinogenicity. Although various common tumor types were found in both sexes, the majority were pituitary and mammary gland adenomas, and adrenal pheochromocytomas, and these occurred at comparable incidences in treated groups and their controls.
The NOAEL for systemic toxicity is 500 ppm (equal to 21.2 mg/kg bw per day based on the significant reduction in growth at 1000 ppm in both sexes. There was no evidence of carcinogenicity in this study (Pavkov and Wyand, 1987).

Study 3:

In a combined chronic toxicity and ca rcinogenicity study, groups of Sprague -Dawley rats (60/sex/dose) were fed diets containing glyphosate (purity 96.5%) at dietary concentrations of 0, 2000, 8000 or 20,000 ppm 24 months. These levels were equal to 0, 89, 362 or 940 mg/kg bw per day for the m ales and 0, 113, 457 or 1183 mg/kg bw per day for the females. An interim sacrifice was conducted on 10 rats/sex/dose at 12 months. Prepared diets were analyzed for stability, homogeneity and dietary concentrations. All animals were observed twice daily fo r mortality and moribundity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week for the first 13 weeks and then every fourth week thereafter. Ophthalmic examinations we re performed at pre -test and just prior to terminal sacrifice. Haematology, blood biochemistry and urinalysis determinations were conducted on 10 animals/sex/dose each at months 6, 12 (interim sacrifice), 18, and 24 (study termination). Ten animals/sex/dose were sacrificed at month 12, and all survivors were sacrificed at month 24. All animals were given a complete gross necropsy. Brain, kidneys, liver and testes with epididymides were weighed. Approximately 40 tissues were preserved and examined microscopically.
Stability analyses indicated that the neat test material was stable throughout the study. The stability and homogeneity of the diet mixtures were determined to be adequate. Analyses to verify dietary concentrations demonstrated that average glyphosate concentrations were 95% of target levels for all dose groups. There were no statistically significant differences in -group survival rates. At the end of the study, the percentages of animals surviving at 0, 2000, 8000, and 20000 ppm were 29, 38, 34, and 34 for males, respectively, and 44, 44, 34, and 36 for females. Various clinical signs were noted throughout the study. However, they were typical of those frequently observed in chronic studies and appeared to be randomly distributed in all groups. Therefore, none were considered to be related to administration of the test material. Statistically significant reductions in body weight were noted in high -dose females from week 7 through approximately the twentieth month. During this time, absolute body weights gradually decreased to 14% below the control value. Body weight gain in high dose females was also consistently reduced compared to control. At the point of maximum body weight depression (20 months), cumulative body weight gain was 23% less than control. Body weight gain in all treated male groups was comparable to controls. There were no statistically significant decreases in food consumption in either sex at any time in the study; significant increases were noted frequently in high dose males.
The ophthalmic examination prior to study termination revealed a statistically significant difference ($p<0.05$) between the incidences of control and high -level males displaying cataractous lens changes (0/15 vs. 5/20). This incidence (25%) was within the range (0 to 33%) observed in previously conducted studies at this laboratory with male CD rats. 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 independent pathologist's examination also revealed a statistically significant increase ($p<0.05$) in cataractous lens changes in high -dose male animals (1/14 control vs. 8/19 high dose) and concluded that there appeared to be a treatment-related occurrence of lens changes affecting high-dose males. Histopathological examination of the eyes by the Monsanto histopathologist revealed the incidences of cataract and/or lens fibre degeneration as shown in Table33. Due to the

small number of rats examined ophthalmologically and affected at termination, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes in high -dose males appears to be exacerbated by treatment.

Table 33: Incidences of cataract and lens fibre degeneration in male rats

	Dose (ppm in diet)*			
	0	**2,000**	**8,000**	**20,000**
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

* Number of rats affected / number of rats examined

There were various changes in haematology and serum chemistry param eters. However, the changes were not consistently noted at more than one time point, were within historical control ranges, small in magnitude, and/or did not occur in a dose -related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically insignificant. There was a statistically significant increase in urine specific gravity in high -dose males at the 6 months. Statistically significant reductions in urine pH were also noted in high -dose males at months 6, 18, and 24 . This may have been related to the renal excretion of glyphosate, which is an acid. Statistically significant increases in liver weight were noted in high -dose males: liver -to-body weight ratio at 12 months, absolute liver weight and liver -to-brain weight ratio at 24 months. There were no other statistically significant changes in organ weights, which occurred in a dose -related manner. Gross abnormalities observed at necropsy were not considered related to glyphosate administration. Histopathological examination revealed an increase in the number of mid -dose females displaying inflammation of the stomach squamous mucosa. This was the only statistically significant occurrence of non -neoplastic lesions. The incidences of this lesion in all groups of anim als are shown in Table 34. Although the incidence (15%) of this lesion in mid-dose females was slightly outside the historical control range (0 to 13.3%) for the laboratory, there was no dose -related trend across all groups of treated females and there was no significant difference in any male group. Therefore, the finding was not considered treatment-related.

Table 34: Incidence of inflammation of the stomach squamous mucosa in the rat

	Concentration in the diet (ppm)			
	0	**2,000**	**8,000**	**20,000**
Males	2/58	3/58	5/59	7/59
Females	0/59	3/60	9/60**	6/59

** $p \leq 0.01$; Fisher Exact Test with Bonferroni inequality

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, shown in Table 35. The historical control range for this tumour at the testing laboratory is 1.8 to 8.5%, but a partial review of studies reported in the literature revealed a prevalence of 0 to 17% in control m ales 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. This indicates th at the distribution of incidences in the four groups was most likely random. The authors also noted that there was considerable inter -group 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). There was no evidence of dose -related pancreatic damage or pre - neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a control male, thus indicating a lack of treatment -induced neoplas tic 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.

EPA-HQ-2018-002024_0004873

Table 35: Incidence of pancreatic islet cell findings

Finding	Sex	Dose Group in ppm*			
		0	2000	8000	20000
Hyperplasia	Males	2/58 (3%)	0/57 (0%)	4/60 (7%)	2/59 (3%)
	Females	4/60 (7%)	1/60 (2%)	1/60 (2%)	0/59 (0%)
Adenoma	Males	1/58 (2%)	8/57** (14%)	5/60 (8%)	7/59 (12%)
	Females	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)
Carcinoma	Males	1/58 (2%)	0/57 (0%)	0/60 (0%)	0/59 (0%)
	Females	0/60 (0%)[a]	0/60 (0%)	0/60 (0%)	0/59 (0%)
Adenoma Carcinoma Combined	Males	2/58 (3%)	8/57 (14%)	5/60 (8%)	7/59 (12%)
	Females	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)

* Number of rats affected / number of rats examined
** Statistically significant at p≤ 0.01 (Fisher exact test with Bonferroni inequality)

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 36). These tumours were not considered to be treatment related since 1) the incidences for these tumours were within the historical control range (1-18%) for the testing facility; 2) absence of pre-neoplastic lesions (i.e., cell hyperplasia or preneoplastic foci); and 3) there was no evidence of progression to malignancy (adenoma to carcinoma).

Table 36: Incidence of hepatocellular tumours in males

Finding	Concentration in the diet (ppm)			
	0	2000	8000	20000
Adenoma	2/44[#*] (5%)	2/45 (4%)	3/49 (6%)	7/48 (15%)
Carcinoma	3/44 (7%)	2/45 (4%)	1/49 (2%)	2/48 (4%)
Adenoma Carcinoma Combined	5/44 (11%)	4/45 (9%)	4/49 (8%)	9/48 (19%)

\# Number of rats affected / number of animals examined, excluding those that died or were sacrificed prior to study week 55.
* Statistically significant at p<0.05 (Cochran-Armitage Trend Test)

An increased incidence of thyroid C -cell adenomas was observed in the 8000 and 20000 ppm dose group in both sexes but did not reach statistical significance compared to the co ntrol animals. There was a statistically significant dose trend for C -cell adenomas and adenomas/carcinomas combined in females as shown in Table 37. The historical control range in the testing laboratory 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% and 0 – 2.9% in males and females, respectively. These tumours are not considered to be related to treatment because: 1) the increased incidences in males were not statistically signific ant; 2) there was no evidence of a progression from adenoma to carcinoma; 3) and there were no dose-related increases in the incidence or severity of preneoplastic lesions (hyperplasia).

Table 37: Incidence of thyroid C-cell tumours in male and females.

Finding	Sex	Concentration in the diet (ppm)			
		0	2000	8000	20000
Adenoma	Males	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
	Females	2/57* (4%)	2/60 (3%)	6/59 (10%)	6/55 (11%)
Carcinoma	Males	0/54 (0%)	2/55[c] (4%)	0/58 (0%)	1/58 (2%)
	Females	0/57 (0%)	0/60 (0%)	1/59[c] (2%)	0/55 (0%)
Adenoma Carcinoma Combined	Males	2/54 (4%)	6/55 (11%)	8/58 (14%)	8/58 (14%)
	Females	2/57* (4%)	2/60 (3%)	7/59 (12%)	6/55 (11%)

Number of rats affected / number of animals examined, excluding those that died or were sacrificed prior to study week 55.

* Statistically significant at $p<0.05$ (Cochran-Armitage Trend Test)

In conclusion, the NOAEL in this study in rats is 8000 ppm; equal to 362 mg/kg bw per day based on decreased in body weight gains in females and catarac tous lens changes in males seen at the LOAEL of 20000 ppm; equal to 940 mg/kg bw per day. It is concluded that glyphosate is not carcinogenic in rats (Strout and Ruecker, 1990).

Study 4:

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 98.9 and 98.7% , two batches) was administered to 85 Sprague -Dawley rats/sex/dose in the diet for 104 weeks in amounts that varied in concentration to deliver 0, 10, 100, 300, and 1000 mg/kg bw per day to both sexes over the course of the study. Designated for the toxicity portion of the study were 35 rats/sex/dose with the remainder designated for the oncogenicity portion of the study. An interim sacrifice was conducted on 15 rats/sex/dose after 52 weeks of glyphosate administration. Animals were in spected twice daily for signs of toxicity and mortality. Clinical examinations were conducted on all animals prior to initiation of the study, and weekly during the study. Palpitation for tissue masses was included. Animals were weighed weekly during weeks 1-13 and once monthly thereafter. Food consumption was measured by cage weekly during weeks 1 -13 and once monthly thereafter. Water consumption was monitored by visual inspection throughout the treatment period. An ophthalmoscopic examination was carried out on 20 males and 20 females from each group designated as the oncogenicity study before treatment commenced and on 20 males and 20 females from the Control and High dose designated oncogenicity study groups at Weeks 25 and 51. In addition all Control an d High dose oncogenicity and toxicity study rats were examined at Week 102. Blood was collected from the retro -orbital sinus of fasted animals for haematology and clinical chemistry while under light ether anesthesia. Samples were obtained from 10 animals/ sex/group in the toxicity study at 14, 25, 51, 78, and 102 weeks. Urine samples were obtained from 10 animals/sex/group at 14, 26, and 53 weeks, and from 10 animals/sex/group in the toxicity study at 14, 25, 51, 78, and 102 weeks. Fifteen males and 15 females from each toxicity study group were killed and necropsied after 52 weeks. All remaining toxicity study and surviving oncogenicity study animals were killed and necropsied after 104 weeks. All premature decedents were also necropsied. Selected organs we re weighed from all interim kill animals and 10 males and 10 females from the oncogenicity study. All collected tissues were examined microscopically on all decedents prior to Week 52, those sacrificed at 52 weeks, and the control and high -dose animals sac rificed at the end of the study. Only the salivary glands were examined on the decedents after 52 weeks and the rats from the other dose groups at terminal sacrifice.

Pale faeces were observed during weeks 16-104 in both sexes at the high dose and in fema les from the low -mid and high -mid dose levels. This sign was not considered to be toxicologically significant. There were no statistically significant differences between each group receiving glyphosate and the control group, in either sex in survival rate s. No treatment -related effect was observed in food consumption, water consumption and haematology, ophthalmoscopic examinations and gross pathology data. Males from the high -dose group had statistically lower mean body weight ($p \leq 0.01$) by 5% to 11% beginni ng Week 2 of the study until Week 104, and at termination was 10% lower (-14% weight gain). Females at the high dose had statistically lower body weight ($p \leq 0.05$) by 5% to 12% beginning Week 20 through Week 80 (with several exceptions), and at termination w as 8% lower (-11% weight gain). Statistically significantly increased alkaline phosphatase activity (ALP) activities (+46% to +72%) were observed in males at the high dose throughout the study except for the 51 week interval when the mean value was 31% higher than control. Elevated ALP activities were observed in females at the high dose (+34% to +53%) throughout the study, and through most of the study at the high -mid dose by +20% to +67%, though not always statistically significant. These changes in the A LP activity are considered as doubtful toxicological significance. Urinalysis data showed reduced pH (5.5-6) in males at the high dose throughout the study.

The absolute liver weight was decreased statistically significantly in females at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight the difference was statistically significant at all three doses. While in males, the absolute liver weight was decreased significantly at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight the difference was not statistically significant. The parotid salivary gland weight was increased significantly in males at 100, 300 and 1000 mg/kg bw per day (56 -111%) after 52 weeks, but not after 104 weeks. The combined weight of the sublingual and submaxillary salivary glands was significantly increased by 13% (22% after correcting for body weight) at 1000 mg/kg bw per day after 52 weeks. In females, the parotid gland was not affected but the sublingu al and submaxillary combined weight was significantly higher by about 15%. The changes in salivary gland weights were accompanied by increased incidence of mild to severe parotid salivary gland cell alterations and slight to moderate mandibular salivary gland cell alterations were observed in both sexes at the 52-week and 104-week intervals. The lesions were described as cells and/or acini that appeared larger and stained in a weakly basophilic manner without showing a tendency toward proliferative or degen erative changes over time. In males, the increased incidence and severity of lesions in the parotid gland were significant ($p \leq 0.01$) at 100, 300, and 1000 mg/kg bw per day at 52 weeks, and significant at 300 and 1000 mg/kg bw per day at 104 weeks. The incre ased incidence of lesions in the mandibular gland were significant at 300 and 1000 mg/kg bw per day at 52 weeks and significant ($p \leq 0.001$) at 100, 300, and 1000 mg/kg bw per day at 104 weeks. In females, the increased incidence of parotid lesions was significant at 300 and 1000 mg/kg bw per day at 52 weeks, and significant at 100, 300, and 1000 mg/kg bw per day at 104 weeks. The increased incidence in the mandibular gland lesions was significant at the high dose at both 52 and 104 weeks. The incidence and/or severity of kidney nephropathy decreased in males at 100, 300, and 1000 mg/kg bw per day at 52 weeks and at the high dose at 104 weeks. Urothelial hyperplasia significantly decreased in females from the high dose group at both the 52-week and 104-week intervals.

All groups had neoplastic lesions, however, No treatment -related neoplastic lesions were observed in male or female rats when histopathology data from treated groups were compared to that of controls at terminal sacrifice (104 weeks).

In conclusion, the LOAEL in male and female Sprague -Dawley rats administered glyphosate for 104 weeks in the diet was 100 mg/kg bw per day based on microscopic lesions in the parotid and mandibular salivary glands. The NOAEL was 10 mg/kg bw per day . There was no treat ment-related increase in tumour incidence at doses up to 1000 mg/kg bw per day (Atkinson et al. 1993b).

Study 5:

In a chronic study in rats, groups of 24 male and 24 female Alpk:APfSD (Wistar -derived) rats were given diets containing glyphosate (puri ty, 95.6%) at a concentration of 0, 2000, 8000 or 20000 ppm (equal to 0, 141, 560, and 1409 mg/kg bw per day for males and 0, 167, 671, and 1664 mg/kg bw per day for females) for 1 year. Analysis of diets showed that the achieved concentrations,

homogeneity and stability were satisfactory throughout the study. The animals were monitored daily for mortality and clinical observations. Body weights and food consumption were measured and at the end of the scheduled treatment period, the rats were killed and subjected to a full examination post mortem. Blood and urine samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no unscheduled deaths during the co urse of the study that could be attributed to the administration of glyphosate. Apart from a small increase in the number of male and female animals in the group receiving glyphosate at 20000 ppm that showed wet or dry urinary staining, there were no other treatment-related clinical observations and no treatment -related ophthalmological findings. Bodyweights of top dose animals were lower than concurrent controls throughout the study (Table 50). Bodyweights of animals receiving 8000 ppm glyphosate were sli ghtly reduced (not significantly in males and significantly only from week 46 in females). There was no effect on bodyweight in animals receiving 2000 ppm glyphosate. The changes in body weights in males and females were not considered biologically significant since the magnitude of change small (less than 10%). (PREVIOUS JMPR CONSIDERED ADVERSE AT 8000 and 20,000 PPM)

Table 38: Inter-group comparison of mean body weight (selected time points)

Weeks	Dietary concentration of glyphosate (ppm)							
	Males				Females			
	0 (control)	2000	8000	20000	0 (control)	2000	8000	20000
2	203.3	202.6	203.1	198.1**	158.0	156.5	155.6	151.7**
10	443.2	445.9	431.7	412.8**	256.2	252.5	251.1	248.3*
30	599.2	593.1	577.3	568.2*	305.6	306.0	298.3	291.8**
46	643.1	640.1	629.4	620.9	338.6	333.5	321.9*	322.1*
52	649.1	642.7	639.4	633.0	349.7	338.6	330.4*	327.3**

** Statistically significant difference from control group mean, 1% level (Student's t-test, 2 sided)
* Statistically significant difference from control group mean, 5% level (Student's t-test, 2 sided)

Food consumption was lower and food utilization was slightly less efficient at 20000 ppm, the reductions being most marked at the start of the study. There was a trend for reduced food intake for females at 8000 ppm, which correlates with the reduction in body-weight gain at this dose in the latter stages of the study.

Table 39: Selected clinical chemistry findings in rats given diets containing glyphosate for 1 year

Parameter/week	Dietary Concentration of Glyphosate Acid (ppm)							
	Males				Females			
	0 Control	2000	8000	20000	0 Control	2000	8000	20000
Cholesterol/14	2.46	2.53	2.31	2.28*	2.13	2.28	2.26	2.21
27	3.09	3.05	2.75*	2.70**	2.62	2.67	2.76	2.78
Triglycerides/14	1.56	1.63	1.28**	1.28**	0.94	0.92	0.89	0.95
27	1.51	1.43	1.15**	0.97**	1.07	1.10	1.13	1.10
Alkaline Phosphatase/14	248	281	342**	429**	161	201*	227**	292**
27	221	250	306**	412**	135	171	200**	254**
53	232	258	291**	379**	87	100	114	160**
Aspartate Aminotransferase/ 27	120	105.3	108.4	113.3	157.1	130.4	116.9*	132.8
53	122.1	123.0	118.3	132.0	133.9	178.7*	198.3**	138.3
Alanine Aminotransferase/14	84.3	92.8	110.9**	109.6**	66.2	79.3	88.2**	90.5**
Creatine Kinase/14	118.2	123.5	127.3	143.7**	96.7	107.5	107.3	124.1**

** Statistically significant difference from control group mean, 1% level (Student's t-test, 2 sided)
* Statistically significant difference from control group mean, 5% level (Student's t-test, 2 sided)
From Milburn (1996)

Some statistically significant differences from control in haematological parameters were seen but there was no evidence of a relationship to dose, the differences were small and not seen consistently at all time points, therefore they were considered to be unrelated to the administration of glyphosate.

Deviations in some clinical chemistry parameters, such as reductions in plasma concentration of cholesterol and triglycerides or a dose-related increase in plasma ALP activity throughout the study as well as occasional increases in the activities of plasma AST, ALT and creatine kinase, were mostly confined to groups receiving the high and intermediate doses (Table 39). In the absence of any histopathological findings these marginal changes are not considered to be of toxicological significance. There was no evidence of any effect of glyphosate on urine parameters. There were no findings at examination post mortem related to treatment. There were no treatment-related effects on organ weights.

At necropsy, there were no gross pathological findings that could be attributed to treatment and no consistent organ weight changes. An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland were seen in both sexes receiving 20000 ppm (Table 40). At 8000 ppm, examples of focal parotid basophilia were of minimal severity and the incidence was slightly above to that in the control animals. No other microscopic findings could be ascribed to administration of glyphosate.

Table 40: Incidence of focal basophilia of parotid acinar cells in rats given diets containing glyphosate for 1 year

Severity	Dietary concentration of glyphosate (ppm)							
	Males				Females			
	0 (control)	2000	8000	20000	0 (control)	2000	8000	20000
Minimal	2	0	3	10	2	0	6	8
Slight	0	0	0	3	0	0	0	5
Moderate	0	0	0	0	0	0	0	2

Similar numbers and types of neoplasms were diagnosed in the control group and in the group receiving glyphosate at 20000ppm, but the duration of the study was not sufficiently long to enable final conclusions to be made with regard to carcinogenicity.

In conclusion, the NOAEL in the 1-year toxicity study in rats was 8000 ppm; equal to 560 mg/kg bw per day based on the increased incidence of basophilia of parotid acinar cells seen at 20000 ppm; equal to 1409 mg/kg bw per day (Milburn, 1996).

Study 6:

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 96.8 and 90.0%, two batches) was administered to 50 Wistar rats/sex/dose in the diet for up to 2 years at a concentration of 0, 100, 1000, or 10,000 ppm (equal to 0, 6.3, 59.4 or 595.2 mg/kg bw per day for males and 0, 8.6, 88.5, or 886 mg/kg bw per day for females). In additional one vehicle control (acetone) with ten rats per sex and one high dose group with 20 rats per sex were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. Veterinary examination was made before and after grouping and at the end of each month of experimental schedule. Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Food consumption was recorded once weekly for each cage group from Week 1 to Week 13 and subsequently over one week in every 4 weeks until termination. Individual blood samples were collected from 20 rats/sex/group at 3, 6, 12, 18 and 24 months. At the scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex/group was subjected to clinical chemistry analysis. Individual urine samples were collected from 10 rats/sex/group at 3, 6, 12, 18 and 24 months. Histopathological examination was carried out on all tissues collected at interim sacrifice, control and high dose groups; all pre-terminally dead and moribund sacrificed rats of the low and mid dose groups and on all lesions of the terminally sacrificed rats from the low and mid dose groups. Selected organs were weighed from 10 rats/sex/dose. A detailed histopathological examination was performed on all sampled tissues of the control and high-dose animals, and on animals that died or were killed in extremis. In addition, gross lesions and masses from low and intermediate dose groups at termination were examined microscopically.

The stability of glyphosate was determined at 2000 and 2000 ppm which demonstrate that prepared diets were fairly stable for 30 days at room temperature with a loss of less than 7%. The analysis of diets indicated that the achieved concentrations were within acceptable range. There were no treatment related effects on mortality, clinical observations, body weights, body weight gains, food consumption, urinalysis and haematology. The following significant dose related changes of the blood chemistry parameters were seen at the high dose: a decrease in GGT level at 12 months in male rats, a decrease in albumin level at 6 months in female rats and increase in AP (alkaline phosphatase) level at 6, 12 and 18 months in female rats. The increase AP in high dose females were 235, 231, 194, and 249 (U/L) at 6, 12, 18 and 24 months, respectively, compared to corresponding control value of 133, 141, 101, 254 for females at 6, 12, 18 and 24 months, respectively.

There were no treatment-related macroscopic findings observed during the study period. There were no treatment-related findings observed in organ weights or relative organ weights. None of the significant microscopic changes, both increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) observed showed dose relationships, hence appeared to be incidental and not related to the treatment with the test compound. At terminal sacrifice, the incidence of cataracts in males were 3/20, 3/20, 1/18 and 6/29 at 0, 100, 1000 and 10,000 ppm, respectively. At terminal sacrifice, the incidence of cataracts in females were 1/24, 1/26, 5/33 and 4/21 at 0, 100, 1000 and 10,000 ppm, respectively. The historical data on neoplasm incidence for the test species indicates that the incidences of various tumours observed in the present study are within the range. The types of tumours seen were also comparable to the historical records. No statistically significant inter group difference between the control and low, mid and high dose treatment groups has been recorded in respect of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sex-wise or for combined sex.

In conclusion, the NOAEL in this carcinogenicity study in rats is 10,000 ppm; equal to 595.2 mg/kg bw per day; the highest dose tested. There was no evidence of carcinogenicity in rats at glyphosate doses up to 10,000 ppm (Suresh, 1996).

Study 7.

In a combined chronic toxicity and carcinogenicity study, groups of 50 Sprague Dawley rats per sex received daily dietary doses of 0, 3000, 15000 and 25000 ppm (0, 0.15/0.21, 0.78/1.06 and 1.29/1.74 g/kg/day [M/F]) Glyphosate technical for two years. In addition, for the control and each dose group 20 rats per sex included for interim sacrifice in Week 52 to study non-neoplastic histopathological changes (chronic toxicity study). Selected dose levels were the same except for the highest dose which was 30000 ppm. Here the dietary doses correspond to 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively. Test diets were prepared weekly by mixing appropriate amounts of the test substance with the basal diet. The stability and homogeneity of the test substance in food was determined in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

No treatment-related clinical signs or deaths were observed in the satellite groups, e.g. the chronic toxicity study. In the carcinogenicity study, e.g. after 104 weeks, male animals of the high dose group exhibited slight but statistically insignificant higher mortalities. No significant toxic signs were observed in treated or control groups. Significantly reduced body weight gain that lasted throughout study until Week 104 was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination. There were no treatment-related effects on food consumption for either sex or group noted during the study. The results show a higher test material intake for females when compared to males for each dose level. The mean intake in the chronic toxicity study for each dose group is 0.18, 0.92 and 1.92 g/kg bw/day (males) and 0.24, 1.13 and 2.54 g/kg bw/day (females) for 3000, 15000 and 30000 ppm, respectively. The mean intake in the carcinogenicity study for each dose group is 0.15, 0.78 and 1.29 g/kg bw/day (males) and 0.21, 1.06 and 1.74 g/kg bw/day (females) for 3000, 15000 and 25000 ppm, respectively.

Ophthalmological examinations revealed no abnormalities. Haematological examination did not reveal any abnormalities attributable to the treatment. Regarding the clinical chemical

investigations, a significant increase in the alkaline phosphatase level was only seen in the high dose of the carcinogenicity study at study termination. Other significant changes observed in haematological, and biochemical parameters were within the range of the historical control data and hence appear to be of no biological significance. Urinalysis did not reveal any abnormalities attributable to the treatment. There were no tr eatment-related macroscopic findings observed during the study period.

Significant and dose-dependent effects in the chronic toxicity study were found in both sexes of the high-dose group. In males, weights of kidneys, brain and testes were increased. In females, in addition to kidneys and brain, the liver weight was increased as well.

Histopathological changes were found at all dose levels including control, hence it is concluded that these are no treatment -related effects. There were no t reatment-related neoplasms observed.

Based on mild toxic effects on body weight gain and the increased organ weights without histopathological changes, the NOAEL in rats after chronic exposure to Glyphosate technical for 24 months is 25,000 ppm (1290/1740 mg/kg bw per day [M/F]) (Bhinde, 1997).

Study 8:

In a combined chronic toxicity and carcinogenicity study groups of 50 Sprague -Dawley rats/sex/group received daily dietary doses of 0, 3000, 10000, and 30000 ppm (0, 104/115, 354/393 and 1127/1247 mg/kg bw per day [M/F]) HR-001. In addition, 30 rats/sex/group were included for interim sacrifices at 26, 52, and 78 weeks.

In the 3000 dose ppm group, significant increases in incidence of decreased spontaneous motor activity, bradypnea, and soiled fur and a si gnificant decrease in incidence of tactile hair loss were observed in males. Analysis of location of the soiled fur demonstrated predominant occurrences of the sign in the external genital region and foreleg. Females in this group showed significant increases in incidence of ptosis and tactile hair loss. In the 10000 ppm dose group, the incidence of tactile hair loss was significantly decreased in males and significantly increased in females when compared to the respective control.

In the 30000 dose group 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 in most of the weeks. In all other groups mortality was comparable to control . Significant increases in incidence of bradypnea, palpable masses, and soiled fur were observed in males when compared to the control. Analysis of location of each mass showed that the ones in the tail were present in 27 males, which was apparently high in incidence compared to 11 of the control. The incidences of masses in other locations were comparable to the control. With respect to soiled fur, the sign was located at the external genital or perianal region. Males in this group also showed significan t decreases in incidence of tactile hair loss, wound, and hair loss. In females, a significant increase in incidence of wetted fur was observed; the sign was mainly seen in the external genital region. Besides the signs mentioned above, loose stool was observed in all cages of this group from Week 24 in males and Week 23 in females until the end of the treatment.

The test compound at the doses tested did not cause treatment or dose related gross and histopathological changes and it is not carcinogenic under the testing conditions.

Based on the effects in this study, the no observable adverse effect level (NOAEL) is 3000 ppm (104/115 mg/kg bw per day) and the LOAEL is 10000 ppm (354/393 mg/kg bw per day) based upon clinical signs (an increase in tactile ha ir loss in females). There was an increased incidence of multiple clinical signs at 30000 ppm (Enomoto, 1997).

Study 9

In a combined chronic toxicity and carcinogenicity study, groups of Fischer F344/DuCrlCrlj rats (50/sex/dose) were fed diets contai ning glyphosate (purity 97.5%) at concentrations of 0, 500, 4000 or 32000 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 sacrifice was conducted on 14 rats/sex/dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet were determined. Clinical observations (including ophthalmoscopy), bodyweights, food 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 killed and subjected to a full examination post mortem. Blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

Prepared diets were stable a t room temperature for 4 months and the test compound was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations were ranged from 80 to 110% of the nominal concentrations. All males and females in the 32000 ppm group showed diarrhoea or soft stool from immediately after the start of administration almost throughout the administration period. Mortality was not affected by the treatment. Statistically significantly reduced body weights were observed throughout the study in high dose males (beginning week 1) and females (beginning week 2). Food consumption in all dosed group decreased or increased (no statistical significance) at various intervals. The only treatment related effects observed in urinalysis are increased urinary proteins in 3 females of the high dose group at 104 week. These changes were thought to be related to the histological changes in the kidney. There were no remarkable changes in males in any dose group, in females in any other dose g roup or at any other examination time. Males and females in the 32000 ppm group showed statistically significant decreases or tendencies toward decreases in erythrocyte count, haematocrit and haemoglobin concentration in Weeks 26, 52 and 78, and males in t his group also showed significant increases in platelet count and leukocyte count in Week 52 and a significant increase in platelet count in Week 78. In the 4000 ppm group, females showed a significant decrease in erythrocyte count in Week 26 (94% of the control value) and males showed significant decreases in erythrocyte count (96% of the control value) and haematocrit (95% of the control value) in Week 52. In males and females in the 500 ppm group, there were no significant differences from those of the 0 ppm group in any examination item. The historical control values for haematological parameters from the performing laboratory were not available, however, the historical control data for Fisher Inbred Strain F344/ DuCrlCrlj were compared with study result s. Through the study, except for at week 104, control group erythrocyte counts and haematocrit values are higher than the literature reported range for this strain of rats. This suggests that erythrocyte and haematology values for the control groups of the TAC study were unusually high, and that statistically significant decreases in test groups may not be toxicologically significant or relevant. Males and females in the 32000 ppm group showed tendencies toward decrease in albumin at each examination time, and the values were statistically significant in males and females in Week 26 and in males in Week 78 compared to those of the 0 ppm group. In addition, males in this group showed significant increases in γ -GTP, alkaline phosphatase and total bilirubin in Week 52. Otherwise the following changes were observed, but they were thought to be unrelated to administration of the test article since they were not observed continuously or they were not observed in the 32000 ppm group: significant decreases in creatin ine, GPT and total bilirubin in males or females in Week 26 in the 32000 ppm group; and significant increases in creatinine, total protein and albumin in females in the 500 ppm group. Ophthalmoscopic examination of the eyes did indicated treatment related effects opacity in 1 female at Week 104 at high dose, which was considered as incidental change. In the 32000 ppm group, a statistically significant increase in the weight of the kidney weight (relative to body weight) was observed in males in the Week 79 scheduled sacrifice group and in males and females in the Week 105 -106 scheduled sacrifice group. Otherwise the following changes were recorded, but they were thought to be changes due to suppressed body weight gain since there were no corresponding abnorm alities in histopathological examination: significant

EPA-HQ-2018-002024_0004881

increases in the relative weights of the brain and liver in males in the Week 79 and Week 105-106 scheduled sacrifice groups and females in the Week 105-106 scheduled sacrifice group and a significant decrease in the absolute weight of the adrenal in males in the Week 105-106 scheduled sacrifice group in the 32000 ppm group; and a significant decrease in the absolute weight of the brain in males in the Week 79 scheduled sacrifice group in the 4000 ppm group. At necropsy, males and females in the 32000 ppm group showed an increase in luminal dilatation of large intestine in the animals that were necropsied on schedule in Week 79 of administration, but there were no histological changes. Thymic involution was increased in the 32000 and 500 ppm groups in the data of all females. Pituitary hypertrophy was not recorded in the 32000 ppm group for all males. However, these effects were thought to be incidental changes since they are age-related changes and the incidence of the occurrence of each lesion was comparable.

In histopathological examination, an increase in glomerulosclerosis was observed in females in Week 105-106 scheduled necropsy group in the 4000 ppm group, and increases in eosinophilic granule/hyaline droplet in tubular epithelium in the kidney in females in Week 79 scheduled necropsy group and in males and females in Week 105-106 scheduled necropsy group, and an increase in glomerulosclerosis in females in Week 105-106 scheduled necropsy group in the 32000 ppm group. Monsanto and TAC, co-sponsored the Pathology Work Group (PWG) to re-evaluate the microscopic kidney findings, specifically glomerulosclerosis, chronic nephropathy and hyaline droplet renal tubule degeneration in female rats. The PWG concluded (Hardisty 2013 Hardisty J. F. 2013. PATHOLOGY WORKING GROUP REVIEW OF THE HISTOPATHOLOGIC CHANGES IN THE KIDNEY A COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDY OF AK-01 BULK SUBSTANCE [GLYPHOSATE] BY DIETARY ADMINISTRATION IN RATS. NIPPON EXPERIMENTAL MEDICAL RESEARCH INSTITUTE CO., LTD, STUDY NO: H-95053, EPL PROJECT NUMBER 911-004) that the kidneys of male and female rats from a 2-year oral (dietary) carcinogenicity study with glyphosate did not confirm the study pathologist's reported conclusions that the incidence of glomerulosclerosis and the presence of eosinophilic granules/hyaline droplets of renal tubule epithelium were related to test article administration. The PWG did not observe any histologic evidence of renal toxicity in the kidney sections examined. The only frequently observed finding in the kidneys of male and female rats was chronic progressive nephropathy which was similar in incidence and severity in control and treated groups. No treatment related tumours were observed in this study.

In conclusion, the NOAEL is 4000 ppm; equal to 201 mg/kg bw per day based on the decreased in body weights, transient haematological effects, diarrhoea, urine parameters, clinical chemistry effects, increased kidney weight relative to body weight seen at 32000 ppm; equal to 1750 mg/kg bw per day; the highest dose tested. It was not carcinogenic in rats at doses upto 32,000 ppm (Takahasi, 1999b).

Study 10:

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 97.6%) was administered to 64 Alpk:APfSD Wistar rats/sex/dose in the diet for up to 2 years at a concentration of 0, 2000, 6000, or 20,000 ppm (equal to 0, 121, 361, and 1214 mg/kg bw per day for males and 0, 145, 437, and 1498 mg/kg bw per day for females). An interim sacrifice was conducted on 12 rats/sex/dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet were determined. Clinical observations (including ophthalmoscopy), bodyweights, food 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 killed and subjected to a full examination post mortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

The mean achieved concentrations of glyphosate in each dietary preparation were within 10% of the nominal concentration and the overall mean concentrations were within 1% of nominal. The diets were homogenously distributed and prepared diets were stable at room temperature for 45 days. Survival in the control, low and mid-dose group males approached 25% by week 104 of the study

(criteria for termination of the study) although survival in the high -dose group was significantly better. Survival in the females was similar across all groups and better than in the lower dose group males. There was a treatment -related increase in the incidence of red -brown staining of tray papers (particularly in males), and isolated observations of red/brown coloure d urine noted in 3 males and 1 female fed 20000 ppm glyphosate. The body weights of the males and females fed 20000 ppm glyphosate were statistically significantly lower than controls throughout the study, however, they were not considered as toxicological ly relevant since maximum decreased in body weights were approximately 5 and 8% for males and females, respectively. Food consumption and food utilization was statistically significantly lower in high dose males and females. Ophthalmoscopic examination did not reveal any treatment related effects. There were no treatment-related observations noted in the functional observational battery, grip strength measurements, motor activity, landing foot splay measurements and time to tail flick. Haematological param eters were not affected by the treatment. There was a statistically significantly increases in alkaline phosphatase activity at all doses in both sexes up to week 79. There was evidence at one or more time points of increases in the activities of plasma al anine aminotransferase, aspartate aminotransferase and total bilirubin but statistically significant only at 6000 and/or 20000 ppm. In the absence of any histopathological findings these marginal changes are not considered to be of toxicological significan ce. Plasma triglycerides and cholesterol were consistently decreased for all or part of the study in males at 20000 ppm. Plasma creatinine values were lower in all treated female groups at week 27 and in females receiving 6000 and 20000 ppm at week 14, but in the absence of any effects later in the study, this is considered to be of no toxicological significance. Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate compared to controls. An increase in the incidence and severity of blo od/red blood cells was present in males and, to a lesser extent, in females fed 20000 ppm glyphosate. There were no consistent, dose -related effects on organ weights that were considered to be indicative of a toxicologically significant effect of glyphosate.

Macroscopic findings were seen in males fed 6000 ppm and/or 20000 ppm and consisted 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. A minor increase in the incidence but not severity of proliferative cholangitis in the liver was present in males fed 20000 ppm glyphosate at interim and terminal kill. Moreover, in males fed 20000 ppm glyphosate acid an increased incidence of hepatitis and periodontal inflammation was observed. There were a number of changes in the kidneys of both sexes fed 20000 ppm glyphosate, notably renal papillary necrosis, with or without papillary mineralisation, and transitional cell hyperplasia. The incidence was greater in males than females. These findings are considered to be related to treatment but are consistent with the feeding of high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation. T he decrease in the incidence of tubular degeneration of the testis in males fed 20000 ppm is considered to be without adverse consequence (Table 41). The incidence of prostatitis was higher than the control groups in all treated males but it was within his torical background levels in all treated groups but, as the control value in this study was low, the relationship to treatment at the high- dose level cannot be entirely dismissed.

Table 41- Inter-group comparison of selected microscopic findings

	Dietary concentration of glyphosate (ppm)							
	Males (N=64)				Females (n=64)			
Finding	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
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

EPA-HQ-2018-002024_0004883

In contrast to the previous one -year feeding study in r ats by Milburn (1996) microscopic changes were seen in the liver and kidneys but not the salivary glands of rats fed 20000 ppm glyphosate even though the study was conducted on the same strain of the rats and in the same laboratory.

There was an increase in the incidence of hepatocellular adenomas in male rats at the high dose when compared to controls (Table 42). This increase was not considered to be treatment -related due to: 1) absence of dose-response relationship; 2) 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 during the relevant time period (1984 –2003) at the testing laboratory; and 5) the 0% incidence in concurrent controls is lower than the average background incidence for liver adenomas in male Wistar rats.

Table 42. Incidence of hepatocellular adenomas in males rats.

Finding	Concentration in the diet (ppm)			
	0	2000	6000	20000
Adenoma	0/52#** (0%)	2/52 (4%)	0/52 (0%)	5/52* (10%)

Number of tumour-bearing animals/Number of animals examined.

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

Study 11:

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 95.7%) was administered to 51 Han Crl:WI (GLx/BRL/HAN) IGS BR Wistar rats/sex/dose in the diet for up to 104 weeks at a concentration of 0, 1500, 5000, or 15000 ppm (equivalent to mean achieved dose levels of, 0, 95.0, 316.9 or 1229.7 mg/kg bw per day). To ensure that a received dos e of 1000 mg/kg bw per day overall was achieved, the highest dose level was progressively increased to 24000 ppm. In addition, three satellite groups with 15 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes. The satellite control group with 12 rats per sex served as veterinary control. The animals were to be used for investigations should any health problems have developed with study animals. No such problems occurred and therefore the observations of these animals have not been included in the report. Clinical signs, functional observations, body weight development and food and water consumption were monitored during the study. Clinical chemistry and haematological examinations were pe rformed on ten animals per sex from the satellite and main groups at 3, 6 and 12 months. Further haematological and clinical chemistry investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. Urinalytical investigations were performed on ten animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months. Necropsy was conducted for all animals surviving until study termination (main groups: 104 weeks; satellite groups: 52 weeks) as we ll for all animals found dead or killed in extremis. Selected organs were weighed from 10 animals/sex/group that were killed at the end of the study and for all animals from satellite groups at termination. Histopathological examination was initially carri ed out on all tissues collected from control and high dose groups; all pre -terminally dead and moribund sacrificed rats and on all lesions and palpable masses of the terminally sacrificed rats from the low and mid dose groups. Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

Stability analysis demonstrated that the prepared diets were stable for at least six weeks. Analysis of the diet indicated that the achi eved dietary concentrations were within acceptable range. No significant treatment related effects were observed on mortality, clinical signs, behavioural assessments, functional performance tests (motor activity, grip strength values), sensory reactivity,

body weights, body weight gains, food consumption, water consumption, palpable masses, ophthalmoscopic examinations, haematology, clinical chemistry, urinalysis, organ weights, and macroscopic findings.

Adipose infiltration of the bone marrow was seen fo r the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, a generally greater effects were seen among male rats dosed at15000 ppm and this attained statistical significance for term inal kill animals. This data indicates the possibility of myeloid hypoplasia as a consequence of treatment for male rats at 15000 ppm. However, given the normal variability of this condition and the influence of other pathological conditions upon marrow ce llularity in ageing rats, the effect was not altogether convincing but cannot be dismissed. A similar effect was not seen among male rats in the remaining treatment groups. There was a higher incidence of higher grades of severity of adipose infiltration s een among premature deaths but among premature deaths for animals of both sexes at 5000 ppm and females only at 1500 ppm. However, the variable duration of exposure and significant background pathology for premature death animals further negates this as an effect of treatment upon marrow cellularity for female rats.

Moreover, at the highest dose level there was a significant difference in the site of mineral deposition within the kidneys compared with controls. Pelvic mineralisation was commonly seen in bot h sexes and was more prevalent among female rats; however corticomedullary mineralisation was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papill ary deposition and an increase in the corticomedullary deposition. At the same time there was a reduction in the incidence of renal pelvic hyperplasia in both sexes; which is considered to be a consequence of the decreased mineral deposition. The effects o n pelvic and corticomedullary mineralisation, and hyperplasia of the pelvic/papillary epithelium were confined to high dose animals with no indication of a similar effect at any other treatment level for either sex.

There was no influence of treatment up on the development of neoplasia in any organ or tissue, and no effect of treatment upon the overall frequency of benign or malignant tumours.

In conclusion, based on the study results the NOAEL in rats after chronic exposure to glyphosate technical for 24 month is 15000 ppm (equal to mean achieved dose level of 1229.7 mg/kg bw per day); the highest dose tested. Glyphosate was not carcinogenic in rats at doses up to and including 15,000 ppm; the highest dose tested (Wood et al. 2009b).

Published Studies

In a published study, the commercial formulation of the glyphosate Roundup Original® (glyphosate 41%, POEA ≅15% Monsanto Company, St. Louis, MO, USA) was used in a 2 -stage cancer model in Swiss mice to evaluate for tumor promotion via topical administration. A known tumor promoter, 12 -o-tetradecanoylphorbol-13-acetate (TPA) was used as a positive control and fo r comparison with glyphosate effects after exposure to a tumor initiator, 7, 12 - dimethylbenz[a]anthracene. Proteomic analysis using 2 -dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyp hosate, 7, 12 - dimethylbenz[a]anthracene (DMBA) and 12 -O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF -1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti -VEGF antibody, peroxiredoxin -2, superoxide dismutase [Cu –Zn], stefin A3, and calgranulin -B) were common and showed similar expression pattern in glyphosate and TPA -treated mouse skin. The study author concluded that glyphosate formulation has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA (George et al., 2010).

2.3 Genotoxicity

Genotoxicity Studies on Glyphosate, its Formulation Products, and Metabolites

EPA-HQ-2018-002024_0004885

Glyphosate and its formulation products have been extensively tested for genotoxic effects using a variety of endpoints in a wide range of organisms. These tests have ranged from standard, validated tests in bacteria and mammalian model organisms to less common and non-validated tests in phylogenetically distant species such as plants, earthworms, clams, frogs, tropical fish, and caiman. In these studies, the test compounds were administered through a variety of routes including parenteral routes used for specialized studies but considered to be largely irrelevant for assessing risks resulting from low-level dietary exposures. The reviewed studies for glyphosate and its metabolites are briefly summarized in the text and summary tables below. Summary tables of studies conducted in non-traditional or phylogenetically distant organisms are shown in Appendix XX. In addition, a number of studies were conducted of humans exposed occupationally or environmentally to glyphosate and/or its formulation products. Many of these involved co-exposures to many different pesticides and were considered uninformative. In a few, glyphosate was considered to be the major agent. These are summarized and briefly discussed below.

Human biomonitoring studies

Micronucleus (MN) frequencies in the peripheral blood lymphocytes of individuals living in three areas in Columbia where glyphosate formulations were sprayed, were evaluated over several months to investigate whether glyphosate exposure was associated with an increase in micronucleus frequencies, and to determine if these persisted over time (Bolognesi et al., 2009). Significant increases in MN were seen several days after spraying occurred. In one case, the induced MN frequencies decreased over time, in another they remained at the same level and in the third, they increased. The observed increases in MN frequencies did not correlate with glyphosate spray rates. In addition, in all three communities, the MN 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 MN 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.

In another study in which the Comet assay was used, the frequency of DNA strand breakage in the peripheral blood lymphocytes of individuals living in an Ecuadorian community at or within 3 km of where glyphosate had been sprayed was reported to be significantly higher than that of individuals living in a community 80 km away where glyphosate was not used (Paz-y-Mino et al., 2007). The samples of the exposed were collected 2 weeks to 2 months after the spraying had occurred. In examining the study, the 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 which is highly prone to technical artifacts during sample preparation. The two populations were located at considerable distance from each other and the background frequencies of DNA breakage in these and other non-exposed Ecuadorian or Columbian communities are also not known. After reviewing the study, 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 chromosome aberrations in peripheral blood lymphocytes was measured in study population that two years previously had been exposed to glyphosate and 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 ele vated but statistically non -significant increase (RR = 1.47) was reported.

Summary

Biomonitoring studies of DNA and chromosomal alterations were conducted in 5 -6 communities by several investigators. In no study was a convincing association between glyphosate exposure and genetic damage seen.

Conventional short-term *in vitro* tests

Bacteria:

Gene mutations, DNA damage and chromosomal alterations

Glyphosate or Roundup was tested in ~33 studies for mutagenicity in bacteria. Most studies were conducted with and without metabolic activation (S9). It was found to be negative in almost all of these with weak positive results being reported in one or two studies. The actual number of tests performed was well over one hundred and fifty as multiple tester st rains with and without S9 were used in most studies. Glyphosate was also reported to be negative in 3 assays measuring DNA repair (rec) in *B. subtillis* and positive in one SOS chromotest assay in *E. coli*. Several studies reported that glyphosate could enh ance DNA strand breaks in cyanobacteria caused by UV radiation.

Mammalian cells in vitro

Gene mutations, DNA damage and chromosomal alterations

Glyphosate or its formulation products were tested for various types of genetic damage in mammalian cells in vitro. The results are briefly summarized as follow. Four studies of gene mutation induced by glyphosate or its formulation product were conducted in mammalian cells in vitro. No increase was seen in any of the four. In contrast, nine of ten studies investigating DNA strand breaks induced by glyphosate or Roundup in mammalian cells were reported as showing positive results. Four of eleven studies of chromosome aberrations were reported as positive. The one reported result without S9 occurred at low con centrations – much lower than the studies reporting negative results. Two studies reported negative results for polyploidy. One study of the glyphosate formulation product Herbazed reported an induction of chromosome aberrations in mouse splenocytes in v itro (see further discussion of Herbazed below). Four of seven studies of MN were positive, 2 were negative and 1 was equivocal. Two of the positives required S9 whereas 2 did not. Seven of eight studies of sister chromatid exchanges induced in periphera l blood lymphocytes 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 u nscheduled DNA synthesis in rat hepatocytes were negative.

In vivo mammalian studies

Oral route

As shown in Table XX, thirty studies investigating chromosomal alterations or micronuclei, one measuring sister chromatid exchange, another unscheduled DNA synthesis, and another measuring dominant lethal mutations in duced by glyphosate or its formulation products were

EPA-HQ-2018-002024_0004887

performed using the oral route of exposure in rodents (29 in mice and four in rats). Fourteen of the studies were conducted using glyphosate (≥ 90% pure) and 19 involved formulation products. The results were negative for 29 of the 33 studies. The majority of the studies were considered good to acceptable quality, industry -sponsored GLP studies that were conducted in compliance with OECD Guideline 474. The four positive studies are briefly described. A small two-fold, but statistically significant, increase in MN was reported by Suresh (1993) in female (but not male) mice treated with two high 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 (1997) and the ICH (2011). The MN 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 3 other positive studies came from one article, a fairly obscure 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 7 or more doses of a glyphosate formulation product called Herbazed (other positive results from that study are presented below). In contrast, in a different repeated do se study conducted by the U.S. National Toxicology Program (Chan et al. 1992), increases in micronuclei were not seen in the bone marrow erythrocytes of male or 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.

<u>Intraperitoneal injection</u>

The JMPR committee concluded that genotoxic effects occurring 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 form ulation products by ip 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. , 2009a, Manas et al. , 2009 and 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) 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 (c.f. Jensen 1991 and Kier, 1992). When positive results were seen and when a direct comparison can be made, the formul ation 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 (see above) 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 fo rmulation and reported that significant toxicity occurred in the liver and kidney when dosing occurred by ip injection. They postulated that the previously reported DNA damage was likely a secondary effect of toxicity.

Bolognesi and colleagues (Peluso et al., 1998) also reported that following the ip administration of Roundup, but not glyphosate, an increase in DNA adducts was detected in the mouse liver and kidney by the sensitive but non-specific ^{32}P postlabeling method. They attributed the adducts to an unknown component of the herbicide mixture. This same group of investigators reported that ip administration of glyphosate and Roundup resulted in an increase in 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.

Non-traditional tests or tests in phylogenetically distant organisms

The results of genotoxicity studies performed in phylogenetically distant organisms or using non-traditional and generally non-validated assays are presented in Appendix XX. 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, 2012b; Navarro and 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 and Martinez, 2014).

Glyphosate metabolites

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 Table XX. The *in vivo* studies have investigated the ability of these metabolites 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 ip injection to male mice. Studies by other investigators using the more relevant oral route of administration did not show an increase in MN 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 and negative 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. Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

Summary:

The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg bw by the oral route, the route most relevant to human dietary exposure, was not associated with an increase in chromosome alterations in the overwhelming majority of studies conducted in rodents, a model considered to be relevant for assessing genotoxic risks to humans. There is no convincing evidence that glyphosate, its formulation products, or metabolites cause mutations in mammals *in vivo* when administered by a physiologically relevant

EPA-HQ-2018-002024_0004889

route. The genotoxic effects that have been reported to occur *in vitro* or in phylogenetically distant organisms have not been seen *in vivo* in appropriately treated mammalian models.

When administered by intraperitoneal injection, mixed, largely negative, results have been reported in studies of chromosomal damage of glyphosate, its formulation products and metabolites. Mixed, and somewhat contradictory, results have been reported in the few studies (all conducted by ip injection) that have investigated DNA adducts induced by glyphosate or Roundup. Results obtained by this route of administration are believed to have limited relevance when estimating risks from human dietary exposure.

The positive results reported by Amer et al. (2006) using both oral and ip routes of administration appear anomalous, and may have been due to impurities or other components present within the Herbazed formulation product.

Mechanistic considerations

Many studies have been performed to identify chemical structural motifs that are associated with carcinogenesis. Neither glyphosate nor its metabolites possess structures that arecommonly associated with mutagenesis or carcinogenesis (Ashby et al., 1989; Ashby and Paton, 1993). However, one study investigating the effects of a series of *alpha*-aminophosphonic acids with structural similarities to glyphosate, reported moderate clastogenic activity in the mouse bone marrow chromosome aberration test when administered by ip injection (Naydenova et al., 2007). In contrast, glyphosate bioassay results in 620 assays screening biological activity including cytotoxicity are reported in PubChem (accessed 4-20-2016). Positive results were seen only 21 of the 620 assay reports, the majority of which appear to be closely related to glyphosate's herbicidal mechanism of action in plants. The few other positives involved protein-ligand binding and inhibition of the metabolic enzyme CYP71B1. These results indicate that, at the concentrations tested, glyphosate has few off-target molecular or cellular effects.

Table 43 Results of In Vivo Human Biomonitoring studies with glyphosate

Endpoint	Test object	Concentration	Puirty %	Results	Reference
Structural chromosome aberrations	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Columbia	Glyphosate containing mixture	Negative	Paz-y-Mino et al. 2011
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Narino, Columbia	Herbicide mixtures containing glyphosate and adjuvant	Equivocal/inconclusive	Bolognesi et al. 2009
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Putumayo, Columbia	Herbicide mixtures containing glyphosate and adjuvant	Equivocal/inconclusive	Bolognesi et al. 2009
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Valle del Cuaca	Round-up 47	Equivocal/inconclusive	Bolognesi et al. 2009
DNA strand breaks/Comet	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Columbia	Round-up Ultra (44%)	Equivocal/inconclusive	Paz-y-Mino et al. 2007
DNA adducts (8-OHdG)	Human peripheral blood cells	Pesticide applicators	Glyphosate	Negative	Koureas et al. (2014)

EPA-HQ-2018-002024_0004890

1
2 Table 44 Results of in vivo genotoxicity studies with glyphosate in other mammalian species

Endpoint	Test object	Concentration	Puirty %	Results	Reference
Oral administration					
Dominant lethal test	Mouse fetuses and resorptions	200 - 2000 mg/kg	Glyphosate (98.7%)	Negative	Rodwell/Wren n (1980) for Monsanto
Chromosome aberrations	Rat bone marrow cells	21 - 188 mg/kg	Glyphosate trimesium SC-0224 (58.5%)	Negative in males at all time points including the 5 day exposure	Matheson (1982) for Stauffer provided by Syngenta
Chromosome aberrations	Mouse bone marrow cells	50 - 5000 mg/kg on 2 days	Glyphosate (96.8%)	Negative in both males & females	Suresh (1994) for Feinchemie Schwebda
Chromosome aberrations	Mouse bone marrow cells	1080 mg/kg bw	Round-up (>90% purity)	Negative	Dimitrov et al. (2006)
Chromosome aberrations	Mouse bone marrow cells	50 & 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	Positive	Amer er al. (2006)
Chromosome aberrations	Mouse spermatocyte s	50 & 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	Positive	Amer er al. (2006)
Micronucleu s	Mouse bone marrow erythrocytes	400 - 1100 mg/kg	Glyphosate trimesium SC-0224 (55.3%)	Negative in both males & females	Majeska (1986) for Stauffer Chemical provided by Snygenta
Micronucleu s	Mouse bone marrow erythrocytes	3 -50 mg/kg diet	Glyphosate (98.6%)	Negative in both males & females	NTP (1992)
Micronucleu s	Mouse bone marrow erythrocytes	50 - 5000 mg/kg bw; administered 2X	Glyphosate (96.8%)	Negative for males; weak positive/equivocal for females at highest dose	Suresh (1993) for Feinchemie Schwebda
Micronucleu s	Mouse bone marrow erythrocytes	5000 mg/kg bw	Glyphosate (95.6%)	Negative in both males & females	Fox (1996) for Zeneca Agrochemicals
Micronucleu s	Mouse bone marrow erythrocytes	2000 mg/kg bw	Glyphosate potassium salt (49% glyphosate acid by analysis) [indicated 59.3% in text]	Negative in males	Jones (1999) for Zeneca Agrochemicals
Micronucleu s	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 78634 (65.2% glyphosate)	Negative	Erexson (2003) for Monsanto
Micronucleu s	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	AK-01 technical (99.1%)	Negative	Inoue (2004) for TAC group
Micronucleu s	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	Glyphosate technical (97.73%)	Negative in both males & females	Honavar (2005) for Caliope
Micronucleu s	Mouse bone marrow erythrocytes	1080 mg/kg bw	Round-up (>90% purity)	Negative	Dimitrov et al. (2006)

Micronucleus	Mouse bone marrow erythrocytes	8 - 30 mg/kg bw	Glyphosate technical Helm (≥95%)	Negative/equivocal	Zoriki Hosomi (2007) for Helm do Brasil Mercantil
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	Glyphosate (99.1%)	Negative	Honarvar (2008) for Syngenta
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 79864 (38.7% glyphosate)	Negative	Xu (2008) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 76171 (31.1% glyphosate)	Negative	Xu (2008b) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 76313 (30.9% glyphosate)	Negative	Xu (2008c) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	2000 mg/kg bw	Glyphosate (A17035A) (280g/L)	Negative in males	Negro Silva (2009) for Syngenta
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 79991 (71.6% glyphosate)	Negative	Xu (2009) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 76138 (38.5% glyphosate)	Negative	Xu (2009b) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 78910 (30.3% glyphosate)	Negative	Xu (2010) amended version of Exexon (2006) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	TROP M (Glyphosate 480) (358.4 g/L glyphosate acid; 483.6 g/L glypohsate IPA salt)	Negative in both males & females	Fluegge (2010) for Milenia Agrociencias
Micronucleus	Mouse bone marrow erythrocytes	2000 mg/kg bw	Glyphosate SL (A13013Z) (500g/L)	Negative in males	Negro Silva (2011) for Syngenta
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw	MON 78239 (36.6% glyphosate)	Negative	Xu (2011) amended version of Erexson (2003) for Monsanto
Micronucleus	Mouse bone marrow erythrocytes	2000 mg/kg bw	Glyphosate (96.3%)	Negative	Roth (2012) for Syngenta
Micronucleus	Mouse bone marrow erythrocytes	2000 mg/kg bw	Glyphosate TGAI (98.9%)	Negative	Patel (2012) for Dow
Micronucleus	Rat bone marrow erythrocytes	500 - 2000 mg/kg bw	Glyphosate technical grade (98.8%)	Negative in both males & females	Fluegge (2009b) for Helm AG
Micronucleus	Rat bone marrow erythrocytes	500 - 2000 mg/kg bw	Glyphosate 75.5 DF (69.1% glyphosate)	Negative in both males & females	Fluegge (2010) for Helm AG

Unscheduled DNA synthesis	Rat liver hepatocytes	150 - 600 mg/kg bw	Glyphosate trimesium ICIA0224 (57.6%)	Negative in males	Kennelly (1990) for ICI Agrochemicals provided by Syngenta
Sister chromatid exchange	Mouse bone marrow cells	50 - 200 mg/kg bw	Herbazed (glyphosate, 84%)	Positive	Amer er al. (2006)
Intraperitoneal Administration					
Chromosome aberrations	Rat bone marrow cells	1000 mg/kg bw	Glyphosate (98%)	Negative in both males & females	Li and Long (1988)
Chromosome aberrations	Mouse bone marrow cells	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	Negative	Amer et al. (2006)
Chromosome aberrations	Mouse spermatocytes	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	Positive	Amer et al. (2006)
Chromosome aberrations	Mouse bone marrow cells	25 & 50 mg/kg bw	Round-up (>41%)	Positive	Prasad et al. (2009)
Micronucleus	Mouse bone marrow erythrocytes	5000 mg/kg bw	Glyphosate (98.6%)	Negative in both males & females	Jensen (1991c) for Cheminova
Micronucleus	Mouse bone marrow erythrocytes	850 - 3400 mg/kg bw	Rodeo formulation (40%)	Negative in both males & females	Kier (1992b) Monsanto
Micronucleus	Mouse bone marrow erythrocytes	100 - 200 mg/kg bw	Glyphosate isopropylamine salt	Negative	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	133 & 200 mg/kg bw as glyphosate isopropylamine salt	Roundup (480 g/L)	Negative	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	68 - 206 mg/kg bw	Glifos (360 g/L glyphosate)	Negative in both males & females	Zaccaria (1996b) for Cheminova Agro
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	Positive	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	450 mg/kg bw; 135 mg/kg as glyphosate	Round-up (30.4%)	Positive	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	188 - 563 mg/kg bw	Glyphosate technical Nufarm (95%)	Negative	Carvalho Marques (1999) for Nufarm du Brazil
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate technical grade	Negative	Chruscielska et al. (2000)
Micronucleus	Mouse bone marrow erythrocytes	90 mg/kg bw	Glyphosate formulation Perzocyd 10 SL	Negative	Chruscielska et al. (2000)
Micronucleus	Mouse bone marrow erythrocytes	50 - 200 mg/kg bw	Glyphosate (Roundup 69?)	Negative	Nascimento et al. (2000)

Micronucleus	Mouse bone marrow erythrocytes	1008 - 3024 mg/kg bw	Glifosato IPA Technico Nufar; glyphosate isopropylamine salt (613 g/kg salt equivalent)	Negative in both males & females	Gava (2000) for Nufarm do Brasil
Micronucleus	Mouse bone marrow erythrocytes	50 - 200 mg/kg bw	Roundup (480 g/L)	Negative	Grisolia (2002)
Micronucleus	Mouse bone marrow erythrocytes	150 - 600 mg/kg bw	Glyphosate technical grade (95.7%)	Negative/equivocal	Durward (2006) for Nufarm Asia
Micronucleus	Mouse bone marrow erythrocytes	15.6 - 62.5 mg/kg bw	Glyphosate technical grade (98%)	Negative in both males & females	Costa (2008) for Jingma Chemicals
Micronucleus	Mouse bone marrow erythrocytes	25 & 50 mg/kg bw	Round-up (>41%)	Positive	Prasad et al. (2009)
Micronucleus	Mouse bone marrow erythrocytes	100 - 400 mg/kg bw	Glyphosate (analytical grade; 96%)	Positive	Manas et al. (2009a)
Micronucleus	Mouse bone marrow erythrocytes	0.148 - 1.28 mg/kg bw	Roundup	Positive	Rodrigues et al. (2011)
DNA strand breaks	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	Positive	Bolognesi et al. (1997)
DNA strand breaks	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Round-up (30.4%)	Positive	Bolognesi et al. (1997)
DNA adducts by ^{32}P postlabeling	Liver and kidney of mice	130 and 270 mg/kg	Glyphosate isopropylammonium salt	Negative	Peluso et al. (1998)
DNA adducts by 32P postlabeling	Liver and kidney of mice	400 - 600 mg/kg	Round-up (30.4%)	Positive	Peluso et al. (1998)
Oxidative DNA adducts (8-OH-dG)	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	Positive	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OH-dG)	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Round-up (30.4%)	Positive	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OH-dG)	Liver and kidney of mice	600 & 900 mg/kg bw	Glyphosate formulation (30.4%)	Negative	Heydens et al. (2008)

1
2

1
2 Table 45 Results of in vitro genotoxicity studies with glyphosate in bacteria

Endpoint	Test object	Concentration	Puirty %	Results	Reference
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537	±S9; 0.1 - 1000 µg/plate	Glyphosate (98.4%)	±S9; Negative	Kier (1978) Monsanto
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537 and 1538	±S9; 0.005 - 50 µl/plate	Glyphosate trimesium SC-0224 (19.2%)	±S9; Negative	Majeska (1982) for Stauffer provided by Syngenta
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537, 1538 and *Escherichia coli* WP2 *uvrA*	±S9; 10 - 5000 µg/plate	Glyphosate (98%)	Negative	Li and Long (1988)
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537, 1538 and *Escherichia coli* WP2 *uvrA*	±S9; 1.6 - 5000 µg/plate	Glyphosate trimesium ICIA 0224	±S9; Negative	Callander (1988) for ICI Agrochemicals provided by Syngenta
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 313 - 5000 µg/plate	AK-01 technical (glyphosate acid) (96.4%)	±S9; Negative	Yanagimoto (1991) for TAC Group
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535 and 1537	±S9; 160 - 5000 µg/plate	Glyphosate (98.6%)	±S9; Negative	Jensen (1991a) for Cheminova Agro
Point mutations	*Salmonella typhimurium* strains TA97, 98, 100, and 1535	±S9; 33 - 10,000 µg/plate	Glyphosate (98.6%)	±S9; Negative	NTP (1992)
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535 and 1537	±S9; 50 - 5000 µg/plate	Rodeo (40% glyphosate	±S9; Negative	Kier(1992b) Monsanto
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537, 1538 and *Escherichia coli* WP2 and WP2 *uvrA*	±S9; 100 - 5000 µg/plate	Glyphosate trimesium TMSC (95%)	±S9; Negative	Callander (1993) for ICI Agrochemicals provided by Syngenta
Point mutations	*Salmonella typhimurium* strains TA98, TA100	±S9; 180 - 1440 µg/plate	Roundup	±S9, weak positive/equivocal	Rank et al. (1993)
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537	±S9; 156 - 5000 µg/plate	HR-001 (95.7%)	±S9; Negative	Akanuma (1995a) for Sankyo
Point mutations	*Salmonella typhimurium*	±S9; 50 - 5000 µg/plate	Glyphosate (95.3%)	±S9; Negative	Thompson (1996) for

	strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA				Mastra and Maruzen
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 and WP2 uvrA	±S9; 100 - 5000 µg/plate	Glyphosate (95.6%)	±S9; Negative	Callander (1996) for Zeneca Agrochemicals
Point mutations	*Salmonella typhimurium* strains TA97a, 98, 100, and 1535	±S9; 1 - 5000 µg/plate	Glifos (360 g/L glyphosate)	±S9; Negative	Vargas (1996a) for Cheminova Agro
Point mutations	*Salmonella typhimurium* strains TA97a, 98, 100, and 102	0.025 - 0.3 µg/plate	Glyphosate formulation Perzocyd 10 SL	±S9; Negative	Chruscielska et al. (2000)
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535, 1537	±S9; 10 - 5000 µg/plate	Glyphosate, technical; (97%)	±S9; Negative	Schreib (2012) for Industrias Afrasa
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535 and 1537	±S9; 648 - 5000 µg/plate	Glyphosate, technical Helm (98%)	±S9; Negative	Riberri do Val (2007) for Helm do Brasil Mercantil
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 3 - 5000 µg/plate	Glyphosate (95.1%)	±S9; Negative	Sokolowski (2007a) forNufarm Asia
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 3 - 5000 µg/plate	Glyphosate (97.7%)	±S9; Negative	Sokolowski (2007b) forNufarm Asia
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 3 - 5000 µg/plate	Glyphosate (95%)	±S9; Negative	Sokolowski (2007c) forNufarm Asia
Point mutations	*Salmonella typhimurium* strains TA97a, 98, 100, 102, and 1535	±S9; 1 - 1000 µg/plate	Glyphosate TC (98%)	±S9; Negative	Mijay (2008) for Jingma Chemicals
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535 and 1537	±S9; 31.6 - 3160 µg/plate	Glyphosate TC (97.5%)	±S9; Negative	Fluegge (2009a) for Helm
Point mutations	*Salmonella typhimurium* strains TA98, 100,	±S9; 3 - 5000 µg/plate	Glyphosate (96.3%)	±S9; Negative	Sokolowski (2009) for Syngenta

	1535, and 1537 and *Escherichia coli* WP2 and WP2 uvrA				
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535, and 1537	±S9; 31.6 - 5000 µg/plate	Glyphosate (>96%)	±S9; Negative	Donath (2010) for Helm AG
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535 and 1537	±S9; 31.6 - 3160 µg/plate	Glyphosate TC (95.2%)	±S9; Negative	Fluegge (2010) for Helm
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 31.6 - 5000 µg/plate	Glyphosate (96%)	±S9; Negative	Schreib (2010) for Feinchemie Schwebda
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 3 - 5000 µg/plate	Glyphosate (>95%) spiked with glyphosine (0.63%)	±S9; Negative	Sokolowski (2010) for Helm AG
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535, and 1537	±S9; 31.6 - 5000 µg/plate	Glyphosate (>95.8%)	±S9; Negative	Wallner (2010) for Helm AG
Point mutations	*Salmonella typhimurium* strains TA98, 100, 102, 1535, and 1537	±S9; 10 - 2000 µg/plate	Glyphosate (>95.4%)	±S9; Negative	Donath (2011a) for Helm AG
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 10 - 5000 µg/plate	Glyphosate (98.8%)	±S9; Negative	Donath (2011b) for Cheminova
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 10 - 5000 µg/plate	Glyphosate (97.8%)	±S9; Negative	Donath (2011c) for Cheminova
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537 and *Escherichia coli* WP2 uvrA	±S9; 1.5 - 5000 µg/plate	Glyphosate (85.8%)	±S9; Negative	Thompson (2014) for Albaugh Europe Sarl
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537 and *Escherichia coli* WP2 *uvrA*	±S9; 10 - 5000 µg/plate	Glyphosate, technical; (94.1%)	±S9; Negative	Schreib (2015) for Cheminova

DNA damage	*Bacillus subtilis* Rec assay H17 and M45	20 - 2000 µg/disk	Glyphosate (98%)	Negative	Li and Long (1988)
DNA damage	*Bacillus subtilis* Rec assay H17 and M45	±S9; 15 - 240 µg/disc	AK-01 Technical (glyphosate acid)(96.4%)	Negative	Yanagimoto (1992b) for TAC Group
DNA damage	*Bacillus subtilis* Rec assay H17 and M45	7.5 - 240 µg/disk	Glyphosate (95.7%)	Negative	Mie Ananuma (1995b) for Sankyo
DNA damage	*Escherichia coli* SOS chromotest	0.1 - 0.25 µg	Roundup	Positive	Raipulis et al. (2009)
DNA strand breaks	Cyanobacteria (*Scytonema javanicum*)	10 µM	Glyphosate	Weak positive	Wang et al. (2012)
DNA strand breaks	Cyanyanobacteria (*Anabaena sp.*)	10 µM	Glyphosate	Positive	Chen et al. (2012)
DNA strand breaks	Cyanyanobacteria (*Microcystis viridis*)	10 µM	Glyphosate	Positive	Chen et al. (2012)
DNA damage	Acellular prophage superhelical PM2 DNA	w/o S9; 75 mM	Glyphosate (98.4%)	Negative	Lueken et al. (2004)

Table 46 Results of in vitro genotoxicity studies with glyphosate in mammalian cells

Endpoint	Test object	Concentration	Purity	Results	Reference
Gene mutation (HPRT)	Chinese Hamster Ovary cells	±S9; 2 - 25 mg/ml	Glyphosate (98%)	Negative, ±S9	Li and Long (1988)
Gene mutation (TK)	Mouse lymphoma cells (L5178Y TK$^{\pm}$)	±S9; 0.094 - 5 mg/ml	Glyphosate trimesium ICIA 0224 (57.6%)	Negative, ±S9	Cross (1988) for ICI Agrochemicals
Gene mutation (TK)	Mouse lymphoma cells (L5178Y TK$^{\pm}$)	±S9; 0.52 - 5 mg/ml	Glyphosate (98.6%)	±S9, negative	Jensen (1991b) for Cheminova Agro
Gene mutation (TK)	Mouse lymphoma cells (L5178Y TK$^{\pm}$)	±S9; 44 - 1500 µg/ml	Glyphosate (95.6%)	±S9, negative	Clay (1996) for Zeneca Agrochemicals
Chromosome aberrations	Chinese Hamster Ovary cells	±S9; 4 - 10 µl/ml	Glyphosate trimesium SC-0224 (55.6%)	Negative, ±S9	Majeska (1985) for Stauffer provided by Snygenta
Chromosome aberrations	Chinese hamster cells (CHL/IU)	±S9; 37.5 - 1200 µg/ml	AK-01 Technical (glyphosate acid)(96.4%)	Negative (-S9); Positive (+S9)	Yanagimoto (1992) for TAC Group
Chromosome aberrations	Human peripheral blood lymphocytes	±S9; 100 - 4000 µg/ml	TMS Chloride (95%) [Glyphosate trimesium]	Equivocal ±S9	Griffitts (1993) for Zeneca Agrochemicals provided by Snygenta
Chromosome aberrations	Chinese hamster lung cells	±S9; 62.5 - 1000 µg/ml	HR-001 (95.7%)	±S9, negative	Matsumoto (1995) for Sankyo
Chromosome aberrations	Human peripheral blood lymphocytes	±S9; 33 - 562 µg/ml	Glyphosaat	Negative	Van de Waart (1995) Notox project

Chromosome aberrations	Chinese hamster lung cells	±S9; 39 - 1250 µg/ml	Glyphosate (technical grade; 95.3%)	±S9, negative	Wright (1996) for Mastra and Maruzen Kako
Chromosome aberrations	Human peripheral blood lymphocytes	±S9; 100 - 1250 µg/ml	Glyphosate (95.6%)	±S9, negative	Fox (1998) Zeneca Agrochemicals
Chromosome aberrations	Human peripheral blood lymphocytes	w/o S9; 5 - 51 µM	Glyphosate (≤98%)	Positive	Lioi et al. (1998a)
Chromosome aberrations	Bovine lymphocytes	17 - 170 µM	Glyphosate	Positive	Lioi et al. (1998b)
Chromosome aberrations	Mouse splenocytes	w/o S9; 0.1 - 50 mM	Herbazed (glyphosate, 84%)	Positive	Amer et al. (2006)
Chromosome aberrations	Human peripheral blood lymphocytes	no S9; 0.2 - 6 mM	Glyphosate (analytical grade; 96%)	Negative	Manas et al. (2009a)
Micronucleus	Bovine lymphocytes	w/o S9; 28 - 560 µM	Glyphosate isopropylamine salt mixture (62%)	Equivocal	Piesova (2004)
Micronucleus	Bovine lymphocytes	±S9; 28 - 560 µg/ml	Glyphosate isopropylamine salt mixture (62%)	±S9,Negative	Piesova (2004)
Micronucleus	Bovine lymphocytes	w/o S9; 28 - 1120 µM	Glyphosate isopropylamine salt mixture (62%)	Negative	Sivikova et al. (2006)
Micronucleus	Human peripheral blood lymphocytes	±S9; 0.5 - 580 µg/ml	Glyphosate (technical grade; 98%)	Negative (-S9); Positive (+S9)	Mladinic et al. (2009)
Micronucleus	Human epithelial cancer cell line TR146	w/o S9; 10 - 20 mg/L	Glyphosate (95%)	Positive	Koller et al. (2012)
Micronucleus	Human epithelial cancer cell line TR146	w/o S9; 10 - 20 mg/L	Round-up	Positive	Koller et al. (2012)
Micronucleus	CHO K1 cells	5 - 100 µg/ml	Glyphosate	w/o S9: negative; w/ S9 positive	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM5757	w/o S9; 75 mM	Glyphosate (98.4%)	Negative alone; Positive in presence of H2O2	Lueken et al. (2004)
DNA strand breaks (Comet assay)	Human fibrosarcoma cell line HT1080	w/o S9; 4 - 6/5 nM	Glyphosate (technical grade)	Positive	Monroy et al. (2005)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM38	w/o S9; 4.5 - 6/5 nM	Glyphosate (technical grade)	Positive	Monroy et al. (2005)
DNA strand breaks (Comet assay)	Human liver HepG2 cell line	w/o S9; 1 - 10 ppm	Round-up (R400)	Positive	Gasnier et al. (2009)

DNA strand breaks (Comet assay)	Human Hep-2 cell line	no S9; 3 - 7.5 mM	Glyphosate (analytical grade; 96%)	Positive	Manas et al. (2009a)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	±S9; 0.5 - 580 µg/ml	Glyphosate (technical grade; 98%)	Positive (±S9)	Mladinic et al. (2009)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	w/o S9; 10 - 2000 mg/L	Glyphosate (95%)	Positive	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	w/o S9; 10 - 2000 mg/L	Round-up	Positive	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks	Mouse spermatognia	60- 180 mg/L	Glyphosate	Positive	Ming et al. (2014)
Sister chromatid exchange	Human peripheral blood lymphocytes	w/o S9; 0.25 - 25 mg/ml	Round-up	Positive	Vigfusson and Vyse (1980)
Sister chromatid exchange	Chinese Hamster Ovary cells	±S9; 4 - 10 µl/ml	Glyphosate trimesium SC-0224 (55.6%)	Negative, ±S9	Majeska (1985) for Stauffer provided by Snygenta
Sister chromatid exchange	Bovine lymphocytes	17 - 170 µM	Glyphosate	Positive	Lioi et al. (1998b)
Sister chromatid exchange	Human peripheral blood lymphocytes	no S9; 0.33 - 6 µg/ml	Glyphosate (analytical grade; 99.9%)	Positive	Bolognesi et al. (1997)
Sister chromatid exchange	Human peripheral blood lymphocytes	no S9; 0.1 - 0.33 µg/ml	Round-up (30.4% glyphosate)	Positive	Bolognesi et al. (1997)
Sister chromatid exchange	Human peripheral blood lymphocytes	w/o S9; 5 - 51 µM	Glyphosate (≥98%)	Positive	Lioi et al. (1998a)
Sister chromatid exchange	Mouse splenocytes	w/o S9; 0.1 - 50 mM	Herbazed (glyphosate, 84%)	Positive	Amer et al. (2006)
Sister chromatid exchange	Bovine lymphocytes	w/o S9: 28 - 1120 µM	Glyphosate isopropylamine salt mixture (62%)	Positive	Sivikova et al. (2006)
Unscheduled DNA synthesis	Rat hepatocytes	0.0000125 - 0.125 mg/ml	Glyphosate (98%)	Negative	Li and Long (1988)
Unscheduled DNA synthesis	Rat hepatocytes	0.2 - 111.7 mM	Glyphosate (≥98%)	Negative	Rossberger (1994) for Feinchemie Webda

1
2

1 Table 47 Results of in vivo genotoxicity with glyphosate in non-mammalian species

Endpoint	Test object	Concentration	Purity %	Results	Reference
Mutation	*Drosophila larvae*	0.1 ppm	Pondmaster	Positive	Kaya et al. (1995)
Mutation	Drosophila larvae	1 ppm	Roundup	Positive	Kaya et al. (1995)
Mutation	*Drosophila* standard cross	0.1 - 10 mM	Glyphosate (96%)	Weak positive	Kaya et al. (2000)
Mutation	*Drosophila* high bioactivation cross	0.1 - 10 mM	Glyphosate (96%)	Negative	Kaya et al. (2000)
DNA strand breaks (Comet assay)	Sipderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Positive	Alvarez-Moya et al. (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L	Glyphosate	Negative	Akcha et al. (2012)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) blood cells	17.9 35.7 µg/L	Glyphosate	Positive	Guilherme et al. (2012b)
DNA strand breaks (Comet assay)	Nile tilapia *Oreochromis niloticus* erythrocytes	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks (Comet assay)	Sipderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Weak positive/inclusive	Alvarez-Moya et al. (2014)
DNA damage	Zebrafish (*Danio rerio*) sperm	5 & 10 mg/L	Glyphosate	Positive	Lopes et al. (2014)
DNA strand breaks (Comet assay)	Sabalo fish (*Prochilodus lineatus*) erythrocytes and gill cells	0.48 & 2.4 mg/L	Glyphosate	Positive	Moreno et al. (2014)
Roundup and other formulations					
Mutation (sex-linked recessive lethal)	*Drosophila* standard cross	1 ppm	Roundup	Positive	Kale et al. (1995)
Mutation (sex-linked recessive lethal)	*Drosophila* standard cross	0.1 ppm	Pondmaster	Positive	Kale et al. (1995)
Chromosome aberrations	Plant meristems of *Crepis capillaris*	0.05 - 1%	Roundup (>90% purity)	Negative	Dimitrov et al. (2006)
Chromosome abnormalities	Mitotic plant meristems of *Hordeum vulgare*	0.1 - 2%	Roundup	Positive	Truta et al. (2011)
Micronucleus	Nile tilapia fish *Oreochromis niloticus erythrocytes*	42 -170 mg/kg bw	Glyphosate (Roundup 69)	Negative	Nascimento et al. (2000)
Micronucleus	*Tilapia rendalli* peripheral erythrocytes	42 - 170 mg/kg bw	Roundup (480 g/L)	Positive	Grisolia (2002)
Micronucleus	Plant meristems of *Crepis capillaris*	0.05 - 1%	Roundup (>90% purity)	Negative	Dimitrov et al. (2006)
Micronucleus	The freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	Positive	Cavas and Konen (2007)

Micronucleus	Neotropical fish (*Prochilodus lineatus)* erythrocytes and gill cells	10 mg/L	Roundup (41%)	Negative	Cavalcante et al. (2008)
Micronucleus	*Caiman latirostris* erythrocytes	50 - 1750 µg/egg	Roundup (66,2%)	Positive	Poletta et al. (2009)
Micronucleus	European eel (Anguilla anguilla) blood cells	58 & 116 µg/L	Roundup (30.8%)	Negative	Guilherme et al. (2010)
Micronucleus	*Caiman latirostris* erythrocytes	3%	Roundup (66.2%)	Positive	Poletta et al. (2011)
Micronucleus and Nuclear abnormalities	Brazilian freshwater fish *Astyanax* sp.	0.006 mL/L	Roundup	Positive	Rossi et al. (2011)
Micronucleus	The fish *Corydoras paleatus* erythrocytes	6.67 µg/L	Roundup (48%)	Negative	de Castilhos Ghisi et al. (2013)
Micronucleus	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	Positive	de Souza Filho et al. (2013)
Micronucleus	*Caiman latirostris* erythrocytes	2.5 - 21 mg/L	Roundup	Positive	Lopez Gonzales et al. (2013)
Micronucleus	Ten spotted fish *Cnesterodon decemmaculatus* erythrocytes	22.9 - 68.8 mg/L	Glyphosate formulation Credit (48%)	Positive	Vera-Candioti et al. (2013a)
Micronucleus	Ten spotted fish *Cnesterodon decemmaculatus* erythrocytes	3.9 - 11.8 mg/L	Glyphosate formulation Panzer (48%)	Weak positive	Vera-Candioti et al. (2013a)
Micronucleus	Indian skittering frog (Euflictis cyanophlyctis) tadpole erythrocytes	1 - 8 mg a.e./L	Roundup (41%)	Positive	Yadav et al. (2013)
Micronucleus	Earthworm (*Pheretima peguana*) coelomocytes	47 - 432 µg cm^{-2}	Glyphosate formulation (36%)	Positive	Muangphra et al. (2014)
Micronucleus	*Channa punctatus* blood cells	8.1 -24.4 mg/L	Roundup (41%)	Positive	Nwani et al. 2014
Micronuclei and meiotic anomalies	Black lentil beans Vinga mungo	Not specified	Glyphosate	Positive	Singh and Srivastava (2014)
DNA strand breaks (Comet assay)	Bullfrog *(Rana catesbeiana*) tadpoles	1.69 - 27 mg/L	Roundup (356 g/L)	Positive	Clements et al. (1997)
DNA strand breaks (Comet assay)	Freshwater mussels (Utterbackia imbecillis)	2.5 & 5 mg/L	Roundup (18%)	Negative	Conners & Black (2004)
DNA strand breaks (Comet assay)	The freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	Positive	Cavas and Konen (2007)

DNA strand breaks (Comet assay)	Neotropical fish (*Prochilodus lineatus)* erythrocytes and gill cells	10 mg/L	Roundup (41%)	Weak positive	Cavalcante et al. (2008)
DNA strand breaks (Comet assay)	*Caiman latirostris* erythrocytes	50 - 1750 µg/egg	Roundup (66.2%)	Positive	Poletta et al. (2009)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) blood cells	58 & 116 µg/L	Roundup (30.8%)	Positive	Guilherme et al. (2010)
DNA strand breaks (Comet assay)	*Caiman latirostris* erythrocytes	3%	Roundup (66.2%)	Positive	Poletta et al. (2011)
DNA strand breaks (Comet assay)	The snail (*Biomphalaria alexandrina*) hematocytes	10 mg/L	Roundup (48%)	Positive	Mohamed (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L active ingredient	Roundup	Negative	Akcha et al. (2012)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) gill and liver cells	58 & 116 µg/L	Roundup (30.8%)	Positive	Guilherme et al. (2012)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) blood cells	58 & 116 µg/L	Roundup (30.8%)	Positive	Guilherme et al. (2012b)
DNA strand breaks (Comet assay)	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	Positive	de Souza Filho et al. (2013)
DNA strand breaks (Comet assay)	Frog (*Eleutherodactylus johnstonei*) blood cells	0.5 - 1.7 mg a.e./cm2	Roundup SL–Cosmoflux 411F (360 g/L glyphosate)	Positive	Meza-Joya et al. (2013)
DNA strand breaks (Comet assay)	The fish *Corydoras paleatus* erythrocytes	6.67 µg/L	Roundup (48%)	Positive	de Castilhos Ghisi et al. (2013)
DNA strand breaks (Comet assay)	Freshwater clam (Corbicula fluminea) hemocytes	2 & 10 ppm	Roundup	Negative	dos Santos & Martinez (2014)
DNA strand breaks (Comet assay)	The common carp (*Cyprinus carpio*) erythrocytes	2 mg/L	Roundup (480 g/L)	Positive	Gholami-Seyedkolaei et al. (2013)
DNA strand breaks (Comet assay)	*Channa punctatus* blood and gill cells	3.25 - 6.51 mg/L	Roundup (41%)	Positive	Nwani et al. 2013
DNA strand breaks (Comet assay)	Earthworm (*Eisenia andrei*) coelomocytes	15 & 30 $\mu g/cm^{-1}$	Roundup FG (71%)	Positive	Piola et al. (2013)
DNA strand breaks (Comet assay)	Earthworm (*Eisenia andrei*) coelomocytes	15 - 240 $\mu g/cm^{-1}$	Glyphosate formulation (85.4%)	Negative	Piola et al. (2013)
DNA strand breaks (Comet assay)	Ten spotted fish *Cnesterodon decemmaculatus* erythrocytes	3.9 mg/L	Glyphosate formulation Panzer (48%)	Positive	Vera-Candioti et al. (2013b)

DNA strand breaks (Comet assay)	Ten spotted fish *Cnesterodon decemmaculatus* erythrocytes	22.9 mg/L	Glyphosate formulation Credit (48%)	Positive	Vera-Candioti et al. (2013b)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) blood cells	116 µg/L	Roundup (30.8%)	Positive	Guilherme et al. (2014)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) liver cells	58 & 116 µg/L	Roundup (30.8%)	Positive	Marques et al. (2014)
DNA strand breaks (Comet assay)	Sabalo fish (*Prochilodus lineatus*) erythrocytes and gill cells	1 & 5 mg/L	Roundup Transorb (480 g/L)	Positive	Moreno et al. (2014)
DNA strand breaks (Comet assay)	Earthworm (*Pheretima peguana*) coelomocytes	47 - 432 µg cm^{-2}	Glyphosate formulation (36%)	Negative	Muangphra et al. (2014)
DNA strand breaks (Comet assay)	Tambaqui (Colossoma macropomum) fish	10 - 15 mg/L	Roundup (360 g/L)	Positive	Braz-Mota et al. (2015)
Nuclear abnormalities	Neotropical fish (*Prochilodus lineatus)* erythrocytes and gill cells	10 mg/L	Roundup (41%)	Negative	Cavalcante et al. (2008)
Nuclear abnormalities	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	Positive	de Souza Filho et al. (2013)
Nuclear abnormalities	European eel (Anguilla anguilla) blood cells	58 & 116 µg/L	Roundup (30.8%)	Equivocal	Guilherme et al. (2010)
Nuclear abnormalites	The freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	Positive	Cavas and Konen (2007)
Nuclear abnormalites	*Channa punctatus* blood cells	8.1 -24.4 mg/L	Roundup (41%)	Positive	Nwani et al. 2014

Table 48 Results of in vitro genotoxicity with glyphosate in non-mammalian species

Endpoint	Test object	Concentration	Purity %	Results	Reference
Chromosome damage	*Allium* root cells	w/o S9; 720 - 2880 µg/L	Glyphosate isopropylamine (96%)	Negative	Rank et al. (1993)
Chromosome alterations	*Allium* root cells	w/o S9; 720 - 2880 µg/L calculated as glyphosate isopropylamine	Roundup	Positive	Rank et al. (1993)
Chromosome alterations	*Allium cepa* onion root tips	0.036 - 0.146%	Springbok, glyphosate isopropylamine formulation (48%)	Positive	Asita et al. (2008)
Chromosome alterations	*Trigonella foenum-graecum*n root tips	0.1 - 0.5%	Glyphosate	Positive	Siddiqui et al. (2012)
Chromosome alterations	*Allium cepa* onion root tips	3%	Glyphosate	Positive	Frescure et al. (2013)

Micronucleus	*Allium cepa* onion root tips	35. 70, 105, 140, 350, 700. 1050 and 1400 µg/g	Glyphosate formulation (21%)	Negative*	De Marco et al. (1992)
DNA strand breaks (Comet assay)	Sipderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Positive	Alvarez-Moya et al. (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L	Glyphosate	Negative	Akcha et al. (2012)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L active ingredient	Roundup	Negative	Akcha et al. (2012)
DNA strand breaks (Comet assay)	Frog (*Eleutherodactylus johnstonei*) blood cells	4.6 - 37 mg a.e./cm2	Roundup SL–Cosmoflux 411F (360 g/L glyphosate)	Positive	Meza-Joya et al. (2013)
DNA strand breaks (Comet assay)	Tilapia (*Oreochromis niloticus*) erythrocytes	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks (Comet assay)	Sipderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.0007 - 0.7 mM	Glyphosate isopropylamine (96%)	Inconclusive	Alvarez-Moya et al. (2014)

Table 49 Results of in vivo genotoxicity studies with AMPA, metabolite of AMPA and formulants

Endpoint	Test object	Concentration	Purity %	Results	Reference
AMPA					
Micronucleus	Mouse bone marrow erythrocytes	100 - 1000 mg/kg bw	AMPA (98.4%)	Negative in both males and females	Kier (1993) Monsanto
Micronucleus	Mouse bone marrow erythrocytes	5000 mg/kg bw	AMPA (99.2%)	Negative in both males and females	Jensen (1993c) for Cheminova
Micronucleus	Mouse bone marrow erythrocytes	200 - 400 mg/kg bw	AMPA (99%)	Positive	Manas et al. (2009b)
N-acetyl-AMPA					
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw (active ingredient, adjusted for purity)	N-acetyl-AMPA (72%; IN-EY252)	Negative in both males and females	Donner (2007) for DuPont
N-Acetyl-glyphosate					
Micronucleus	Mouse bone marrow erythrocytes	500 - 2000 mg/kg bw (active ingredient, adjusted for purity)	N-acetyl-glyphosate (63%; IN-MCX20)	Negative in both males and females	Donner (2006) for DuPont

Other related chemicals					
Chromosome aberrations	Mouse bone marrow cells	10 & 100 mg/kg bw	Series of alpha-aminophosphonic acids	Positive	Naydenova et al. (2007)
Polyoxyethyleneamine					
DNA Breakage (Comet assay)	Neotropical fish *Prochilodus lineatus* blood cells	0.15 - 1.5 mg/L	Polyoxyethylene amine	Positive	Navarro and Martinez (2014)

1
2 Table 50 Results of in vitro genotoxicity studies with AMPA, metabolite of AMPA and formulants

Endpoint	Test object	Concentration	Purity %	Results	Reference
AMPA					
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537 and *Escherichia coli* WP2 *uvrA*	±S9; 200 - 5000 µg/plate	AMPA (99.3%)	±Negative	Akanuma (1996) for Sankyo
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537, 1538 and *Escherichia coli* WP2 *uvrA*	±S9; 1.6 - 5000 µg/plate	AMPA (>99%)	±Negative	Callander (1988) for ICI Agrochemicals
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, and 1537	±S9; 310 - 5000 µg/plate	AMPA (99.2%)	±Negative	Jensen (1993) for Cheminova Agro
Gene mutation	Mouse lymphoma cells (L5871Y)	±S9; 0.31 - 5.0 mg/ml	AMPA (99.2%)	±Negative	Jensen (1993) for Cheminova Agro
Chromosome aberrations	Human peripheral lymphocytes	no S9; 0.9 - 1.8 mM	AMPA (99%)	Weak positive	Manas et al. (2009b)
Micronucleus	CHO K1 cells	±S9; 0.005 - 0.1 µg/L	AMPA	Positive	Roustan et al. (2014)
Micronucleus	CHO K1 cells	±S9; 5 - 100	Glyphosate + AMPA	Negative	Roustan et al. (2014)
Micronucleus	European eel (Anguilla anguilla) blood cells	11.8, 23.6 µg/L	AMPA	Negative	Guilherme et al. (2014b)
DNA strand breaks (Comet assay)	Human Hep-2 cell line	no S9; 2.5 -7.5 mM	AMPA (99%)	Positive	Manas et al. (2009b)
DNA strand breaks (Comet assay)	European eel (Anguilla anguilla) blood cells	11.8, 23.6 µg/L	AMPA	Positive	Guilherme et al. (2014b)

Unscheduled DNA synthesis	Rat hepatocytes	5 - 2500 µg/ml	AMPA (94.4%)	Negative	Bakke (1991) for Monsanto
Unscheduled DNA synthesis	Rat hepatocytes	0.078 - 10 mM	AMPA (99.9%)	Negative	Neslany (2002) for Calliope SAS
Nuclear abnormalities	European eel (Anguilla anguilla) blood cells	11.8, 23.6 µg/L	AMPA	Positive	Guilherme et al. (2014b)
N-acetyl-AMPA					
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537 and *Escherichia coli* WP2 *uvrA*	±S9; 50 - 5000 µg/plate	N-acetyl-AMPA (76%; IN-EY252)	Negative	Wagner (2007) for DuPont
Chromosome aberrations	Human peripheral blood lymphocytes	±S9; 191 - 1530 µg/mL	N-acetyl-AMPA (76%; IN-EY252)	Negative	Gudi (2007) for DuPont
Gene mutation (HPRT)	Chinese hamster Ovary cells	±S9; 100 - 1531 µg/mL (active ingredient, adjusted for purity)	N-acetyl-AMPA (72%; IN-EY252)	Negative	Glatt (2007) for DuPont
N-Acetyl-glyphosate					
Point mutations	*Salmonella typhimurium* strains TA98, 100, 1535, 1537 and *Escherichia coli* WP2 *uvrA*	±S9; 100 - 5000 µg/plate	N-acetyl-glyphosate sodium salt (84.3%)	Negative	Mecchi (2004) for Verdia
Gene mutation (HPRT)	Chinese hamster Ovary cells	±S9; 250 - 2091 µg/mL (active ingredient, adjusted for purity)	N-acetyl-glyphosate sodium salt (63%)	Negative	Glatt (2006) for DuPont
Chromosome aberrations	Chinese hamster Ovary cells	±S9; 960 - 2800 µg/mL	N-acetyl-glyphosate sodium salt (84.3%)	Negative	Murli (2004) for Verdia

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

Study 1.

In a three generation reproduction study , glyphosate (purity considered 100%) was administered to 12 male and 24 female CD rats/dose level in the diet at doses of 0, 3, 10, and 30 mg/kg bw per day starting 63 days prior to mating. Animals were mate d on a 1 male to 2 female basis. The first litters (F1A, F2A, and F3A) from each mating were raised to weaning and then

EPA-HQ-2018-002024_0004907

terminated. Second matings (F1B and F2B) were selected to become parents for subsequent generations or to be subjected to complete gross necropsy (F3B). Tissues were also evaluated microscopically (10/sex/group) from the control and high-dose parental animals for all generations and F3B offspring. This study was conducted prior to GLP regulation.

Analytical results demonstrated that glyphosate was stable and homogeneity distributed in the diet. Analysis of various batches showed an average of 98.0 + 6.8% of the target concentration. No treatment-related adverse effects were observed on mortality, clinical signs, body weights, food consumption, food efficiency, organ weights, or histopathological changes for parental animals of either generation. There were no adverse effects 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 bodyweights or development.

There were no adverse effects noted in the study; therefore, the NOAEL for parental, reproductive, and offspring toxicity is 30 mg/kg bw per day (Schroeder, 1981).

Study 2

In a two-generation reproduction study, glyphosate (purity, 97.67%) was administered to 30 Sprague-Dawley rats/sex/dose level in the diet at doses of 0, 2000, 10,000, and 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, animals were paired within each dose group on a 1:1 basis to produce the F1 litters. At weaning, 30 rats/sex/dose were selected to form the F1 generation (referred to as F1A in study report), similarly exposed (approximately 14 weeks), and mated twice to produce F2A and F2B generations. On day 4 post-partum, litters were standardized (4 males and 4 females when possible). Offspring not selected for mating, F2A and F2B pups, and adult females which had littered were sacrificed on or after day 21 of lactation. Adult males were sacrificed after completion of the mating phase. Organs were retained from all parental animals and 1 pup/sex/litter from F2A and F2B. 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 to 96.7% of target levels. No treatment-related adverse effects were observed on mortality, food consumption, organ weights, or histopathological changes for parental animals of either generation. The incidence of soft stool was increased for high-dose adult animals in both generations. Reduced bodyweights were noted in parental animals of both generations. In the F0 generation, bodyweights were approximately 8-10% lower than controls at termination while bodyweights were 10-13% lower than controls in the F1 generation at termination.

There were no adverse effects observed for mating performance, pregnancy rate, or duration of pregnancy in either generation. When compared to control, a slight reduction in average litter size was observed in pups from FO dams at the highest dose group. An even smaller difference was noted after the first Fl mating. Statistical significance was not achieved in slight reduction in average litter size. The F1a adults were re-mate to produce the F2b generation. There was no dose-related decrease in litter size in this second mating. Since the reductions in litter size were not statistically significant, and because they were not consistently observed in all generations, their relationship to treatment could not be conclusively established. Therefore, it is concluded that litter size and viability were not affected by treatment. No adverse effects were noted for offspring bodyweights or development.

The NOAEL for parental toxicity is 10,000 ppm; equal to 666 mg/kg bw per day, based on decreased bodyweights and increased incidence of soft stool at 30,000 ppm (equal to 1983 mg/kg bw per day). There were no effects on reproductive parameters or offspring measurements; therefore, the NOAEL for reproductive and offspring toxicity is 30,000 ppm; equal to 1983 mg/kg bw per day (Reyna 1990).

Table 51. Incidence of soft stool in parental animals[1]

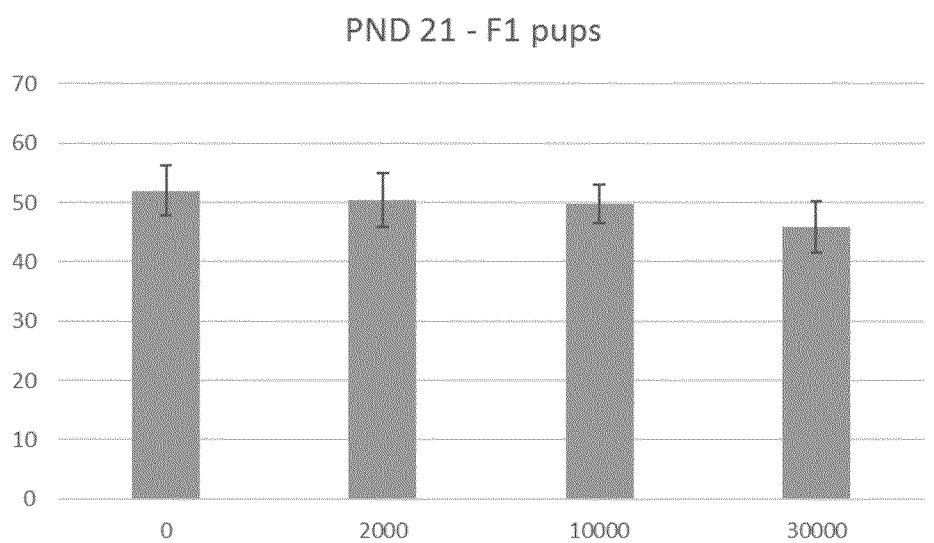
	Dose (ppm)			
	0	2,000	10,000	30,000
F0 generation - males				
Number of animals	0	0	0	30
Number of occurrences	0	0	0	457
F0 generation - females				
Number of animals	0	0	0	22
Number of occurrences	0	0	0	116
F1 generation -males				
Number of animals	0	0	1	30
Number of occurrences	0	0	1	698
F1 generation -females				
Number of animals	0	0	0	29
Number of occurrences	0	0	0	537

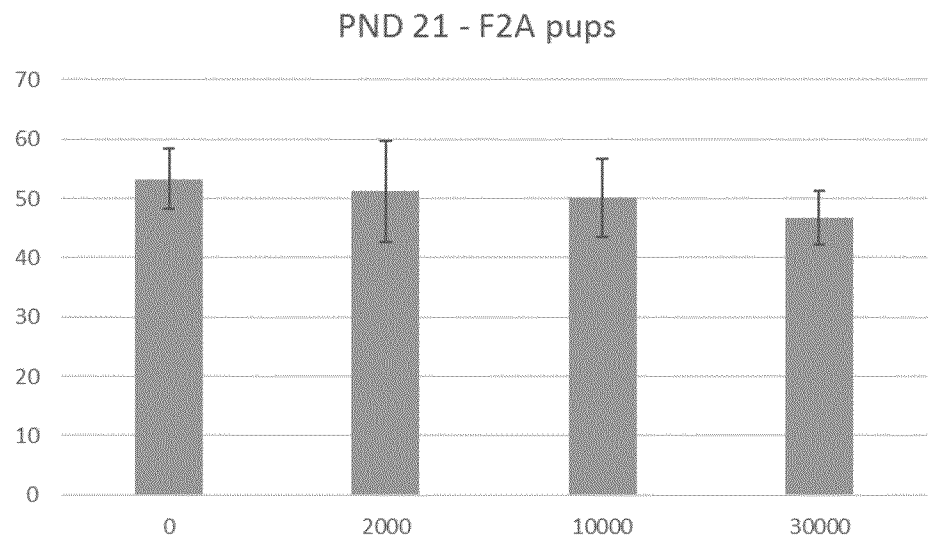
[1] n = 30

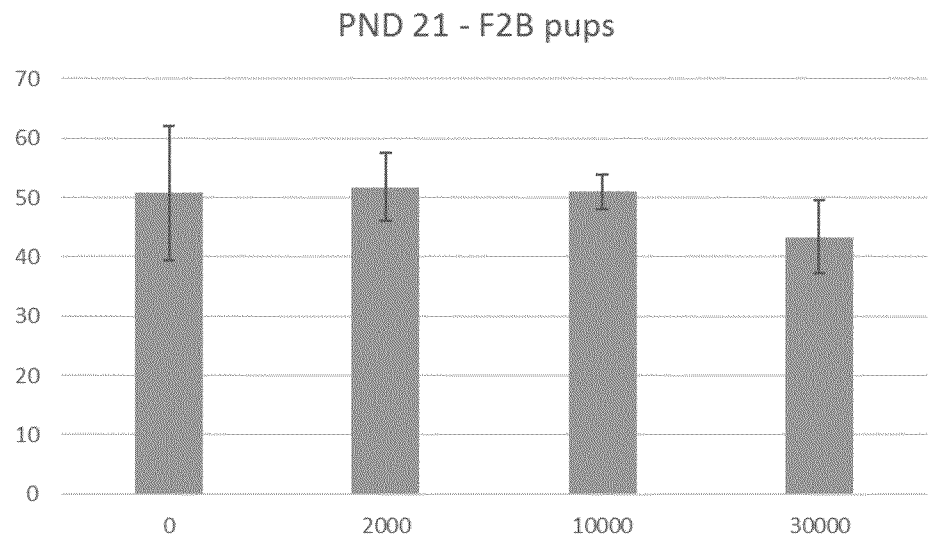
Table 52. Terminal bodyweights in parental animals[1]

	Dose (ppm)			
	0	1200	6000	30000
F0 generation				
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%)
F1 generation				
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%)

[1] Values presented are means ± standard deviations. Percent change from controls in parentheses.







(Reyna 1990)

Study 3

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 glyph osate technical (purity, 99.2%) at a concentration of 0, 1,000, 3,000 or 10,000 ppm (equal to 0, 66.4, 196.8, or 668.1 mg/kg bw per day for males and 0, 75.3, 226.0, or 752.3 mg/kg bw per day for females) for 70 days before their first mating and until ter mination. Each generation was mated twice, changing partners for the second mating and avoiding sister/brother matings throughout. On post -natal day 4, litters were standardized (4 males and 4 females when possible). Culled pups and those not selected f or mating were sacrificed and subjected to gross pathological examinations. Treatment was continued for parental animals until day 21 of weaning of the second litter when animals were sacrificed for organ weighing, gross pathological examination, and hist opathological examination. Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analyzed when an effect was seen in a tissue at the highest dose.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, food consumption, food efficiency, or organ weights for parental animals of either generation. There were no adverse effects 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 bodyweights 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 53). Changes were described as hypertrophy of acinar cells with prominent granular cytoplasm (minimal severity). Increased incidence of the effects was evident at the highest dose tested.

Table 53. Incidence of minimal cellular alterations in salivary glands.

	Dietary concentration (ppm)							
	Males				Females			
	0	1,000	3,000	10,000	0	1,000	3,000	10,000
F0 generation								
Animals examined	27	28	28	26	28	27	28	28
Parotid gland	2	2	3	12	0	2	5	17
Submaxillary gland	0	-	-	0	0	1	4	14
F1 generation								
Animals examined	24	24	23	23	24	23	24	23
Parotid gland	1	0	4	11	0	0	4	9
Submaxillary gland	0	-	-	0	0	0	0	3

- = not examined

The NOAEL for parental toxicity is 3,000 ppm ; equal to 196.8 mg/kg bw per d ay, based on increased incidence of histopathological effects observed in the parotid (males and females) and submaxillary (females only) salivary glands in both generations at 10,000 ppm ; equal to 668.1 mg/kg bw per day. There were no effects on reproduc tive parameters or offspring measurements; therefore, the NOAEL for reproductive and offspring toxicity is 10,000 ppm ; equal to 668.1 mg/kg bw per day (Brooker, 1992).

Study 4

In a two-generation reproduction study, glyphosate (purity, 96.8%) was admini stered to 30 Wistar rats/sex/dose level in the diet at doses of 0, 100, 1000, and 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 r eported 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 th e F1 litters. On day 4 post -partum, litters were standardized (4 males and 4 females when possible). At weaning, 30 males and 30 females from each dose group were selected to form the F1 generation. Unselected offspring were sacrificed and subjected to macroscopic evaluation. The offspring selected for the F1 generation were dosed for at least 10 weeks and paired within dose group to produce F2 litters. All parental animals, non -selected pups from F1, and all pups from F2 were necropsied. Tissue collection was only conducted for parental animals.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, food consumption, food efficiency, organ weights, or histopathological changes for parental animals of either generation. There were no adverse effects 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 bodyweights or development.

There were no adverse effects noted in the study; therefore, the NOAEL for parental, reproductive, and offspring toxicity is 10,000 ppm ; equivalent to 660 mg/kg bw per day (Suresh, 1993a).

Study 5

In a two-generation reproduction study, glyphosate (purity 94.61%) was administered to 24 Sprague Dawley [Crl:CD(SD)] rats/sex/dose level in the diet at doses of 0, 1200, 6000 and 30,000 ppm (equal to 83.6, 417 and 2150 mg/kg bw per day for males and 0, 96.9, 485, 2532 mg/kg bw per day for fem ales) 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 F1 litters. On day 4 post-partum, litters were standardized (4 males and 4 females when possible). At weaning, 24 males and 24 females from each dose group were selected to form the F1 generation. Unselected offspring were sacrificed and subjected to gross necropsy. The offspring selected for the F1 generation were dosed for at least 10 weeks and paired within dose group to produce F2 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 F0 males; 0, 97, 485, and 2532 mg/kg bw per day for F0 females; 0, 92, 458, and 2411 mg/kg bw per day for F1 males; and 0, 105, 530, and 2760 mg/kg bw per day for F1 females.

No treatment -related adverse effects were observed on mortality, body weights, food consumption, food efficiency, or histopathological changes for paren tal animals of either generation. The incidence of loose stool was increased for high -dose parental animals in both generations. Additionally, the incidences of cecum of distension was increased in high -dose parental animals in both generations. 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 54. Incidence of loose stool in parental animals

	Pre-mating				Mating/gestation				Lactation/post-weaning			
	Dose (ppm)				Dose (ppm)				Dose (ppm)			
	0	1200	6000	30000	0	1200	6000	30000	0	1200	6000	30000
F0 generation												
Males	0/24	0/24	0/24	3/24	0/23	0/24	0/24	2/24	N/A	N/A	N/A	N/A
Females	0/24	0/24	0/24	1/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	6/24
F1 generation												
Males	0/24	0/24	0/24	13/24	0/23	0/24	0/23	0/24	N/A	N/A	N/A	N/A
Females	0/24	0/24	0/24	4/24	0/23	0/23	0/21	0/19	0/23	0/23	0/21	2/19

N/A = not applicable

Table 55. Incidence of cecum distension in parental animals

	Dose (ppm)			
	0	1200	6000	30000
F0 generation				
Males	0/24	0/24	0/24	21/24
Females	0/24	0/24	0/24	24/24
F1 generation				
Males	0/24	0/24	0/24	19/24
Females	0/24	0/24	0/24	17/24

EPA-HQ-2018-002024_0004912

Table 56. Mean offspring bodyweights

PND	Dose group (ppm)							
	0	1200	6000	30,000	0	1200	6000	30,000
	F1 Pups – male				F2 Pups – male			
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**
	F1 Pups – female				F2 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*

Values presented are means ± standard deviation.
Statistics reported from study report.
* significantly different from control at p≤0.05.
** significantly different from control at p≤0.01.
*** significantly different from control at p≤0.001.

Table 57. Incidence of cecum distension in offspring

	Dose (ppm)			
	0	1200	6000	30000
F1 pups	0/136	0/141	0/143	89/141
F2 pups	0/182	0/183	0/164	111/149

There were no adverse effects observed for mating performance, pregnancy rate, or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. Offspring bodyweights were decreased in both generations for the high-dose starting typically on post-natal day 14. Gross pathological examinations found an increased incidence of cecum distension in offspring at the high-dose for both generations.

The NOAEL for parent al toxicity is 6,000 ppm ; equal to 417 mg/kg bw per day, based on increased incidence of loose stool and cecum distension in both generations at 30,000 ppm ; equal to 2150 mg/kg bw per day . There were no effects on reproductive parameters; therefore, the N OAEL for reproductive is 30,000 ppm; equal to 2150 mg/kg bw per day . The NOAEL for offspring toxicity is 6,000 ppm ; equal to 417 mg/kg bw per day, based on decreased pup body weights and increased incidence of cecum distension in both generations at 30,00 0 ppm; equal to 2150 mg/kg bw per day (Takahashi 1997).

Study 6

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 a concentration of 0, 1,000, 3,000 or 10,000 ppm (equal to 0, 99.4, 292.6, or 984.7 mg/kg bw per day for males and 0, 104.4, 322.8, or 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 littering and females were terminated on or soon after day 29 of lactation. Following euthanasia, parental animals were subjected to organ weighing, gross pathological examination, and histopathological examination. Offspring not selected for mating were sacrificed on day 29 post -partum. At termination on day 29

post partum, 1 pup/sex/litter was used for organ weight determination and 2 pups/sex/litter were given macroscopic examinations. All remaining pups were sacrificed with no further examination.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, food consumption, food efficiency, organ weights, or histopathological changes for parental animals of either generation. There were no adverse effects observed for mating performance, pregnancy rate, or duration of pregnancy in either generation. Litter size and viability were not affected bytreatment. The bodyweights of F1A pups were lower in comparison to the control group from day 8 onwards, but a similar effect was not seen in the F2A pups. There was no treatment -related effect on total litter weight (Table 58).

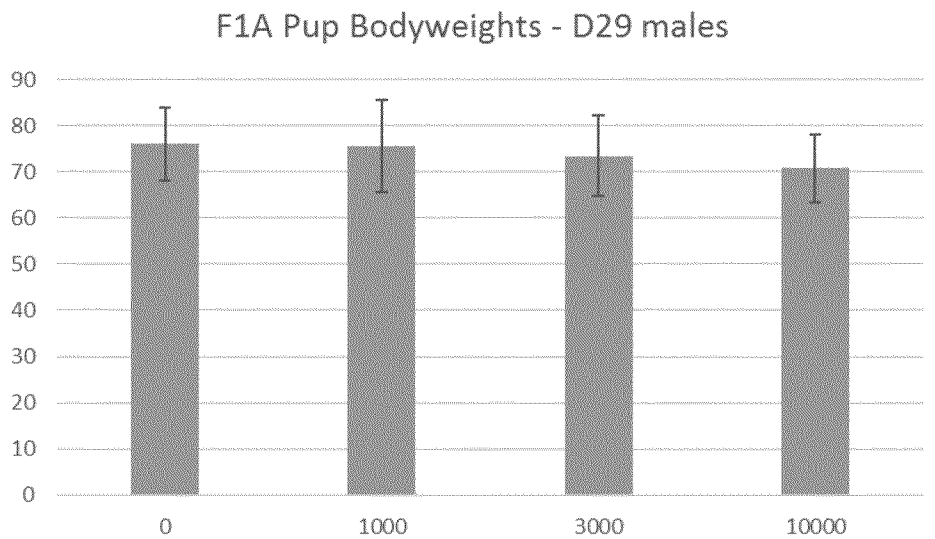
Table 58: Intergroup comparison of pup bodyweights (g) – (adjusted mean from Day 1 onwards) – F1A/F2A litters

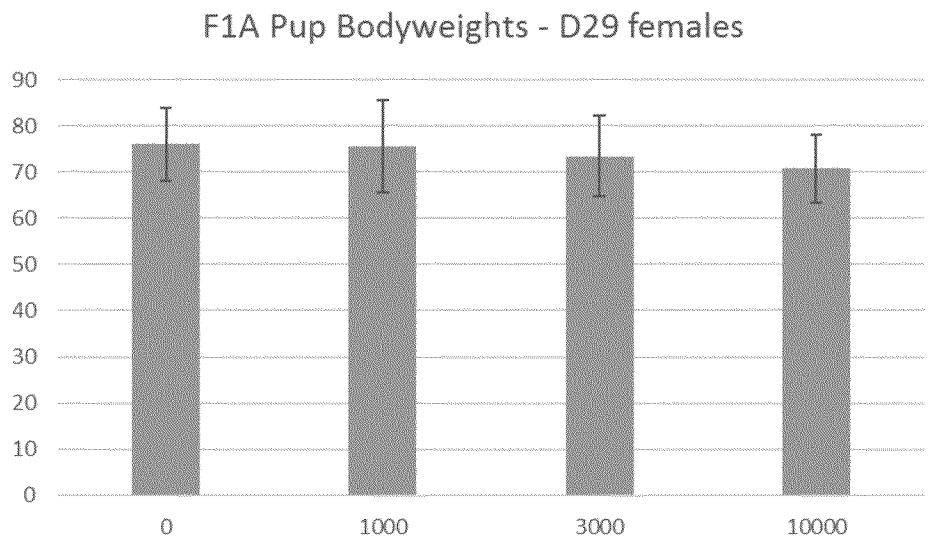
	Dietary concentration of glyphosate (ppm)							
	Males				**Females**			
Day	**0**	**1000**	**3000**	**10000**	**0**	**1000**	**3000**	**10000**
F1A								
1	5.8	6.1	6.0	6.1	5.4	5.8	5.6	5.7
5	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
8	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
15	26.8	26.1	25.8	24.6*	26.1	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**
F2A								
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
29	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4

;nssificant difference from control group mean, 1% level (Student's t-test, 2 sided)
* Statistically significant difference from control group mean, 5% level (Student's t-test, 2 sided)

There were no adverse effects noted in the study; therefore, the NOAEL for parental and reproductive toxicity is 10,000 ppm ; equal to 984.7 mg/kg bw per day . The NOAEL for offspring toxicity is 3000 ppm; equal to 292.6 mg/kg bw per day based on reduced pup weights in F1A generation seen at 10,000 ppm; equal to 984.7 mg/kg bw per day ((Moxon 2000).

No adverse effects were noted for offspring bodyweights or development.





(Moxon 2000)

Study 7

In a two -generation reproduction study , glyphosate (purity, 95.7%) was administered to 28 Sprague Dawley [Crl:CD(SD) IGS BR] rats/sex/dose level in the diet at doses of 0, 1500, 5000 and 15,000 ppm (equ al to 0, 104, 351, and 1063 mg/kg bw per day in males and 0, 1 26, 423, and 1 273 mg/kg bw per day in females) for two successive generations with one litter per generation. After 10 weeks of treatment, ani mals were paired within each dose group on a 1:1 basis to produce the F1 litters. At weaning, 24 males and 24 females from each dose group were selected to form the F1 generation. Surviving adult females and unselected offspring were terminated on day 21 post-partum, followed by termination of adult males. The offspring selected for the F1 generation were dosed for at least 10 weeks and paired within dose group to produce F2 litters. Adult females and their offspring were terminated on day 21 post -partum, followed by termination of adult males. All adult animals and offspring were subjected to macroscopic examinations and organs taken from all parental animals for weights. A small subset of organs were taken from one male and one female offspring from the F0 and F1 pairings (where available). Tissues from control and high-dose F0 and F1 animals were subjected to histopathological examination. Since there were indications of changes in the adrenal glands of F1 animals, microscopic examination was extended to include all dose groups.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, food consumption, food efficiency, organ weights, or histopathological changes for parental animals of either generation. There were no adverse effects observed for mating performance, pregnancy rate, or duration of pregnancy in either generation. Litter size, viability, and offspring bodyweights were not affected by treatment. A delay in attaining complete preputial separation (PPS) was noted in F1 male pups in the high -dose (2.9 days) along with a 10% increase in bodyweight at attainment (Table 59). There were no effects of treatment on the age or weight at attainment of vaginal opening.

Table 59. Age and weights at attainment of preputial separation for F1 males

Dose (ppm)	Number of animals	Age at completion (days)[1]	Bodyweight at attainment (g)[1]
0	24	43.0±2.3	210±23
1,500	24	43.3±1.6	216±22
5,000	24	43.5±2.3	219±22
15,000	24	45.9±3.1	230±28

[1] Values are means ± standard deviations.

There were no effects for parental animals or on reproductive parameters; therefore, the NOAEL for parental and reproductive toxicity is 15,000 ppm ; equal to 1063 mg/kg bw per day . The NOAEL for offspring is 5,000 ppm ; equal to 351 mg/kg bw per day, based on delayed age and increased weight at attainment of preputial separation at 15000 ppm; equal to 1063 mg/kg bw per day (Dhinsa 2007).

(b) Developmental toxicity

Rats

Study 1

In a pre-GLP developmental toxicity study, glyphosate (purity 98.7%) suspended in a 0.5% aqueous Methocel was administered to 2 5 copulated CD female rats/dose by oral gavage at dose levels of 0, 300, 1000, or 3500 mg/kg bw per day from days 6 through 19 of gestation (GD6-19). On day 20 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

Soft stool, diarrhoea, red nasal dischar ge, reduced activity and rales (abnormal respiratory noise) were noted in the 3500 mg/kg bw per day dosage group. Six rats in the 3500 mg/kg bw per day group died by gestation day 17. A reduced mean body weight gain was noted over the treatment period in the 3500 mg/kg bw per day group due to a loss in mean maternal weight over the first three days of treatment. There were no significant differences in the mean number of viable foetuses, implantations, postimplantation losses, corpora lutea, or mean foetal body weight between the 300 and 1000 mg/kg bw per day dosage groups and the control group. The mean number of total implantations, viable foetuses and mean foetal body weight were significantly decreased in the 3500 mg/kg bw per day dosage group compared to controls. In addition, there was a significant increase in early resorptions, causing a slight increase in post implantation losses, in the 3500 mg/kg bw per day group.

At 3500 mg/kg bw per day, the number of litters with malformations was identical to t hat of the control group, but there was an increase in the number of foetuses with malformations. However, since the number and type of malformations observed were similar to those observed in historical

control data, it was concluded that they were not tr eatment related. In the 3500 mg/kg bw per day there was an increased number of foetuses with unossified sternebrae. While it was treatment related, it is considered to be a developmental variation instead of a frank teratogenic malformation. No malformations were observed in the 300 and 1000 mg/kg bw per day dosage groups.

The NOAEL for maternal is 1000 mg/kg bw per day based on mortality, soft stool and reduced body weight gain seen at 3500 mg/kg bw per day. The NOAEL for developmental toxicity is 1000 mg/ kg bw per day based on decreased in t he mean number of total implantations, viable foetuses, mean foetal body weight, increased in early resorptions and increased number of foetuses with unossified sternebrae at 3500 mg/kg bw per day (Tasker, Rodwell and Jessup, 1980a).

Study 2

In a developmental toxicity study, glyphosate (purity 98.6%) suspended in a 1.0% aqueous solution of methylcellulose was administered to 2 5 copulated (Crl: CD (SD) BR VAF/Plus female rats/dose by oral gavage at dose levels of 0, 30 0, 1000, or 3500 mg/kg bw per day from days 6 through 15 of gestation (GD6 -15). On day 20 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

At the highest dose, clinical abnormalities included salivation, loose stools and noisy respiration. The latter was also observed in two animals at the intermediate dose on one occasion. There were 2 maternal mortalities at 3500 mg/kg bw per day following signs of respiratory distress. Body weight gain was markedly reduced at the highest dose (by 16–81% of control values, days 6–20 of gestation) and marginally reduced at the intermediate dose (by 86–97% of control values, days 6 – 20 of gestation). Food consumption was slightly decreased at the highest dose during the dosing period (75 –94% of control values, days 6 –15 of gestation), but was comparable with controls thereafter. Water intake was increased at the highest dose (139–205% of control values, days 6–15 of gestation). No treatment-related changes were observed at any dose at necropsy.

A total of 23, 23, 25 and 22 dams had live young at day 20 in the control group, and at 300, 1000 and 3500 mg/kg bw per day, respectively. There was no significant influence of treatment on embryonic losses, litter size or sex ratio, but the litter weights and mean fetal weights were reduced at the highest dose, the latter being sta tistically significant (90% and 94% of control values, respectively). The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher at the highest dose and slightly higher at the intermediate dose; the incidences on the basis of foetuses (litters) were 1 (1), 0 (0), 3 (2), and 28 (11) for the 0, 300, 1000 and 3500 mg/kg bw per day, respectively. In addition, reduced ossification was seen slightly more frequently a t the highest and intermediate doses. The percentage of foetuses showing skeletal anomalies (variations) was significantly increased at the two higher doses, but the percentage of foetuses affected at the intermediate dose exceeded the historical background range (21.9–27.2%) only slightly (Table 60).

Table 60: Intergroup comparison of foetal skeletal anomalies (From Brooker et al. (1991)

	Dose level of glyphosate (mg/kg bw per day)			
	0 (control)	**300**	**1000**	**3500**
Number examined	155 (23)	143 (23)	166 (25)	142 (22)
Total number	19 (11)	36 (16)	46 (19)	55 (19)
Mean %	11.7	22.6	28.4*	35.7**
Historical range	21.9 – 27.2			

** Kruskal-Wallis H-statistic followed, if significant, by intergroup comparison with control (distribution-free Williams' test) significant at * P<0.05 ** P<0.01 (n) – number of litters

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

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

Study 3

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 dose levels of 0, 30, 300, or 1000 mg/kg bw per day from days 6 through 15 of gestation (GD6-15). On day 20 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treatment -related changes in mortality, bodyweight, food consumption, or macroscopic findings for dams. An increased incidence of slightly loose stool 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 following the last dosing.

There were no effects on number, growth, or survival of foetuses. Any external, visceral, or skeletal abnormalities were not considered tr eatment-related since the effects were also seen in the control group, incidences of the effects were low, and/or there was no dose-response for the effect.

The NOAEL for maternal toxicity is 300 mg/kg bw per day based on the increased incidence of slightly loose stool observed in dams at 1000 mg/kg bw per day . There were no developmental effects; therefore, the NOAEL for developmental toxicity is 1000 mg/kg bw per day (Hatakenaka, 1995).

Study 4

In a developmental toxicity study , glyphosate acid (purity 95.6%) in deonizied water was administered to 24 time-mated female Alpk:APfSD (Wistar-derived) rats/dose by oral gavage at dose levels of 0, 250, 500, or 1000 mg/kg bw per day from days 7 through 16 of gestation (GD6 -28). On day 22 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

One control animal was killed on day 7 d ue to incorrect dosing. There were no treatment -related changes in clinical observations, bodyweight, food consumption, or macroscopic findings for dams.

There were no effects on number, growth, or survival of foetuses. Additionally, there were no treatment-related external, visceral, or skeletal abnormalities observed.

There were no maternal or developmental effects; therefore, the NOAEL for maternal and developmental toxicity is 1000 mg/kg bw per day (Moxon 1996b).

Rabbits

Study 1

In a developmental toxicity study, glyphosate (purity 98.7%) suspended in a 0.5% aqueous Methocel solution was administered to 16 Dutch Belted female rabbits/dose by oral gavage at dose levels of 0, 75, 175, or 350 mg/kg bw per day from days 6 through 27 of gestation (GD6-27). On day 28 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities. This study was conducted prior to GLP.

There was an increased incidence of mortality in the high-dose group. Spontaneous deaths in the control, low-, mid-, and high-dose groups were 0/16, 1/16, 2/16, and 10/17, respectively. A slight increase was noted in the 175 mg/kg bw per day dose group in the incidence of soft stool and

diarrhoea (individual data not reported). In the 350 mg/kg bw per day dose group, either soft stool, diarrhoea, or both of these clinical signs were observed in each animal at least on ce during the treatment period. An increased incidence of nasal discharge was also noted in the high -dose group (individual data not reported). There were no treatment -related changes in bodyweight or macroscopic findings for dams.

Due to the increased m ortality at the high -dose, the number of animals available for evaluation of developmental effects was not sufficient for this dose (6 pregnant females). The number of pregnant dams were also low for the remaining doses (12, 15, and 11 in the control, low -, and mid-dose groups, respectively); therefore, evaluation of developmental effects is limited for this study. The available data for the control, low -, and mid -dose groups indicate that there were no treatment related adverse effects on the number, gro wth, or survival of foetuses. Any external, visceral, or skeletal abnormalities were not considered treatment-related.

The NOAEL for maternal toxicity is 1 75 mg/kg bw per day based on increased incidence of clinical signs (soft stool and diarrhoea) and mortality at 350 mg/kg bw per day. Individual data were not provided for the clinical signs at 175 mg/kg bw per day ; however, the increase in incidence was only considered slight at this dose. Due to the reduced number of pregnant dams, developmental effects could not be evaluated; however, it should be noted that the available data indicate that there was no evidence of developmental effects (Tasker, Rodwell and Jessup, 1980b).

Study 2

In a developmental toxicity study, glyphosate (purity 95%) suspended in a 0.1% aqueous gum acacia solution was administered to 15 New Zealand White strain female rabbits/dose by oral gavage at dose levels of 0, 125, 250, and 500 mg/kg bw per day , respectively, from days 6 through 18 of gestation (GD6-18). On day 29 of gestat ion, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treat ment-related adverse changes in mortality, food consumption, or macroscopic findings for dams. Two abortions were noted in the high-dose group. There was a slight decrease in bodyweight gain noted in the high-dose group as well.

There were no treatment -related adverse effects on the number, growth, or survival of foetuses. The mean number of viable implants per litter was lower than the other treatment groups and accordingly the mean number of non -viable implants per litter was higher than the other treatments groups; however, when taking into account the variability for these measurements, the changes are not considered adverse.

There were no differences in incidences of external, visceral, or skeletal variations/malformations in foetuses in the low - and mid-dose groups as compared to the control group. At the high -dose, incidences of variations/malformations were higher than the control group; however, in many cases the increase was minimal or similar to the other groups when evaluated on a litter basis.

Table 61. Incidence of fetal malformations and variations

	Dose level (mg/kg bw per day)			
Fetal findings	0	125	250	500
Number of litters examined	13	14	14	12
Number of foetuses 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 centra 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 center	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)
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)

The NOAEL for maternal toxicity is 250 mg/kg bw per day based on abortions observed at 500 mg/kg bw per day. The NOAEL for developmental toxicity is 250 mg/kg bw per day based on increased incidence of variations/malformations observed at 500 mg/kg bw per day. 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 and Patil, 1989).

Study 3

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 strain rabbits/dose by oral gavage at dose levels of 0, 50, 150, or 450 mg/kg bw per day, respectively, from days 7 through 19 of gestation (GD7-19). On day 29 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treatment-related adverse changes in bodyweight, food consumption, or macroscopic findings for dams. At the high-dose, 1 animal was found dead on day 20 following signs of abortion on day 19, soft/liquid faeces, and a reduction in food intake and bodyweight loss from the start of treatment. The incidence of soft/liquid faeces was increased at the high-dose (13 out of 20 animals).

There were no treatment-related adverse effects on the number, growth, or survival of foetuses. At termination, the number of pregnant females was reduced in treatment groups with 18, 12, 15, and 13 pregnant females available for evaluation in the control, low-, mid-, and high-doses; therefore, 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 out of 13 pregnant animals; however, it was barely outside of the historical control range from studies conducted during the same period and there was a reduced number of litters for evaluation at this dose.

The NOAEL for maternal toxicity is 150 mg/kg b w per day based on clinical signs (soft/liquid faeces) at 450 mg/kg bw per day . Due to the reduced number of pregnant dams at the low- and high-doses, developmental effects could not be evaluated; however, it should be noted that the available data indicate that there was no evidence of teratogenic effects. The EU review states The NOAEL for developmental toxicity is considered to be 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).

Study 4

In a developmental toxicity study, glyphosate acid (purity 96.8%) suspended in a 0.5% aqueous carboxymethylcellulose solution was administered to 26, 17, 16, and 16 presumed mated New Zealand White strain rabbits/dose by oral gavage at dose levels of 0, 20, 100, or 500 mg/kg bw per day, respectively, from days 6 through 18 of gestation (GD6 -18). On day 28 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treatment -related adverse changes in bodyweight, food consumption, or macroscopic findings for dams . Mortalities were recorded as 2, 0, 4, and 8 animals in the control, low-, mid-, and high -doses, respectively. The deaths in the control were definitively attributed to gavage error. There was an increased incidence of soft stool/liquid faeces observed at the high-dose (12/15 animals). Other clinical signs observed at the high -dose included rales, weakness, dyspnea, and ocular discharge; however, the incidence of these effects was low and some may be an indication of gavage error. At necropsy, various findings were noted in the lungs and trachea in the mid - and high-dose animals, which further suggests possible gavage errors and/or issues with animal husbandry.

There were no treatment -related adverse effects on the number, growth, or survival of foetuses. At termination, the number of pregnant females was reduced in treatment groups with 20, 13, 12, and 6 pregnant females available for evaluation in the control, low -, mid-, and high -doses; therefore, evaluation of developmental effects is limited for t his study. Total litter loss was recorded for one female in the high-dose group.

Any external, visceral, or skeletal abnormalities were not considered treatment-related. Major visceral malformations primarily affected the heart, but occurred in single incidences and/or showed no dose -response except for the dilated heart; however, interpreting the dose -response is difficult given the limited number of litters available, especially at the high-dose.

Based on the uncertainties regarding gavage errors a nd mortalities across doses in this study and the reduced number of pregnant females, the study is considered unacceptable (Suresh, 1993b).

Study 5

In a developmental toxicity study, glyphosate (purity 97.56%) suspended in a 0.5% aqueous solution of sodium carboxymethylcellulose was administered to 18 artificially inseminated Japanese white rabbits (Kbl:JW)/dose by oral gavage at dose levels of 0, 10, 100, or 300 mg/kg bw per day from days 6 through 18 of gestation (GD6 -18). On day 27 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treatment -related changes in bodyweight, food consumption, or macroscopic findings for dams. One dam died on GD20 without showing any clinical signs and cause of death was undetermined. In the 300 mg/kg bw per day group, an increased incidence of loose stool was observed during the dosing period in 4 of the 17 pregnant females. Two of these animals continued to display this effect during the post-dosing period and one of them aborted on GD26.

EPA-HQ-2018-002024_0004921

There were no effects on number, growth, or survival of foetuses. All observations of external or visceral malformations were sporadic in nature and not considered treatment -related. Skeletal malformations and variations were also not considered treatment -related since thee effects were also seen in the control group, incid ences of the effects were low, and/or there was no dose -response for the effect.

The NOAEL for maternal toxicity is 100 mg/kg bw per day based on the increased incidence of loose stool observed in dams at 300 mg/kg bw per day . There were no developmental effects; therefore, the NOAEL for developmental toxicity is 300 mg/kg bw per day (Hojo 1995).

Study 6

In a developmental toxicity study, glyphosate (purity 95.3%) suspended in a 1% carboxymethyl cellulose vehicle was administered to 18 mated New Zealan d White strain female rabbits/dose by oral gavage at dose levels of 0, 50, 200, or 400 mg/kg bw per day from days 7 through 19 of gestation (GD7-19). On day 29 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorded. All foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treatment -related changes in bodyweight, food consumption, or macroscopic findings for dams. At the high dose, one female was found dead prior to dosing on day 19 and one female was killed *in extremis* on day 20. One death occurred in the control and mid -dose groups as well. 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 was not observed.

There were no -treatment related adverse effects on the number, growth, or survival of foetuses. Increases in late fetal deaths and pos t-implantation loss were noted at the high -doses; however, it is not considered adverse taking into consideration the variability in the measurements. Additionally, the increase can mainly be attributed to one animal with 9 late deaths. There were no treatment-related external, visceral, or skeletal abnormalities observed.

The NOAEL for maternal toxicity is 200 mg/kg bw per day based on increased incidence of diarrhoea at 400 mg/kg bw per day. There were no developmental effects; therefore, the NOAEL for developmental toxicity is 400 mg/kg bw per day (Coles and Doleman, 1996).

Study 7

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionis ed water was administered to 20 time-mated New Zealand White strain female rabbits/dose by ora l gavage at dose levels of 0, 100, 175, or 300 mg/kg bw per day from days 8 through 20 of gestation (GD8 -20). On day 30 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lutea, implantations, and live foetuses recorde d. All foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no treatment -related adverse changes in mortality, bodyweight, food consumption, or macroscopic findings for dams. The incidence of diarrhoea and few faeces were increased at the mid- and high-doses. The incidence of staining in the genital area was also increased at the high-dose.

Table 62. Incidence of clinical signs in maternal animals

	Number of rabbits affected			
	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

There were no -treatment related adverse effects on the number, growth, or survival of foetuses. R educed mean fetal weight was noted at the high -dose.; however, it was not considered adverse taking into account the variability of the measurements. Additionally, the decrease could be attributed to two litters with lower weights. Any external, visceral, or skeletal abnormalities were not considered treatment-related.

Table 63 Intergroup comparison of mean body weight (g) selected timepoints (adjusted mean for all days except day8)

Dosing period/day		**Dose level of glyphosate (mg/kg bw per day)**			
		0 (control)	**100**	**175**	**300**
Pre-dosing	Day 8	3924	3771	3822	3815
During dosing	Day 9	3845	3837	3834	3823
	Day 11	3885	3873	3874	3854
	Day 13	3917	3905	3902	3880
	Day 15	3975	3982	3939	3896
	Day 17	4049	4053	3982	3923*
	Day 19	4085	4061	4005	3927**
Post dosing	Day 23	4177	4118	4049*	3951**
	Day 26	4236	4210	4169	4093*
	Day 30	4313	4294	4256	4183

** Statistically significant difference from control group mean, 1% level (Student's t-test, 2-sided)
* Statistically significant difference from control group mean, 5% level (Student's t-test, 2-sided)

The NOAEL for maternal toxicity is 100 mg/kg bw per day based on increased incidence of clinical signs (few faeces and signs of diarrhoea) at 175 mg/kg bw per day . There were no developmental effects; therefore, the NOAEL for developmental toxicity is 300 mg/kg bw per day (Moxon 1996b).

EPA-HQ-2018-002024_0004923

(Moxon 1996)

2.6 Special studies

(a) Neurotoxicity

Rats

In an acute neurotoxicity study, groups of fasted (24 hours), approximately 42 day old Alpk:APfSD ra ts (10/sex/dose) were given a single oral dose of glyphosate (purity 95.6%) in deionized water at doses of 0, 500, 1000, or 2000 mg/kg bw and observed for 2 weeks. Neurobehavioral assessment (functional observational battery and motor activity testing) wa s performed in all animals in week -1, on day 1 (approximately 6 hours after dosing), day 8 and day 15. At study termination, 5 animals/sex/dose were euthanized and perfused. Of the perfused animals, the control and highest dose groups were used for neur opathological examinations and brain and peripheral nervous system tissues subjected to histopathological evaluation.

Administration of a single dose of glyphosate produced treatment -related clinical signs of general toxicity at 2000 mg/kg bw. At approxi mately 6 hours after dosing on day 1, three females in the 2000 mg/kg bw dose group were observed with decreased activity, subdued behavior, hunched posture and/or hypothermia. Diarrh oea was also seen in an additional female at this dose. Full recovery was established by day 2. These clinical signs do not reflect signs of neurotoxicity and are mostly likely associated with administration of excessively high doses of glyphosate. There were no treatment related effects observed on mortality, body weight, or brain weight. Similarly, neuropathological and histopathological examinations displayed no treatment -related effects. Functional observational battery and motor activity testing revealed no treatment -related effects. Although overall motor activity wa s lower than controls at 2000 mg/kg bw for both sexes on day 1, these differences were not statistically significant or dose-dependent.

The lowest-observed-adverse-effect level (LOAEL) for this study was 2000 mg/kg bw based on clinical signs of general tox icity (decreased activity, subdued behavior, hunched posture, hypothermia and diarrhoea). The no -observed-adverse-effect-level (NOAEL) for this study is 1000 mg/kg bw (Horner, 1996a).

In a subchronic neurotoxicity study, glyphosate (purity 95.6%) was adm inistered to 12 Alpk:APfSD rats/sex/group in the diet at dose levels of 0, 2000, 8000, or 20000 ppm (equal to 0, 155.5, 617.1, 1546.5 mg/kg bw per day for males and 0, 166.3, 672.1, 1630.6 mg/kg bw per day for females) for 13 weeks. Neurobehavioral assess ment (functional observational battery and motor activity testing) was performed in all animals at weeks -1, 1, 5, 9, and 14. At study termination, 6 animals/sex/group were euthanized and perfused. Of the perfused animals, the control and highest dose groups were used for neuropathological examinations and brain and peripheral nervous system tissues subjected to histopathological evaluation.

Overall mean body weight ($p<0.05$; 92.8% of the controls) and food utilization ($p<0.01$) were reduced in males recei ving 20000 ppm glyphosate with no treatment related effect on food consumption. Group mean bodyweight was also lower than controls in males receiving 8000 ppm from weeks 6 to 14 (not statistically significant). There were no treatment related effects observed on mortality, clinical signs, or brain weight. Functional observational battery and locomotor activity testing revealed no treatment -related effects. Neuropathological and histopathological examinations of the peripheral and nervous system did not yield any treatment -related effects from glyphosate administration.

The lowest -observed-adverse-effect level (LOAEL) was not observed. The no -observed-adverse-effect-level (NOAEL) for this study is 20000 ppm ; equal to 1546.5 mg/kg bw per day (Horner, 1996b).

In an acute delayed neurotoxicity study, twenty hens (hybrid brown laying strain – Lohmann Brown) were given a single oral dose of glyphosate (purity 95.6%) at a dose of 2000 mg/kg bw. In addition 12 negative and 12 positive control hens were dosed wi th distilled water or 1000 mg/kg bw of tri-ortho-cresyl phosphate (TOCP), respectively. This was followed by an observation period of 21/22 days. The hens were examined for any clinical signs twice per day and a daily assessment of ataxia was made. They were weighed weekly. Brain acetylcholinesterase (AchE), brain neuropathy target esterase (NTE) and lumbar spine NTE measurements were made on 3 hens, 48 hours after dosing. At the end of the observation period, 6 hens from each treatment group were selec ted for macroscopic examination post mortem and histopathological examination. After perfusion through the heart with fixative, the following tissues were taken, processed and examined histopathologically.

No treatment related mortality was observed in the study. There was no evidence of clinical ataxia in any of the negative controls or in any of the hens dosed with glyphosate. Five of the hens dosed with TOCP developed clinical ataxia, starting between days 11 and 21. There was no effect on bodyweights for hens dosed with glyphosate but TOCP -dosed hens showed an overall weight loss. Acetylcholinesterase was reduced by 6% in glyphosate treated hens and 19% in TOCP treated hens. There was no effect on NTE levels in brain or spinal cord for the glyphosate -treated hens but in the positive controls there was an 84% reduction in brain NTE levels and a 78% reduction in spinal cord NTE levels compared to the negative controls. No macroscopic abnormalities were seen in any of the hens examined. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment -related changes in glyphosate -treated hens. Hens dosed with TOCP showed significant axonal degeneration in spinal cord, peripheral nerve and cerebellum, demonstrating the validity of the test system.

In conclusion, oral administration of a single dose of 2000 mg/kg bw of glyphosate did not produce any clinical signs of delayed neurotoxicity, no significant reduction in acetyl cholinesterase, lack of histopathological findings. The NOAEL for acute delayed neurotoxicity of glyphosate in hens was 2000 mg/kg bw (Johnson, 1996).

Immunotoxicity

Mice

In an immunotoxicity study, glyphosate (purity 85.2 %) was administered to female B6C3F1/Crl mice (10/dose) in the diet at dose levels of 0, 500, 1500, or 5000 ppm (equal to 0, 150.1, 449.1, or 1447.5 mg/kg bw per day , respectively) for 28 days. The positive control group (10 females) was administered 50 mg/kg bw per day of cyclophosphamide (10 mL/kg at a concentration of 5 mg/mL) by intraperitoneal injection from study Days 24-27. On the study Day 24, all animals in all groups received a single intravenous dose of 7.5 x 107 sheep red blood cells (SRBC) in 0.2 mL of Earle's Balanced Salt Solution (E BSS) with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). At sacrifice, selected organs were removed and weighed (spleen and thymus). The T -cell dependent antibody response (TDAR) to sheep red blood cell (SRBC) was measured with antibody -forming cell (AFC) assay.

There were no premature deaths and no treatment-related clinical signs. No treatment related effects on food and water consumption, mean body weights , organ weights and macroscopic findings in all treated groups. Positive control group treated with cyclophosphamide had no significant difference in body weight from the vehicle control group; but statistically significant decreased ($p<0.01$) in absolute and relative spleen and thymus weights.

EPA-HQ-2018-002024_0004925

The systemic NOAEL was 5000 ppm; equal to 1448 mg/kg bw per day, the highest dose tested.

There were no statistically significant differences observed in anti -SRBC AFC responses for Specific Activity (AFC/106 spleen cells) and Total Spleen Activity (AFC/spleen) in treated groups when compare to the vehicle control group. Positive con trol group had statistically significant ($p<0.05$) decrease in spleen cell numbers, mean specific activity, and mean total spleen activity. This confirmed the ability of the test system to detect immuno -suppressive effects and confirmed the validity of the study design. The Natural Killer (NK) cells activity was not evaluated in this study.

The NOAEL for immunotoxicity was 5000 ppm ; equal to 1448 mg/kg bw per day , HDT (Haas, 2012).

Effects on Salivary Gland:

Groups of 24 male Alpk: AP $_f$SD (Wistar-derived), Sprague-Dawley (Charles River CD) and Fischer 344 rats were administered diets containing 0 or 20,000 ppm glyphosate acid for 28 consecutive days. Eight animals from each group were sacrificed of Day 29 and the remaining rats were retained without treatment for an addition 4 (8 rats/group) or 13 weeks (8 rats/group).

Dietary exposure to 20,000 ppm glyphosate acid resulted in significant reductions in bodyweight and minor reductions in food consumption in AP and CD rats, but not in F344 rats. Salivary gl and weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the 4 week dietary exposure period. Microscopic examination of the salivary glands showed the most pronounced effected occurred in the F344 strain where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells occurred in the AP and CD strains.

After four weeks on the control diet there was significant recovery of the salivary gland changes in the F344 strain, while AP and CD rats were indistinguishable from their corresponding controls.

After 13 weeks on the control diet slightly more F344 glyphosate treated rats showed minor focal changes in the salivary gland compared to their controls and group mean salivary gland weights were increased slightly (Allen, 1996).

The purpose of this study was to evaluate the effects of a low pH diet on the parotid salivary glands of rats. There were five groups, each containing 10 male Crl:CD® (SD) rats, which were dosed for 56 consecutive days. A low pH diet containing 14,000 ppm citric acid was offered continuously to Group 4. A high pH diet with 21,400 ppm trisodium citrate dihydrate (with an equivalent citrate ion concentrate to Group 4) was offered continuously to Group 5. Group 2 (controls) received the basal diet. Citric acid in deionized water was administered orally by gavage at a dose level of 791 -1316 mg/kg bw per day to Group 3, with dosing calculated to maintain approximately equivalent citric acid dose levels to Group 4. Group 1 animals were gavaged with deionized water.

Test substance-related effects consisted of statistically significant higher parotid salivary gland weights in Group 4, as compared to their Group 2 contr ols. There were non -statistically significant higher parotid salivary gland weights in Groups 3 (gavage citric acid) and 5 (trisodium citrate dihydrate diet).

The report states that with the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are considered to be adaptive responses to local irritation from the low pH diet in the oral cavity rather than adverse effects((Haas, 2010).

The purpose of this study was to investigate rat strain susceptibility to the effec ts of glyphosate acid on the salivary glands when administered orally in the diet for 28 consecutive days and to monitor recovery over an additional 4 or 13 weeks.
Administration of diets containing 20,000 ppm glyphosate acid to male rats for 4 weeks produ ced minor strain differences in terms of systemic toxicity (changes in bodyweight and food consumption) and marked differences in the severity of effect on the parotid salivary gland. Significant reductions in body weight with minor reductions in food consumption were seen in AP and CD rats but not in the F344 rat. In contrast, salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats. Microscopic examination showed the most pronounced effect was in F344 rats where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but lesser effects involving small foci of cells only occurred in the AP and CD strains.

Complete recovery of effects was apparent in AP and CD rats following the 4 week recovery period. Although significant recovery of salivary gland change was evident in F344 rats this may not have been complete after a 13 week recovery period (Wood, 1996).

For the *in vivo* study five male and five female Sprague -Dawley (CD) rats were dosed with glyphosate Technical (95.3%) at a dose level of 5000 mg/kg bw with similar sized control groups receiving vehicle only. Approximately one hour after dosing control and treated animals were examined for either haematological changes, elect rocardiographic changes or behavioral/functional changes. *Ex vivo* studies were evaluation of the isolated guinea pig ileum and isolated rat gastrocnemius muscle using saturated solutions of the test material.

Results:

In Vivo Study: There were no differences in response between treated and control animals.

Ex Vivo Studies: Glyphosate Technical caused a contractile response to isolated guinea pig ileum similar to that seen with Acetyl Choline. The effect seen was negated when the ileum was pre - incubated with Atropine sulfate (Wood, E, 1996).

Irritation

A study was conducted in beagle dogs to evaluate the irritating effects of glyphosate formulation containing IPA salt (41%) and surfactant (15%) on stomach and ileum and compared with hydrochloric acid. In this study, a teflon coated catheter was inserted into intestinal duct for administration of test solutions. Each test article was left for 30 minutes. After the 30 minute of application, the stomach and intestine were washed with physiological saline. Tissues were examined histopathologically. Based on the histopathological findings, the study author concluded that the muscosal damage in the stomach and intestine caused by glyphosate formulation was mild equivalent to that caused by 0.25 N hydrochloric acid. The intestine was more severly damaged than the stomach in every case (Mizuyama, 1987).

Endocrine disruption

Executive Summary : Assessment of Endocrine Activity of Glyphosate in relation to Human Health

Information derived from the US EPA evalu ation of Glyphosate within the US Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery was specifically utilised, together with other scientifically relevant information, including general toxicity data and open literature studies of sufficient quality, were considered in this weight of evidence (WoE) assessment.
The Tier 1 battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This

EPA-HQ-2018-002024_0004927

interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In determining whether glyphosate interacts with E, A or T hormone pathways, the number and type of effects induced, the magnitude of responses, and the pattern of responses observed across studies, taxa, and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

This evaluation re-examines the data evaluates by the EDSP Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) weight-of-evidence (WoE) analysis of the potential interaction of glyphosate with the E, A or T hormone pathways, as conducted on September 17, 2014, and concurs with the overarching conclusions.

For the estrogen pathway, there was no evidence of potential interaction of glyphosate with the estrogen pathway in the EDSP Tier 1 in vitro assays [i.e, ER binding, ER transactivation assay (ERTA), aromatase and steroidogenesis assays]. Whilst glyphosate has been reported to show estrogen receptor (ER) agonism in vitro with estrogen-dependent human breast cancer cells (Thongprakaisang et al., 2013), there are confounding issues with this study, and other in vitro ER studies with glyphosate have not demonstrated an interaction (e.g. Kojima et al 2004).

Additionally, glyphosate was negative in the Tier 1 in vivo mammalian assays (i.e., uterotrophic or female pubertal assays). In the fish short-term reproduction assay (FSTRA), the non-treatment-responsive decrease [only significant at mid-treatment] in vitellogenin (VTG) was seen in isolation in the absence of any treatment-related effects in the other estrogen-related endpoints such as gonado-somatic index (GSI), gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology. In the open literature, glyphosate did not increase plasma VTG in levels in juvenile rainbow trout (Xie et al., 2005). There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies (decreases in offspring body weight observed in one avian reproduction study). Therefore, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate.

For the androgen pathway, there was no evidence of interaction of glyphosate with the androgen pathway in the Tier 1 in vitro, via androgen receptor (AR) binding, and glyphosate was negative in an AR transactivation assay (Kojima et al., 2004, 2010). However there is conflicting evidence for the aromatase and steriodogenesis assays which were negative for glyphosate alone in the US EPA evaluation and a murine in vitro model (Forgacs et al 2012), but positive for the co formulants in another laboratory (Benechour et al 2007, Defarge et al 2016), with mechanistic underpinning via both the regulatory protein StAR, and the P450scc cleavage enzyme first shown by Walsh et al 2000. 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 separation (PPS) at 1234 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 (1000 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).

For the thyroid pathway, there was no convincing evidence of potential interaction of glyphosate. There were no treatment-related effects on thyroid hormones (T4 and TSH), thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. There were no thyroid-related effects observed in the female pubertal assay. In the amphibian metamorphosis assay (AMA), there were no developmental effects or alterations in thyroid histopathology. No thyroid-related effects were noted in any of the Part 158 studies.

For glyphosate, little data is available to inform upon other endocrine mediated effects, for example in relation to retinoids, Vitamin D receptor, metabolic syndrome, obesogens, glucocorticoids etc. This is a major data gap. In non mammalian models there are two endocrine relevant pathways that have been reported: evidence of retinoic acid dysfunction has been observed in tadpoles exposed to glyphosate formulation. One academic (non industry funded) report looking at stress responses via inhibition of cortisol response in fish of selected pesticides is notable because glyphosate did not present a stress

response inhibition, unlike most of the other test pesticides (Koakoski et al 2014). Whilst glyphosate was not included in the recent Toxcast screens due to solubility issues, mechanistic information on the induction of receptors such as AhR (Takeuchi et al 2008), PPARs (Vaino et al 1983; Kojima et al 2010) and PXR (Kojima et al 2010) are all negative.
Toxcast endocrine receptor/mechanistic data on the co formulants …
Adverse endocrine impacts due to glyphosate poisoning in humans, clinically, have not been reported by poison centres (Bradberry et al 2004), and no recent updates are available.

EDSP studies (May want to delete it since these studies are summarized in a table).

In Vitro assays

Androgen receptor binding

Willoughby, J.A. (2012a). Glyphosate: Androgen Receptor Binding (Rat Prostate Cytosol) Screening Assay. CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V -100334ARB, March 8, 2012. Unpublished.

In an *in vitro* androgen receptor (AR) competitive binding assay, the binding of a single concentration (1 nM) of [^{3}H]-R1881 (reference androgen) in the presence of increasing concentrations (10^{-10} to 10^{-3} M) of glyphosate (purity 95.93%) was measured. Sprague Dawley rat ventral prostate cytosol was the source of the AR for the study. Low-salt TEGD buffer was used as the vehicle for glyphosate. A total of 3 runs were performed, and each run included dexamethasone as a weak positive control, and R1881 as the ligand reference standard.

The saturation binding curves showed a dissociation constant (K_d) for [^{3}H]-R1881 of 0.613±0.041 nM and an estimated B_{max} of 0.817±0.049 fmol/100 µg protein for the batch of prostate cytosol used in the study. In the competitive b inding runs, the estimated mean log IC_{50}s for R1881 (strong positive control) and the weak positive control (dexamethasone) were −9.0 and −4.6 M, respectively, and t he mean relative binding affinity (RBA) for the weak positive control, dexamethasone, was 0 .004%. At glyphosate concentrations of 10^{-10} to 10^{-3} M, specific binding of [^{3}H]-R1881 was 92.4 -101.3% with the exception of one concentration (10^{-9} M) in Run 1, which had an average binding of 66.5%. Review of the data indicated that this value was a result of a single replicate with a specific binding of 7.5%. Excluding this value yielded a mean specific binding of 96.0%, which concurs with the other runs. Since the specific binding was >75% at all concentrations of glyphosate in all runs, no IC_{50} or RBA values were estimated. Based on the results from the three runs, glyphosate does not competitively bind to the AR (Willoughby, 2012).

Estrogen receptor binding

Willoughby, J.A. (2012b). Glyphosate: Estrogen Receptor Binding (Rat Uterine Cytosol). CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V-100364ERB, March 8, 2012. Unpublished

In an estrogen receptor (ER) binding assay, the binding of a single concentration of [^{3}H]-17β-estradiol (1 nM) in the presence of increasing concentrations (10^{-10} to 10^{-3} M) of glyphosate (purity 95.93%) was measured. TEGD buffer was used as the solvent vehicle for glyphosate. A total of 3 runs were performed, and each run included 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control, and 17-β-estradiol as the natural ligand reference chemical.

The K_d for [^{3}H]-17β-estradiol was 0.331 ± 0.061 nM and the estimated Bmax was 74.55 ± 3.03 fmol/100 µg protein for the prepared rat uterine cytosol. The K_d for each run was within the expected range of 0.03 to 1.5 nM. In the competitive binding experiment, the estimated mean log IC_{50}s for 17β-estradiol and 19-norethindrone were −9.0 and −5.5 M, respectively. The mean relative binding affinity (RBA) was 0.032% for 19 -norethindrone, compared to the natural ligand. Glyphosate was

EPA-HQ-2018-002024_0004929

tested over a concentration range (10^{-10} to 10^{-3} M) that fully defined the top of the curve. Across all runs, the lowest average percent radiolabeled estradiol binding in the presence of glyphosate was >81% (*i.e.* showed less than 25% displacement) at concentrations up to 10^{-3} M. Based on the results from the three runs, glyphosate does not competitively bind to the estrogen receptor (Willoughby, 2012b).

Estrogen receptor transcriptional activation

Willoughby, J.A. (2012 c) Estrogen Receptor Transcriptional Activation (Human Cell Line (HeLa -9903)) Screening Assay with Glyphosate. CeeTox, Kalamazoo, MI. Laboratory Report No.: 6500V-100334ERTA, March 8, 2012. Unpublished.

In an estrogen receptor (ER) transcriptional activation assay, hERα-HeLa-9903 cells cultured *in vitro* were exposed to glyphosate (purity 85.14%) at logarithmically increasing concentrations from 10^{-10} to 10^{-3} M in cell culture media for 24 hours in three independent runs. The experiments were performed using 96-well plates and each glyphosate concentration was tested in 6 wells/plate in each run. The solvent vehicle was culture media for glyphosate and DMSO (0.1%) for the reference chemicals. Cells were exposed to the test agent for 24±2 hr to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor was measured using a luciferase assay.

Glyphosate was tested up to the limit dose, with no precipitation or cytotoxicity observed at any tested concentration. At concentrations up to 10^{-3} M, the relative transcriptional activation of glyphosate was ≤2.4%. Glyphosate was only able to reach a maximum of 0.8-2.4% of the positive control (PC) 1 nM 17β-estradiol when tested up to the highest concentration. Because the RPC_{Max} (maximum level of response induced by a test chemical, expressed as a percentage of the response induced by the positive control) was less than the PC_{10} (concentration of a test chemical at which the response is 10% of the response induced by the positive control in both assay runs, glyphosate was considered negative for estrogen receptor transcriptional activation in this test system (Willoughby, 2012c).

Aromatase

Wilga, P.C. (2012). Glyphosate: Human Recombinant Aromatase Assay. CeeTox, Inc., Kalamazoo, MI. Laboratory Study No.: 6500V-100334AROM, March 9, 2012. Unpublished.

Glyphosate (purity 95.9%) was evaluated for its potential to inhibit aromatase activity by incubating with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H(N)]-androst-4-ene-3,17-dione; [^{3}H]ASDN) at log concentrations of 10^{-10} to 10^{-3} M glyphosate. The solvent vehicle was 0.1 M phosphate buffer for glyphosate, ethanol for ASDN, and dimethyl sulfoxide (DMSO) for 4-OH ASDN, with a final assay volume of ≤1% DMSO. Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting (LSC). Each run included a full activity control, a background activity control, a positive control series (10^{-10} to 10^{-5} M) with a known inhibitor (4-hydroxyandrostenedione; 4-OH ASDN), and the test chemical series (10^{-10} to 10^{-3} M) with three repetitions per concentration.

Aromatase activity in the full activity controls was 0.676 ± 0.072 nmol·mg-protein^{-1}·min^{-1}. The response of each full activity control within a run was between 90 to 110% of the average full activity. Activity in the background controls ranged 0.23 to 0.38% and averaged 0.30% of the full activity control. For the positive control substance (4-OH ASDN), the estimated log IC_{50} averaged −7.29 M and the Hill slope was −0.96. For glyphosate, aromatase activity averaged 0.673 ± 0.066 nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration of 10^{-10} M and 0.741 ± 0.100 nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration of 10^{-3} M. The average aromatase activity was

≥99.67% of the control at all tested glyphosate concentrations for all runs. The results indicate that glyphosate does not inhibit aromatase activity (Wilga, 2012).

Steroidogenesis

Hecker, M., Hollert H., Cooper, R., *et al*. (2011). The OECD validation program of the H295R steroidogenesis assay: phase 3. Final inter -laboratory validation study. Environ. Sci. Pollut. Res. (2011) 18:503-515.

The purpose of this study was to validate the use of a standardized steroidogenesis assay as detailed in OECD Guideline for the Testing of Chemicals: Draft Proposal for a New Guideline 4XX – The H295R Steroidogenesis Assay. In this validation study, 28 chemicals were selected as a screen for potential effects of endocrine -disrupting chemicals on the production of testosterone (T) and 17β -estradiol (E2). These chemicals were selected based on their known or suspected endocrine activity, or lack thereof, and included inhibitors and inducers of different potencies as well as positive and negative controls. In this steroidogenesis assay, H295R cells cultured in vitro in 24 -well plates were incubated with glyphosate (purity and lot # not provided) at seven concentrations between 0.0001 and 100 µM for 48 hours in triplicate for three independent experiments. A quality control (QC) plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentrations; positive controls included the known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production. T and E2 levels were measured using radioimmunoassays or ELISA; responses of the QC plates measured by these assays were confirmed by LC -MS. In this validation study, the laboratories demonstrated that glyphosate does not affect testosterone or estradiol levels in this assay(Hecker, et al., 2011).

In Vivo assay

Hershberger assay

Stump, D. G. (2012 a). A H ershberger Assay of Glyphosate Administered Orally in Peripubertal Orchidoepididymectomized Rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Report No.: WIL-843003, January 6, 2012. Unpublished.

In a Hershberger Assay screening for androgen ic activity, glyphosate (purity 85.1) in 0.5% methylcellulose (w/v) was administered daily via oral gavage (5 mL/kg) to groups of six 54 - or 55-day old, castrated male Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 (limit dose) mg/kg bw per day. The androgenic positive control group consisted of 6 castrated rats exposed to 0.2 mg/kg/day of testosterone propionate (TP) by subcutaneous (s.c.) injection. To screen for potential anti androgenic activity, glyphosate in 0.5% methylcellulos e (w/v) was administered daily via oral gavage to groups of six 54 - or 55-day old, castrated male Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 mg/kg/day in conjunction with a daily dose of reference androgen TP at 0.2 mg/kg/day by s.c. injection. The anti androgenic positive control group consisted of 6 castrated rats exposed to 0.2 mg/kg/day TP by s.c. injection and 3 mg/kg/day flutamide (FT) via oral gavage. TP alone was used as the anti androgenic negative control. For both components of the assay, body weights were determined daily. The animals were dosed for 10 consecutive days and terminated approximately 24 hours after the final dose. At necropsy, the five androgen -dependent tissues were collected and weighed.
In the androgen agonist assay, there were no treatment -related effects on body weights, overall body weight gains, or the weights of accessory sex organs for any glyphosate dose group. Animals in the positive TP control group had increased ($p<0.01$) accessory sex orga n weights as follows: 437% in seminal vesicles; 728% in ventral prostate; 200% in levator ani -bulbocavernosus (LABC); 361% in Cowper's gland; and 45% in glans penis. The performance criteria indicated that this assay was performing as expected.

In the anti-androgen assay, there were no treatment-related effects on body weights, overall body weight gains, or the weights of accessory sex organs for any glyphosate dose group. Animals dosed with TP + FT (positive control) had decreased ($p<0.01$) accessory sex organ weights as follows: 76% in seminal vesicles; 80% in ventral prostate; 63% in LABC; 70% in Cowper's gland; and 29% in glans penis. The performance criteria indicated that this assay was performing as expected.

Statistically significant changes were not seen in two or more of the five androgen sensitive tissue weights. Glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay (Stump, 2012a).

Uterotrophic assay

Stump, D. G. (2012b). A Uterotrophic Assay of Glyphosate Administered Orally in Ovariectomized Rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Report No.: WIL-843002, January 6, 2012. Unpublished.

In a Uterotrophic Assay conducted to screen for potential estrogenic activity, glyphosate (purity 85.1%) in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six ovariectomized female Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300, or 1,000 (limit dose) mg/kg bw per day on post-natal days (PND) 66/67 to 68/69. The positive control group was treated with a daily dose of 17α-ethynyl estradiol (EE) at 3 µg/kg/day by oral gavage. Body weights were determined daily. All animals were terminated and necropsied on PND 69/70 approximately 24 hours after the final dose administration to determine wet and blotted uterine weights.

All animals survived until scheduled termination and no treatment-related clinical findings were observed in glyphosate dosed animals. Body weights, body weight gains, and uterine weights in the glyphosate groups were comparable to the vehicle control. As expected, in positive control (EE) group the absolute wet and blotted uterus weights were increased by 758% and 256% respectively.

Glyphosate is negative in the uterotrophic assay (Stump, 2012b).

Male pubertal assay

Stump, D.G. (2012c) A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Male Rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Project ID: WIL-843005, April 10, 2012. Unpublished.

In a Male Pubertal Assay), 15 Crl:CD(SD) Sprague-Dawley rats/dose group were treated daily via oral gavage (5 mL/kg) with glyphosate (purity 95.93%) in 0.5% methylcellulose at doses of 0, 100, 300 or 1000 mg/kg bw per day (limit dose) from post-natal day (PND) 23 to 53. Animals were examined for preputial separation (PPS) daily beginning on PND 30, and age and weight at day of attainment were recorded. Following sacrifice on PND 53, blood was taken for total thyroxine (T4), testosterone, thyroid stimulating hormone (TSH), and clinical chemistry analysis. The hormones were analyzed by radioimmunoassay (RIA) or chemiluminescence.
Treatment-related clinical findings were limited to rales in 9/15 and 14/15 males in the 300 and 1000 mg/kg/day groups, respectively, approximately 4 hours post-dosing. This finding persisted in the daily examinations in 7/15 males at 1000 mg/kg/day throughout the study. On PND 53, final body weights in the 300 and 1000 mg/kg/day groups were decreased ($p<0.05$) by 7-10%. A treatment-related delay in the mean age at attainment of complete PPS was noted at 1000 mg/kg/day (48.0 days) compared to controls (45.9 days). However, it was determined that the delay in attainment of complete PPS at this dose was a result of the treatment-related decrease in body weight, rather than a direct anti-androgenic effect. No compound-related effects on organ weights were observed at any dose. No treatment-related effects on T4, TSH, or testosterone levels were observed at any dose. At 1000 mg/kg/day, there was a slight increase in the number of animals with colloid area Grade 4 (5 treated vs. 1 control) and Grade 5 (1 treated vs. 0 controls). There were no treatment-related effects

on follicular cell height at any dose compared to controls. There were no treatment-related findings in the testes, epididymides or kidneys.

In conclusion, glyphosate did not affect the maturation and produced any thyroid toxicity at doses up to 1000 mg/kg bw per day (Stump, 2012c).

Female pubertal assay

Stump, D.G. (2012 d) A Pubertal Development and Thyroid Function Assay of Glyphosate Administered Orally in Intact Juvenile/Peripubertal Female Rats. WIL Research Laboratories, LLC, Ashland, OH, Laboratory Project ID: WIL-843007, April 10, 2012. Unpublished.

In a Female Pubertal Assay, 15 Crl:CD(SD) Sprague -Dawley rats/dose group were treated daily via oral gavage with glyphosate (purity 95.93%) in 0.5% methylcellulose at doses of 0, 100, 300 or 1000 mg/kg bw per day (limit d ose) from post -natal day (PND) 22 to 42. Animals were examined for vaginal opening (VO) daily beginning on PND 22, and age and weight at day of attainment were recorded. Following sacrifice on PND 42, blood was collected for clinical chemistry analyses, including total thyroxine (T4) and thyroid stimulating hormone (TSH) levels, which were analyzed using an electrochemiluminescent immunoassay (T4) and a magnetic [125I]rTSH gamma counter immunoassay (TSH).
One animal in the control group was sacrificed *in extremis* on PND 27 due to impairment of the right forelimb (due to possible mechanical injury). There were no treatment -related differences in age of attainment of VO, body weight at VO, final body weights, or body weight gains in the treated groups relative to controls. One female each in the control and 300 mg/kg/day groups failed to attain VO. There were no statistically significant differences in mean age at first vaginal estrus, mean cycle length, or percent cycling. The cycle status at necropsy was similar among all groups. Serum T4 and TSH were not affected by treatment, and no adverse treatment -related effects on any clinical chemistry parameter were observed at any dose. There were no treatment -related microscopic findings in the thyroid, ovaries, uterus, or kidneys at any dose.

In conclusion, glyphosate did delay the maturation and no treatment related effects were seen in thyroid toxicity (Stump, 2012d).

Key for symbols:

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
2: no influence on likelihood
? : Influence on likelihood too difficult to assess
Ranges of symbols may be used to indicate more uncertainty about a line of evidence (e.g. ↑/↑↑).

EPA-HQ-2018-002024_0004933

1 **Table 6 4 :Summary table of supporting information to the US EDSP data in relation to**
2 **Glyphosate and endocrine endpoints**

Endpoint Pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion	Reference Conclusion	Reference
Estrogen pathway						
US EDSP Tier 1 data 2014/2015: *In vitro:* ER binding-negative; TG 455 ER STTA Hela Assay-Negative *In vivo:* mammalian assays :i.e., uterotrophic and female pubertal assays and mammalian toxicity studies.	Glyphosate	US validated assays. In vitro assays well characterised and OECD TGs. ER STTA: uses HeLa cell line which has ER alpha not ER beta. ER alpha 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	EDSP 2015
In vitro: ER agonism in estrogen-dependent human breast cancer cells	Glyphosate (>98%) Accustandard.	Validated assay	Cell line contains both ER alpha and ER beta. Luciferase reporter system used with combinations of an isoflavone present in soy: genistein. Phytoestrogens such as genistein are known to overstimulate luciferase, and also are stronger ligands for ER beta. Non-receptor-mediated luminescence signals have been reported at phytoestrogen concentrations higher than 1 μM due to the over-activation of the luciferase	Low	Positive	Thongprakaisang et al., 2013

			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 ER TA assay systems . See Annex 2 of OECD TG 455. Tested with ER antagonist ICI 182780 to inhibit the estrogen mediated response. This can exclude the possibility of dioxin like interference of co-formulant contaminant 1,4 Dioxane with AhR interactions affecting the ER. However it was not tested with an ERα specific antagonist, such as Methylpiperidino pyrazole CAS № 289726-02-9 (MPP), which would determine the relative activities of each ER (Evans, N., L. E. GRAY, AND V. S. WILSON. Validation of T47D-KBluc cell assay for detection of estrogen receptor agonists and antagonists.			

			Presented at Society of Toxicology (SOT) Annual Meeting, San Francisco, CA, March 11 - 15, 2012.)			
In vitro: hER alpha and hER beta (ant)agonism in reporter gene transfected CHO cells	Glyphosate (>95-100%) whether formulation given is not specified in the paper			Med	Negative	Kojima et al 2004, 2010
In vitro: hER alpha and hER beta transient transfection into human hepatocarcinoma Hep G2 cells	Glyphosate formulations and Glyphosate parent chemical	Non validated assays, but well recognised and reliable hepatic cell line	Formulations reduced transcription of ER alpha and ER beta in ERE transiently transfected HepG2 cells, but glyphosate parent did not.	Med	Parent-negative Formulations-positive	Gasnier et al 2009
In vivo fish short-term reproduction assay (FSTRA)		Validated assay), the non-treatment-responsive decrease [only significant at mid-treatment] in vitellogenin (VTG) was seen in isolation in the absence of any treatment-related effects in the other estrogen-related endpoints such as gonado-somatic		Med	Negative	EDSP 2015

		index (GSI), gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology.				
In vivo Rainbow trout VTG assay	glyphosate and glyphosate plus surfactants	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) Xie et al., 2005.
E pathway: overall conclusion	**<u>No convincing evidence of a potential interaction with the estrogen pathway for glyphosate.</u> The one in vitro study that is positive, has not been reproduced by another laboratory.**					
Androgen pathway						
US EDSP Tier 1 data 2014/2015: *In vitro:* negative; both for AR binding and the aromatase assay, *In vivo:* mammalian assays : Hershberger and male	glyphosate	Standardised and validated assays	AR binding assay not a validated OECD TG. But other validated AR assays not available in 2014/2015. Aromatase assay highest soluble test concentration of glyphosate was 10^{-3} M. The in vivo Tier 1 FSTRA and mammalian assays (i.e., Hershberger and	High	Negative, but sperm count and delay in preputial separation effects seen at very high doses, greater than 1000mg/kg bw per day	EDSP 2014/2015

pubertal assays			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 separation (PPS) at 1234 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 (1000 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			Med	Negative	Kojima et al., 2004, 2010.
In vitro: hAR transient transfection into human hepatocarcinoma Hep G2 cells, aromatase evaluation within the Hep G2	Glyphosate and formulations	Non validated assays, but well recognised and reliable hepatic cell line. Method for aromatase activity	MDA-MB453-kb2 cell line has a high content of glucocorticoid receptor (GR) in addition to AR. The characterisation of the cell line and discussion of such confounding factors is not considered in the paper. Whilst	Low	Positive	Gasnier et al 2009*

cells, and MDA-MB453-kb2 cells		evaluation is also part of OECD TG 456 for steroidogenesis.	glyphosate and formulations reduced androgen receptor transcription in this cell line, there appears to have been no control with androgen specific responses, to exclude glucocorticoid specific responses.			
Steroidogenesis *In vitro:* Transformed and human aromatase transfected cDNA in human embryonic kidney 293 cells and placental-derived JEG3 cells, *ex vivo:* normal human placenta and equine testis.	Glyphosate and formulations	Relevant cell models, but limited characterisation 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, for example due to degradation of the enzyme, and therefore performance criteria are specified in guideline OPPTS 890.1200 in order to demonstrate that the assay is functioning correctly. This is addressed in the US EDSP data, but is not evident in the papers from the laboratory of Seralini, although the reference to 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) is used as a positive control chemical in each experiment. Whilst the correct	Low-Med	Positive	Benachour et al 2007*

EPA-HQ-2018-002024_0004939

			positive control was used, profiency testing is not reported in the paper. Compliance with the performance criteria should be checked before evaluating results from this assay. A positive result in guideline OPPTS 890.1200 requires demonstration of inhibition of aromatase activity that fits a 4-parameter nonlinear regression model and such that the concentration response curve crosses 50% inhibition. The concentration response curve allows the determination of potency i.e. IC50 (concentration at which the activity of aromatase is reduced to 50% of control values). In some cases, variability may be due to limited solubility of a chemical.			
Steroidogenesis *In vitro:* placental-derived JEG3 cells	Glyphosate and formulation ingredients Top dose 100ppm		The co-formulants were each tested independently, and are reported to inhibit aromatase activity at concentrations 20%-67% below the NOEC, where glyphosate alone did not significantly inhibit aromatase at these levels. See also comment above regarding proficiency testing of the assay.	Low-Med	Positive	Defarge et al 2016* *: all from the same Seralini laboratory
Steroidogenesis *In vitro:* BLTK1	Glyphosate (300µM)	Relevant and well characterised leydig	No effect on basal or recombinant human chorionic gonadotrophin	Med-High	Negative	Forgacs et al 2012

Murine Leydig cells		cell model				
Steroidogenesis *In vitro:* Steroidogenic Acute Regulatory protein (StAR) in a mouse MA-10 Leydig tumor cell	Glyphosate formulation (containing 180g/L Glyphosate)	Relevant and well characterised cell model.	Statistically significant reduction ($p<0.01$) of $(Bu)_2cAMP$with the Glyphosate formulation, was observed after a 2 hour treatment. Statistical significance ($p<0.01$) also reported for P450scc the enzyme that is responsible for the conversion of cholesterol to pregnenolone, and initiating the synthesis of all steroid hormones.	Med-High	Positive	Walsh et al 2000
A pathway: overall conclusion	**<u>No convincing evidence of a potential interaction with the androgen receptor pathway for glyphosate.</u> Decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial separation (PPS) at 1234 mg/kg bw per day in the two-generation reproduction study in rats 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 of an impact upon the steroidogenesis pathway, via P450scc, and StAR. Also for the glyphosate co-formants. <u>This requires further investigation</u>.**						
Thyroid						
US EDSP Tier 1 data 2014/2015: *In vitro:* No assays conducted. *In vivo* test battery: There were no treatment-related effects on thyroid hormones (T4 and TSH), thyroid weights or thyroid histopathology in the male pubertal	Glyphosate	Relevant and validated test methods.	No convincing evidence of potential interaction of glyphosate.	High	Negative	EDSP 2014/2015

assay in the absence of overt toxicity. There were no thyroid-related effects observed in the female pubertal assay. In the amphibian metamorphosis assay (AMA), there were no developmental effects or alterations in thyroid histopathology. No thyroid-related effects were noted in any of the Part 158 studies.						
T pathway: overall conclusion	**No convincing evidence of a potential interaction with the thyroid pathway for glyphosate.**					
Other endocrine mechanisms						
Retinoid system *In vivo Xenous Laevis embryo* model and chicken embryos	360 pg and 5000 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 glyphosphate formulations. Suspected to be resulting from a dysfunctional retinoic acid or Sonic hedgehog pathway. Further information needed.	Med-High	Positive: increase in endogenous retinoic acid activity.	Pagnelli et al 2010

Cortisol *In vivo* fish study *Rhamdia quelen* fingerlings	Glyphosate formulation 360g L^{-1}	Stress response of *Rhamdia quelen* fingerlings acute exposure at 45, 90. 135 and 180d	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	Positive	Koakoski et al 2014
Hypolipdemia and peroxisome proliferation *In vivo* rat	Glyphosate formulation 300 mg/kg single daily dose for 2 weeks 5 animals per dose 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 $\leq 10^{-5}$M.	Recognised assay		Med	Negative	Takeuchi et al 2008
In vitro mPPARalpha, mAhR, hPXR	Glyphosate.		Review, insufficient detail given. Concentration tested not given for negative test chemicals	Low	Negative	Kojima et al 2010
Other endocrine pathways: overall conclusion	Suggestion of adverse effect upon retinoic acid pathways and cortisol stress pathways. The latter also suggesting a plausible, but not demonstrated interaction with the glucocorticoid pathways. Data gap. Further investigation required.					

References

US EPA EDSP 2014/2015 not sure how to cite

Benachour N, Séralini GE (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chem Res Toxicol, 22(1):97 –105. doi:10.1021/tx800218n PMID:19105591

Benachour N, Sipahutar H, Moslemi S, G asnier C, Travert C, Séralini GE (2007). Time - and dose-dependent effects of Roundup on human embryonic and placental cells. Arch Environ Contam Toxicol, 53(1):126–33. doi:10.1007/s00244-006-0154-8 PMID:17486286

Defarge, N., Takács, E., Lozano, V. L., Mesnage, R., Spiroux de Vendômois, J., Séralini, G.-E., & Székács, A. (2016). Co -Formulants in Glyphosate -Based Herbicides Disrupt Aromatase Activity in Human Cells below Toxic Levels. *International Journal of Environmental Research and Public Health*, *13*(3), 264. http://doi.org/10.3390/ijerph13030264

Escande A., et al. (2006). "Evaluation of Ligand Selectivity Using Reporter Cell Lines Stably Expressing Estrogen Receptor Alpha or Beta", Biochem. Pharmacol., 71, 1459-1469.

Koakoski, G., Quevedo, R. M., Ferreira, D., et al. (2014). Agrichemicals chronically inhibit the cortisol response to stress in fish. *Chemosphere 112*, 85-91. http://dx.doi.org/http://dx.doi.org/10.1016/j.chemosphere.2014.02.083

Kuiper G.G., et al. (1998). "Interaction of Estrogenic Chemicals and Phytoestrogens with Estrogen Receptor Beta", Endocrinol., 139, 4252-4263.5373.

Forgacs AL, Ding Q, Jaremba RG , Huhtaniemi IT, Rahman NA, Zacharewski TR (2012). BLTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. Toxicol Sci, 127(2):391–402. doi:10.1093/toxsci/kfs121 PMID:22461451

Gasnier C, Benachour N, Clair E, Travert C, Langlois F, Laurant C et al. (2010). Dig1 protects against cell death provoked by glyphosate -based herbicides in human liver cell lines. J Occup Med Toxicol, 5(1):29 doi:10.1186/1745-6673-5-29 PMID:20979644

Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE (2009). Glyphosate -based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology, 262(3):184 –91. doi:10.1016/j. tox.2009.06.006 PMID:19539684

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004). Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ Health Perspect, 112(5):524–31. doi:10.1289/ehp.6649 PMID:15064155

Kojima H, Takeuchi S, Nagai T (2010). Endocrinedisrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor J Health Sci, 56(4):374– 86. doi:10.1248/jhs.56.374

Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010). Glyphosate -based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. Chem Res Toxicol, 23(10):1586–95. doi:10.1021/tx1001749 PMID:20695457

Takeuchi S, Iida M, Yabushita H, Matsuda T, Kojima H (2008). In vitro screening for a ryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450–1A induction by propanil, diuron and linuron. Chemosphere, 74(1):155–65. doi:10.1016/j.chemosphere.2008.08.015 PMID:18835618

Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem Toxicol, 59:129 –36. doi:10.1016/j. fct.2013.05.057 PMID:23756170

Vainio H, Linnainmaa K, Kähönen M, Nickels J, Hietanen E, Marniemi J et al. (1983). Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. Biochem Pharmacol, 32(18):2775–9. doi:10.1016/0006-2952(83)90091-6 PMID:6626247

Walsh LP, McCormick C, Martin C, Stocco DM (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environ Health Perspect, 108(8):769–76. doi:10.1289/ ehp.00108769 PMID:10964798

Xie L, Thrippleton K, Irwin MA, Siemering GS, Mekebri A, Crane D et al. (2005). Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. Toxicol Sci, 87(2):391–8. doi:10.1093/toxsci/kfi249 PMID:16049272

Glyphosate/Intestinal Microbiota Effects

Scientific searching for glyphosate

We used several trusted search engines that provide a personal and customized way to search research materials on the chemicals. The search engines include

1) Google Scholar (http://scholar.google.com/)
2) PubMed (http://www.ncbi.nlm.nih.gov/pubmed)
3) WEB OF SCIENCE (https://apps.webofknowledge.com)
4) BioOne (http://www.bioone.org/)
5) ScienceDirect (http://www.sciencedirect.com/).

Basically, we used a keyword searching strategy using the keywords (diazinon, glyphosate, malathion, microbiome, microbiota, gut microbiota, gut microbiome, gastrointestinal microbiota, gastrointestinal microbiome, etc.) and the Boolean Operators (AND, OR, and NOT).

Glyphosate/Intestinal Microbiota Effects

Knowledge Gaps

- ***No specific information that addresses whether or not glyphosate affects the microbiota in the human gastrointestinal tract.***
- ***No specific studies on the effects of glyphosate on the mammalian gut microbiota in mouse, rat, rabbit, or humans, i.e., lack of in vivo studies–all reports are in vitro tests***
- ***No data on whether glyphosate bioaccumulates in the human gastrointestinal tract***
- ***No data that directly show that intestinal microbiota has the ability to metabolize glyphosate***
- ***No data on the microbiological activity of the glyphosate metabolites, i.e., aminomethylphosphonic acid***
- ***No studies that contain measurements of the amount of glyphosate residues in the gastrointestinal tract***

Background

Glyphosate (N-(phosphonomethyl) glycine) is an active ingredient of herbicides, including Roundup®, used for the control of a broad spectrum of grasses and broadleaf weed species in agricultural and industrial fields. Glyphosate specifically inhibits 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS), an enzyme of the shikimate pathway that governs the synthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan in higher plants, algae, bacteria and fungi (Banta et al. 2009). Its herbicidal action is generated by chelating manganese required in the reduction of the flavin mononucleotide (FMN) co-factor EPSPS (Cerdeira and Duke, 2006). Since bacteria have EPSPS and produce amino acids via the shikimate pathway, there is potential for

glyphosate residues to dis rupt and have antimicrobial effects on the microbial community in the human gastrointestinal tract. However, based on a review of the published scientific literature, there have not been any studies that specifically address whether or not glyphosate affects the microbiota in the human gastrointestinal tract or in mouse and rat animal models. Interestingly, what is known is that intestinal microbiota from dairy cows and poultry, selected bacterial pathogens and probiotic bacteria can be affected differently by residual levels of glyphosate.

Special Studies on Microbiological Effects of Glyphosate

Shehata et al. (2013) determined the effect of glyphosate on the growth and viability of poultry microbiota and pathogens that included *Salmonella enteritidis, Sa lmonella gallinarum, Salmonella typhimurium, Clostridium perfringens, Clostridium botulinum, Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis, Escherichia coli, Lactobacillus* spp., *Campylobacter* spp., *Staphylococcus aureus, Staphylococcus haemolyticus* and *Staphylococcus lentus*. The minimal inhibitory concentration (MIC) of glyphosate was determined in triplicate in 24 - well microtiter plates. 100 µl of the tested bacteria (10^5 cfu/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 and 5.0 mg/ml). Plates containing glyphosate and bacteria were incubated at 37ºC. MIC values were determined by quantitative analysis of bacteria on agar plates (Table 65).

C. perfringens, S. gallinarum, S. typhimurium, S. enteritidis and *E. coli* were highly resistant to glyphosate (MIC value of 5 mg/ml). *L. casei, L. buchneri, L. harbinensis, S. aureus, S. lentus* and *S. haemolyticu*s were moderately resistant to glyphosa te (MIC values of 0.60 to 0.30 mg/ml). On the other hand, with the exception of *Lactobacillus* spp., all tested bacteria including *E. faecalis, E. faecium* and *B. badius; B. cereus;* and *B. adolescentis* were highly sensitive to glyphosate with MIC values of 0 .15, 0.30 and 0.075 mg/ml, respectively. Also, pathogenic *E. coli* and *E. coli* 1917 strain Nissle were found also to be resistant to glyphosate (MIC of 5 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 (Table 66).

In summary, Shehata et al. (2013a) reported that glyphosate showed differences in sensitivity between potential pathogens and beneficial microbiota from chickens. A reduction of beneficial bacterial species in the gastrointestinal tract microbiota by ingestion of glyphosate -contaminated feed could disturb the normal gut bacterial community.

Table 1 Inhibitory effect of glyphosate on different bacteria

Genus/species	MIC value of glyphosate (mg/ml)[a]	Bacterial count[b]	
		Treated at MIC value (mean ± SD, $n = 3$)	Untreated (mean ± SD, $n = 3$)[c]
Bacillus badius	0.150	2.24 ± 0.49	8.90 ± 0.44
Bacillus cereus	0.300	2.75 ± 0.68	8.08 ± 0.12
Bacteriodes vulgatus	0.600	3.54 ± 0.31	7.37 ± 0.10
Bifidobacterium adolescentis	0.075	3.87 ± 0.50	8.67 ± 0.48
Campylobacter coli	0.150	3.07 ± 0.50	9.00 ± 0.70
Campylobacter jejuni	0.150	3.90 ± 0.50	9.54 ± 0.97
C. perfringens	5.000	3.37 ± 0.89	8.30 ± 0.28
C. botulinum type A	1.200	4.00 ± 0.50	8.16 ± 0.32
C. botulinum type B	1.200	3.56 ± 0.45	7.60 ± 0.57
E. coli	1.200	3.15 ± 0.24	8.00 ± 0.34
E. coli 1917 strain Nissle	1.200	2.35 ± 0.24	7.26 ± 0.21
Enterococcus faecalis	0.150	2.00 ± 0.45	8.49 ± 0.58
Enterococcus faecium	0.150	2.01 ± 0.34	7.06 ± 0.95
Lactobacillus buchneri	0.600	4.00 ± 0.88	8.00 ± 0.22
Lactobacillus casei	0.600	4.74 ± 0.56	8.28 ± 0.35
Lactobacillus harbinensis	0.600	5.30 ± 0.44	8.40 ± 0.32
Riemerella anatipestifer	0.150	4.00 ± 0.50	7.88 ± 0.50
Salmonella Enteritidis	5.000	2.35 ± 0.26	8.28 ± 0.16
Salmonella Gallinarum	5.000	2.15 ± 0.33	8.68 ± 0.20
Salmonella Typhimurium	5.000	2.75 ± 0.68	8.03 ± 0.16
Staphylococcus aureus	0.300	5.74 ± 0.58	9.00 ± 0.10
Staphylococcus haemolyticus	0.300	5.74 ± 0.32	8.08 ± 0.16
Staphylococcus lentus	0.300	3.90 ± 0.44	8.08 ± 0.14

[a] Minimal inhibitory concentration (MIC) of Glyphosate
[b] Mean of quantitative bacterial counts expressed as reciprocal $\log_{10}$
[c] Bacterial counts without glyphosate treatment (control)

Shehata et al. 2013 [a]

Clair et al. (2012) evaluated Roundup® an d its glyphosate ingredient for their effects on the growth and viability of three food -associated microorganisms, *Geotrichum candidum, Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus* that are widely used as starters in traditional and industrial dairy technologies. They 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. The fungus *Geotrichum candidum* was more sensitive, with an MIC value of 0.100 mg/ml.

Table 66 MIC and MMCfor three microorganisms (*Geotrichum candidum, Lactococcus lactis subsp.cremoris and Lactobacillus delbrueckii subsp. bulgaricus*) after 24 h of incubation in growth media supplemented with Roundup or equivalent amount of glyphosate. (From Claire et al. 2012)

Strain	Glyphosate in Roundup® (g/l)	MIC (ppm)	MMC (ppm)
G.candidum ATCC204307	400 450	100 625	1000 1000
L. lactis subsp. cremoris ATCC19257	450	312	625
L. delbrueckii subsp. bulgaricus CFL1	450	1000	1250

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

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

Krüger et al. (2013) reported on the toxicity of glyphosate to the most prevalent *Enterococcus* spp. in the gastrointestinal tract. Minimal inhibitory concentration tests (MIC) and minimal bactericidal concentration tests (MBC) were done with glyphosate. The lowest concentration of glyphosate and Roundup to show bactericidal or bacteriostatic effects was d etermined in 96 -well microtiter plates. Serial dilutions of glyphosate from 10 to 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 containing diluted glyphosate and *Enterococcus* were incubated overnight at 37°C before plating aliquots on citrate acid-Tween carbonate agar. Bacterial growth on each agar plate was evaluated. Glyphosate and the Roundup herbicide formulation at 0.1 mg/ml to 10 mg/ml inhibited the growth of *E. faecalis* but did not inhibit the growth of *C. botulinum* nor the production of botulinum neurotoxin. Krüger et al. (2013) proposed that glyphosate may be a significant factor in the observed increased risk of *Clostridium botulinum* infection in cattle in German y over the past 10 –15 years. Glyphosate toxicity to *Enterococcus* spp. leads to an imbalance in the gut favoring 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 67 Influence of glyphosate and roundup on the growth of *C. botulinum* type B and *E. faecalis* (from Kruger et al., 2013)

Herbicide concentration (mg/ml)	Glyphosate[a]			Herbicide formulation[b]		
	C. botulinum Type B (cfu/ml) (mean±SD)[c]	BoNT (ng/ml)[d]	*E. faecalis* (cfu/ml) (mean±SD)[d]	*C. botulinum* Type B (cfu/ml) (mean±SD)	BoNT (ng/ml)	*E. faecalis* (cfu/ml) (mean±SD)
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

a Glyphosate (N-Phosphonomethyl) glycine
b Herbicide formulation (Roundup)
c *C. botulinum* type B (10^4/ml) cultured anaerobically in reinforced clostridal medium (RCM) containing different concentrations of glyphosate or herbicide formulation for 5 days. *C.botulinum* quantified using the most probable number (MPN) estimation method. Data express as reciprocal $\log_{10}$.
d *C. botulinum* type B quantified by ELISA.
e E/ faecalis cultured aerobically in RCM containing different concentrations of glyphosate or herbicide formulation for 8 hour and quantified on citrate-acid-tween-carbonate (CATC) agar. . Data express as reciprocal $\log_{10}$.

In a study by Shehata et al. (2014), the neutralization ability of the antimicrobial effect of glyphosate by different humic acids was investigated. The MIC of glyphosate for different bacteria, such as *B. badius*, *B. adolescentis, E. coli, E. coli* 1917 strain Nissle, *E. faecalis, E. faecium, S. enteritidis and S. typhimurium,* were determined in the presence and absence of 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 neutra lized 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 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. Other humic acids had neutralizing activities, with MIC values ranging from 0.3 to 0.6 mg/ml. There findings indicate that humic acids inhibit the antimicrobial effect of glyphosate on different bacteria. 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).

Do glyphosate residues enter the human colon and remain microbiologically active?

A review of the published scientific literature does not show measurements of the amount of glyphosate residues in the intestinal tract. However, several phar macokinetic, toxicokinetic and bioavailability studies indicate that glyphosate is poorly absorbed after oral administration. Toxicokinetics of glyphosate after single 100 mg kg^{-1} intravenous (i.v.) and 400 mg kg^{-1} oral doses were studied in rats by Anadón et al. (2009). The oral bioavailability of glyphosate was 23.21% in rats, which was lower than those of studies in which [^{14}C]-glyphosate was administered at the oral dose of 1 0 mg kg^{-1} and approximately 30–36% of the dose was absorbed (Ridley and Mirley, 1988; Howe et al., 1988; Brewster et al., 1991). An NTP study (NTP, 1992) showed that approximately 19 – 23% of the administered 1000 mg kg^{-1} dose was absorbed, as determined by urinary excretion data. Colvin and Miller (1973) had reported previously a poor oral absorption of ^{14}C-labeled glyphosate. When a single oral dose of glyphosate (6–9 mg kg^{-1}) was administered to New Zealand white rabbits, 80% of the material appeared in th e faeces (Colvin and Miller, 1973). Glyphosate is poorly metabolized in rats, and the metabolite aminomethyl phosphonic acid (AMPA) represented 6.49% of the parent drug plasma concentration. A similar metabolic characterization was indicated by Brewster et al. (1991). The production of this metabolite could have been the result of intestinal microbial action (Rueppel et al., 1977; Mueller et al., 1981). Taken together, the fraction of the oral dose of glyphosate bioavailable to the intestinal microorganisms could range from 70 to 80% and be microbiologically active. The microbiological activity of the minor metabolite AMPA has not been determined.

Do the intestinal microbiota have the ability to metabolize glyphosate?

A review of the literature does not in dicate that intestinal bacteria representative of the human gastrointestinal tract have been tested for the ability to degrade glyphosate. However, the microbial capacity for glyphosate degradation has been shown in terrestrial and aquatic environments (Balthazor and Hallas, 1986, Rueppel et al., 1977, Sprankle et al., 1975; Mueller et al., 1981, Franz et al., 1997; Zaranyika and Nyandoro, 1993; Kryuchkova et al., 2014). Glyphosate is metabolized by several bacteria in soil to give sarcosine, which is then converted to glycine and ammonia by sarcosine oxidase. An alternative metabolic pathway involves the formation by glyphosate oxidoreductase of

aminomethylphosphonic acid (AMPA), which is a minor metabolite in colon tissue in rats (Brewster et al., 1991). Therefore, based on the enzymatic repertoire of the intestinal microbiota, there is potential for these microorganisms to metabolize glyphosate.

REFERENCES:

Ackermann W, Coenen M, Schrodl W, Shehata AA, Krüger M (2015) The influence of glyphosate on the microbiota and production of botulinum neurotoxin during ruminal fermentation. Current Microbiology, 70(3):374-382.

Anadón A, Martínez-Larrañaga MR, Martinez MA, Castellano VJ, Martinez M, Martin MT, Nozal MJ, Bernal JL (2009) Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. Toxicology Letters, 190(1): 91–95.

Balthazor TM, Hallas LE (1986) Glyphosate-degrading microorganisms from industrial activated sludge. Applied and Environmental Microbiology, 51:432–434.

Banta GT, Hansen PE, Jacobsen OS (2009) The influence of organic matter on sorption and fate of glyphosate in soil – comparing different soils and humic substances. Environmental Pollution, 157 (10):2865–2870.

Brewster DW, Warren J, Hopkins II WE (1991) Metabolism of glyphosate in Sprague–Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. Toxicological Sciences, 17(1):43–51

Cerdeira, A, Duke, S (2006) The current status and environmental impacts of glyphosate-resistant crops: a review. Journal of Environmental Quality, 35(5):1633-1658.

Clair E, Linn L, Travert C, Amiel C, Séralini GE, Panoff JM (2012) Effects of Roundup and glyphosate on three food microorganisms: Geotrichum candidum, Lactococcus lactis subsp. cremoris and Lactobacillus delbrueckii subsp. bulgaricus. Current Microbiology, 64:486–491.

Colvin LB, Miller JA (1973) Residue and metabolism. The dynamics of accumulation and depletion of orally ingested N-phosphonylmethylglycine-14C. Unpublished report. Monsanto Company, St. Louis, MO.

Franz JI, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide. ACS Monograph Series, 189:1–678.

Howe RH, Chott RC, McClanahan RH (1988) Metabolism of glyphosate in Sprague–Dawley rats. II. Identification, characterization, and quantitation of glyphosate and its metabolites following intravenous and oral administration. Unpublished report. Monsanto Environmental Health Laboratory, St. Louis, MO.

Krüger M, Shehata AA, Schrodl W, Rodloff (2013) Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum. Anaerobe, 20:74-78.

Kryuchkova YV, Burygin GL, Gogoleva NE, Gogolev YV, Chemyshova MP, Makarov OE, Fedorov EE, Turkovskaya OV (2014) Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7. Microbiological Research, 169(1):99-105.

Mueller MM, Rosenberg C, Siltanen H, Wartiovaara T (1981) Fate of glyphosate and its influence on nitrogen-cycling in two Finnish agriculture soils. Bulletin of Environmental Contamination and Toxicology, 27:724–730.

National Toxicology Program (NTP) (1992) Technical report on toxicity studies of glyphosate (CAS No. 1071-83-6) administered in dosed feed to F344/N rats and B6C3F mice . Toxicity Report Series Number 16, NIH Publication 92 -3135, July 1992. U.S. Department of Health and Human Services, Research Triangle Park, NC.

Piccolo A, Gatta L, Campanella L (1995) Interactions of glyphosate herbicide with a humic acid and its iron complex. Annali di Chimica, 85:31–40.

Piccolo A, Celano G, Conte P (1996) Adsorption of glyphosate by humic substances. Journal of Agricultural and Food Chemistry, 44:2442–2446.

Ridley WP, Mirley K (1988) The metabolism of glyphosate in Sprague –Dawley rats. I. Excretion and tissue distribution of glyphosate and its metabolites following intravenous and oral administration. Unpublished report. Monsanto Environmental Health Laboratory, St. Louis, MO.

Rueppel M, Brightwell BB, Schaefer J, Marvel JT (1977) Me tabolism and degradation of glyphosate in soil and water. Journal of Agricultural and Food Chemistry, 25:517–528.

Shehata AA, Kühnert M, Haufe S, Krüger M (2014) Neutralization of the antimicrobial effect of glyphosate by humic acid in vitro. Chemosphere, 104:258–261.

Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M (20 13a) The Effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Current Microbiology, 66(4):350-358.

Shehata AA, Schrödl W, Neuhaus J, Krüge r M, (2013b) Antagonistic effect of different bacteria on Clostridium botulinum types A, B, C, D and E in vitro. Veterinary Record, 172(2):47–47.

Shehata AA, Schrödl W, Krüger M (2012) Glyphosate suppresses the antagonistic effect of Enterococcus spp. on Clostridium botulinum anaerobe. In: Tagung der Fachgruppe Bakteriologie und Mykologie, Leipzig, Germany, DVG, 181–182.

Sprankle P, Meggitt WF, Penner D (1975) Adsorption, mobility, and microbial degradation of glyphosate in soil. Weed Science 23(3):229-234.

Zaranyika MF, Nyandoro MG (1993) Degradation of glyphosate in the aquatic environment - an enzymatic kinetic-model that takes into account microbial -degradation of both free and colloidal (or sediment) particle adsorbed glyphosate. Journal of Agricultural and Food Chemistry, 41 (5): 838-842.

Evaluation of epidemiological evidence for risk assessment for Glyphosate

1. Evaluation process

The pre-agreed evaluation process and Tier 1 screening criteria are shown in Figure 1 below.

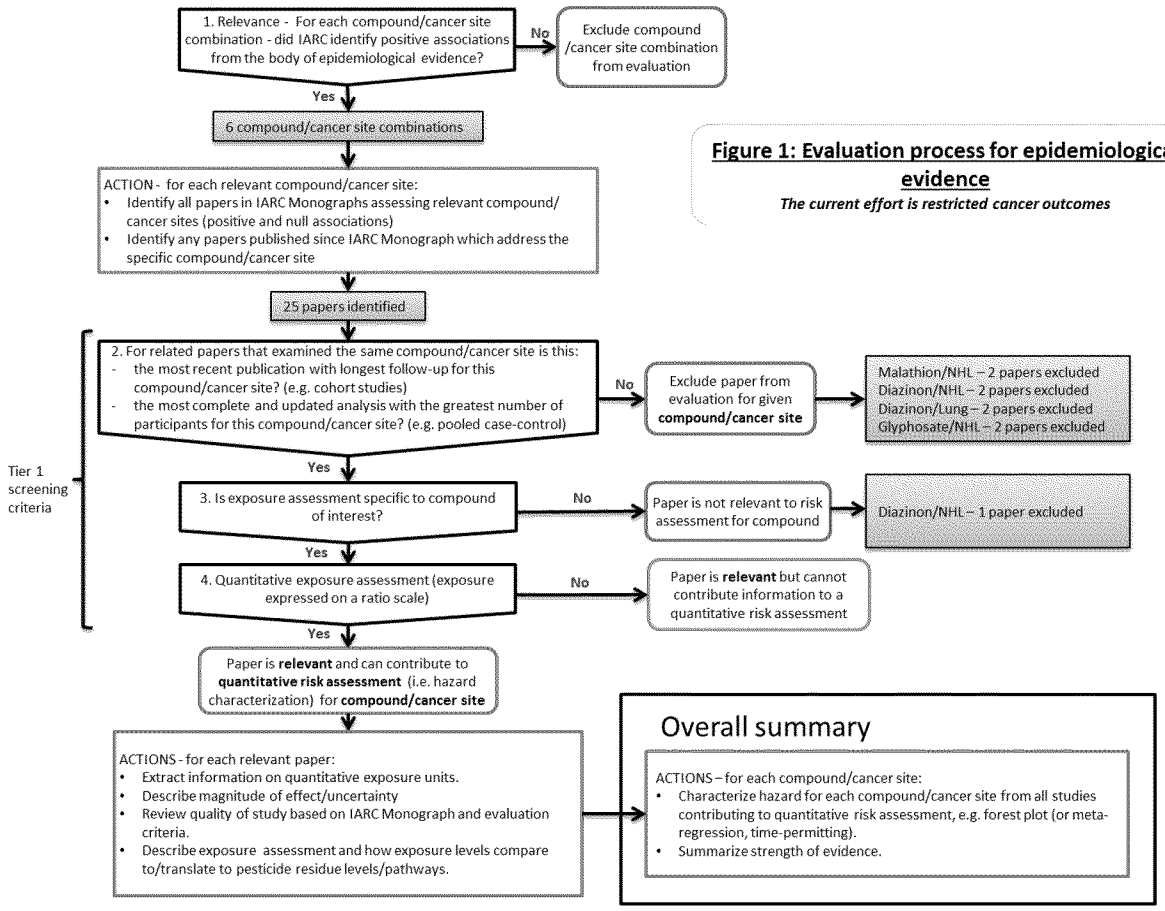


Figure 1: Evaluation process for epidemiological evidence

The current effort is restricted cancer outcomes

42. Identification of compound/cancer sites and screening of papers

We restricted our assessment to studies of cancer outcomes, as per our given remit. We did not evaluate the body of epidemiological evidence for non-cancer outcomes; numerous studies have assessed risks for neurodevelopmental, neurodegenerative, and reproductive outcomes, among other health outcomes. Restricting the assessment to non-cancer outcomes was partly driven by feasibility reasons: a clinically relevant adverse effect size (or an acceptable level of risk) for a non-cancer outcome must be defined, and the methodologies for hazard identification and characterization based on observational epidemiologic findings of non-carcinogenic adverse effects are less well-established than those for cancer (Clewell and Crump 2005; Nachman et al 2011).

The IARC Monographs on Malathion, Diazinon and Glyphosate referred to a total of 45 epidemiological studies. We also identified 2 studies published since the IARC Monographs that evaluated at least one of malathion, diazinon or glyphosate in relation to cancer outcomes (Lerro et al 2015, Koutros et al 2015).

The 45 publications referred to in the IARC Monographs and the 2 publications since (Lerro et al 2015, Koutros et al 2015), covered a total of 48 compound/cancer site combinations. Here we focused our evaluation on the 6 compound/cancer site combinations for which IARC identified positive associations from the body of epidemiological evidence, i.e. those associations noted in Section 6.1 of the Monographs, and which underpin IARC's evaluation of *limited evidence* in humans for the carcinogenicity of malathion, diazinon and glyphosate. The definition for *limited evidence of carcinogenicity* used by IARC is as follows: "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence" (IARC 2015). The 6 compound/cancer site combinations are:

A. Glyphosate / NHL

When identifying relevant publications we noted that there were stand-alone analyses for specific sub-types of NHL (of which there are many subtypes). We did not evaluate the risk separately for sub-types of NHL, as there was insufficient evidence (too few studies or small numbers of cases), or for other haematopoietic and lymphoid tumours, as the positive associations identified by IARC were for total NHL.

There were 25 publications for these 6 compound/cancer site combinations. There were 7 studies which were excluded from at least one evaluation for a given compound/cancer site during Tier 1 screening, either because they were not specific to the pesticide in question, or because the publication had been superseded by a later publication on the same cohort which included longer follow-up time, or there was a more complete analysis on the same study population with a greater number of participants.

The screening (Tier 1) and evaluation of these 25 publications is summarised in Figure 1 and Table 1.

183. Overview of studies included in evaluation

The IARC Monograph on Malathion (IARC 2015) already provides a good overview of the epidemiological studies which have assessed pesticide exposures and cancer risk. Therefore, only a brief summary (largely based on the IARC Monograph) of the studies contributing to the current evaluation is provided here to give context.

The **Agricultural Health Study (AHS)** is a prospective cohort study of ~52,000 pesticide applicators (predominantly farmers) and their spouses (n=~32,000) from Iowa and North Carolina, USA, enrolled in 1993-1997. It has examined a range of cancer outcomes, and published updated analyses with longer periods of follow-up (e.g. De Roos et al 2005, Beane Freeman et al 2005, Koutros et al 2013, Alavanja et al 2014, Jones et al 2015, Lerro et al 2015). Information on participants' use of 50 pesticides and other determinants of exposure was collected retrospectively via baseline and 2 follow-up questionnaires. Cumulative life time exposure estimates were calculated. Validation studies have been conducted to assess the reliability and accuracy of exposure intensity scores (a component of the exposure assessment) (Coble et al 2005, Hines et al 2008; Thomas et al 2010). The impact of exposure misclassification in this study was to bias risk estimates towards null (Blair et al 2011).

The **United States Midwest case-control studies** are 3 population-based case-control studies of cancer conducted in Nebraska (Hoar Zahm et al 1990), Iowa and Minnesota (Brown et al 1990; Cantor et al 1992), Kansas (Hoar et al 1986), which have subsequently been pooled (748 cases/2236 controls) for analysis of non-Hodgkin's lymphoma (white males only) (Waddell et al 2001; De Roos et al 2003; Lee et al 2004). Information on participants' occupational use of pesticides was collected retrospectively via questionnaire. There were some differences in case ascertainment and exposure assessment methods between the 3 studies. For 39% of the pooled study population, proxy respondents were used (Waddell et al 2001), for whom recall of specific pesticide use could be problematic and subject to recall bias which may differ for cases and controls. De Roos et al (2003) (same study population as Waddell et al 2001) performed an extensive evaluation and adjustment for other pesticides.

The **Cross-Canada case-control study of Pesticides and Health** is a population-based case-control study of haematopoietic cancers in men diagnosed during 1991-1994 across 6 Canadian provinces (McDuffie et al 2001). It includes 517 NHL cases and 1506 controls. A questionnaire was administered by post, followed by a telephone interview for those that reported pesticide exposure of 10 hours/year or more, and for a 15% random sample of the remainder. The study was not restricted to pesticide exposure experienced by a specific occupational group (McDuffie et al 2001). Further analyses stratified by asthma/allergy status—to assess possible effect modification by immune system modulation—have been conducted (Pahwa et al 2012). The study has a large sample size and detailed information of pesticide exposures; however, the proportion exposed to pesticides was low.

EPA-HQ-2018-002024_0004953

The three sets of studies above were deemed as high quality and highly informative by the IARC Working Group (IARC 2015).

A number of other case-control studies of pesticide exposure and cancer risk were included in this evaluation: the Florida Pest Control Worker study (Pesatori et al 1994), nested case-control studies within the United Farm Workers of America cohort study (Mills et al 2005), a population-based case-control study of prostate cancer in British Columbia, Canada (Band et al 2011), and case-control studies of NHL/haematopoietic cancers from Sweden (Hardell et al 2002, Eriksson et al 2008), and France (Orsi et al 2009). The IARC Working Group (IARC 2015) noted substantial limitations in these studies, either in relation to exposure assessment, scope for and variation in exposure misclassification, lack of detail reported in publication which hindered interpretation, lack of specificity due to high correlations between use of different pesticides, and limited power.

4. Strengths and Limitations of studies included in evaluation

The included studies predominantly examined the occupational pesticide exposures of farmers and other pesticide applicators, with the vast majority of research being on males only. None of the studies assessed exposure via food consumption or ambient exposure from agriculture (e.g. spray drift). The scientific evidence available is therefore limited in its generalizability and the extent to which it can be translated to general population exposure scenarios and levels that would be associated with pesticide residues. Nonetheless, these observational epidemiological studies provide insight into real-world exposure scenarios, and allow for observation of the species of interest (humans), over long follow-up time periods relevant to cancer.

The number of high quality studies is relatively small. Typically the number of exposed cases in studies is small, particularly when evaluating specific pesticides, which limits study power.

Relatively few studies have assessed exposure quantitatively, meaning the epidemiological evidence available to inform/establish dose-response relationships is very limited. Exposure misclassification is a potential issue for all studies. This is expected to be largely non-differential for cohort studies (i.e. the Agricultural Health Study), resulting in attenuation of risk estimates. All except one of the studies included are case-control studies, and these may be affected by recall bias - i.e. cases and controls recall past pesticide exposure with differing accuracy, leading to differential exposure misclassification which can bias risk estimates either towards or away from the null. As a cohort study, the Agricultural Health Study avoids recall bias.

Given that studies focussed on occupational exposures amongst farmers/pesticide applicators it is unlikely that they were exposed to only one specific pesticide, so confounding, possible effect modification and additive/multiplicative effects due to co-exposures are all concerns. However, many studies were able to adjust risk estimates for other pesticide co-exposures, which yields more accurate risk estimates.

There are some issues in terms of comparing studies and evaluating the consistency of evidence overall. Results of studies may appear heterogeneous, but usually there too few studies to really assess consistency and heterogeneity. Exposure assessment methods and referent groups vary between studies.

Finally, changes in disease classifications (particularly Non-Hodgkin's Lymphoma) or screening/diagnosis rates (prostate cancer) over time, may limit comparability between studies.

5. Publication bias

We did not undertake a formal analysis of publication bias, because the number of studies [risk estimates from non-overlapping study populations] available were few, and it is advised that funnel

plot tests for asymmetry sho uld be used only where there are at least 10 studies, because otherwise there is insufficient statistical power to distinguish true asymmetry from chance (Higgins & Green 2011, Sterne et al 2011). Other formal objective statistical tests require a larger number of studies still, typically at least 30 studies, 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, in our opinion it is unlikely that results would go unpublished.

6. Summary of evidence for the 6 compound/cancer site associations

We considered several aspects of each study and of all studies combined in this evalu ation, 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, dose -response, residual confounding, and consistency (Guyatt et al 2008; Morgan et al 2016).

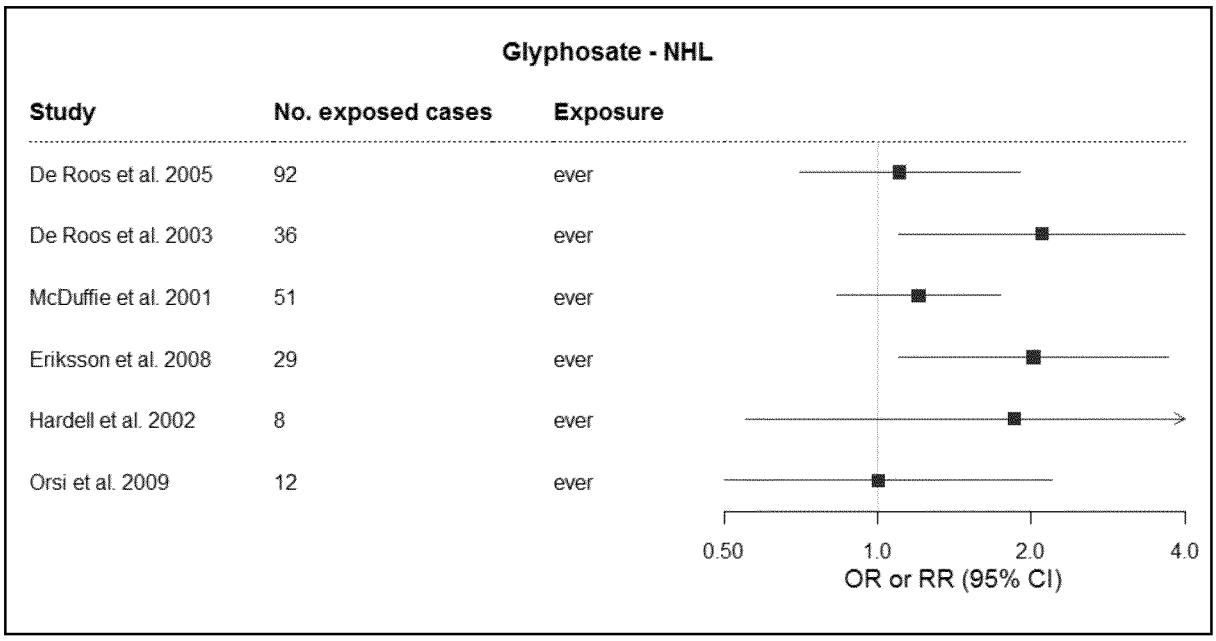
The findings for each study are summarized in Table 1, and findings for non -quantitative exposure assessment (predominantly **ever vs. never use**) are shown in forest plots below.

A. Glyphosate / NHL

Our evaluation included 7 studies (De Roos et al 2005, Lee et al 2004, De Roos et al 2003, McDuffie et al 2001, Eriksson et al 2008, Hardell et al 2002, Orsi et al 2009) and one meta-analysis (Schinasi & Leon 2014). 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 report significant elevated risk of NHL associated with ever vs. never use of glyphosate (OR: 2.1 (1.1 -4.0); and a borderline non -significant 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 modificatio n 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 >2 days/year was associated with a significant elevated risk (OR: 2.12 (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) report significant elevated risk of N HL associated with ever use (OR: 2.02 (1.10–3.71)) and use of glyphosate for > 10 days/year (OR: 2.36 (1.04 -5.37)), and there is indication of an exposure-response relationship. A pooled study of 2 Swedish case control studies reports a non-significant elevated risk of NHL for ever use of (OR: 1.85 (0.55 -6.2)), however with only 8 exposed cases it had limited power to detect associations (Hardell et al 2002). Orsi et al (2009) finds no evidence of association. Schinasi & Leon (2014) report a meta risk ra tio of 1.5 (95% CI, 1.1–2.0) for ever vs. never use of glyphosate. The meta-analysis includes the AHS (De Roos et al 2005) and 5 out of the 6 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).

Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case -control studies and the meta -analysis. However, it is notable that the AHS (De Roos et al 2005), which is the only cohort study and is large and of high quality, found no evidence of association or exposure-response.

EPA-HQ-2018-002024_0004955



7. Hazard characterization

7.1. What quantitative exposure information is available?

- None of the studies evaluated have used exposure units which reflect dose, i.e. the amount of the compound that a person has come into contact with externally or internally.

[Additional information - exposure concentration information is available in some epidemiological studies of non-cancer outcomes e.g. neurodegenerative, neurodevelopmental, and reproductive outcomes (e.g. Eskenazi et al. 2004, Omoike et al. 2015). Some urinary biomarkers have been used to assess exposure, e.g. malathion dicarboxylic acid (MDA) for malathion, and 2-isopropyl-4-methyl-6-hydroxypyrimidine for diazinon. However, the half-lives for these biomarkers are short (hours to days). Repeat sampling is preferable to reduce the within-person variability. Furthermore, some OP metabolites are detected on or in food, and may not represent metabolites produced following absorption. These exposure assessment methods are feasible for some non-cancer outcomes because the critical window for exposure is relatively short. However, in studies of cancer outcomes, which have a long latency, and thus a long potential exposure window, it is not feasible and would be prohibitively expensive].

- Most studies categorised exposure as Never Exposed vs. Ever Exposed.
- Where quantitative exposure metrics were used, these predominantly reflected duration of exposure – either a) days of use per year; or b) lifetime exposure days; or a combination of duration and intensity of exposure, c) intensity-weighted lifetime exposure days. A single study used d) 'exposure level' estimated as a product of work duration, concentration level, frequency and prevalence of exposure – however no units are reported (We will check with the authors re units).

[Definitions:
<u>b) lifetime exposure days</u> - defined as number of years of use x number of days used per year.
<u>c) intensity-weighted lifetime exposure days</u> - defined as number of years of use x number of days used per year x personal protective equipment use reduction factor x 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]. This exposure algorithm was developed by the Agricultural Health Study (AHS) to capture duration and intensity of exposure, 'intensity-weighted lifetime days of use' (Dosemeci et al 2002, Coble et al 2011). The exposure algorithm, and specifically the estimated rank order of exposure, has been validated in several studies, in which

EPA-HQ-2018-002024_0004956

estimated intensity scores were shown to be correlated with biomarker levels and dermal swipe levels (e.g. Coble et al. 2005, Thomas et al 2010).]

7.2. What can the available quantitative exposure information tell us?

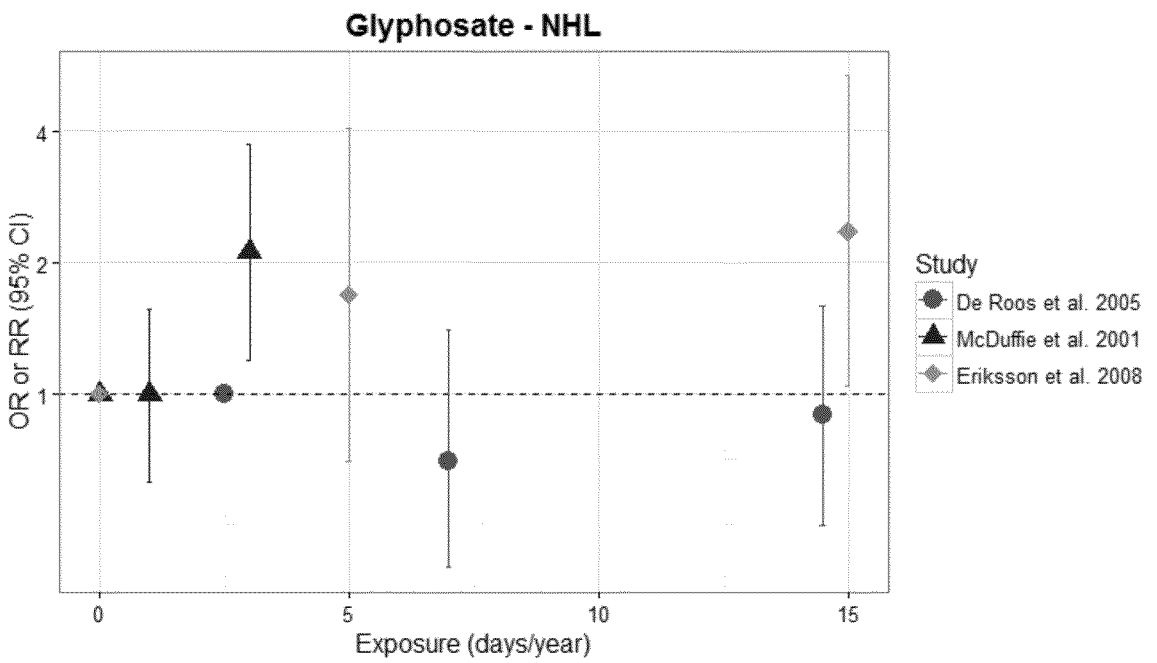
- The studies available do provide dose-response curves of the associations studied.
- However, exposure is measured mainly with dur ation metrics, so we will refer to these as **duration–response** curves.
- Exposure duration metrics provide information relevant to long -term human exposure and cancer latency, which is not available from the toxicological literature.
- Additional work (to be pe rformed by hygienists, occupational health physicians and toxicologists) would be required to translate, if/where feasible, these metrics into plausible external exposure/intake/dose equivalents.

7.3. Information available for quantitative risk assessment for glyphosate/cancer site combination

A. Glyphosate / NHL

3 studies used quantitative exposure metrics, however 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). De Roos et al (2005) found no evidence of association in the AHS cohort, but McDuffie et al (2001)and Eriksson et al (2008) found an significant elevated risks of NHL for those using glyphosate for >2 days per year and >10 days per year respectively (their highest exposure categories), as shown below. We requested from the authors data on median, minimum and maximum value of 'days of use per year' for the participants in each of the lifetime days of exposure and Intensity-Weighted days of exposure categories, in order to be able to translate their findings onto a comparable scale, as shown in the exposure -response plot below. [NOTE: While this enabled us to plot multiple studies in one plot, this reduced the amount of infor mation in the exposure metric: the risk estimates reflect associations with an intensity -duration metric (lifetime exposure days), and we present a metric which reflect only intensity and not duration (days/year).]

The plot provides some suggestion of an exposure-response relationship between glyphosate exposure and risk of NHL, however it is notable that the evidence from the AHS (De Roos et al 2005) does not support this pattern.



Footnote: With the obtained median number of days/year (from the original LED metric) plotted for De Roos et al. 2005.

De Roos et al 2005: Agricultural Health Study cohort

Cumulative exposure days	**NHL RR (95% CI)**	Additional data provided by AHS
1-20	1.0	Median days/year=2.5 Minimum=2.5 Maximum=14.5
21-56	0.7 (0.4-1.4)	Median days/year=7.0 Minimum=2.5 Maximum=49.5
57-2,678	0.9 (0.5-1.6)	Median days/year=14.5 Minimum=2.5 Maximum=200
p-trend	0.73	
Intensity-weighted exposure days	**NHL RR (95% CI)**	
0.1-79.5	1.0	Median days/year=2.5 Minimum=2.5 Maximum=49.5
79.6-337.1	0.6 (0.3-1.1)	Median days/year=7.0 Minimum=2.5 Maximum=105
33.7-18,241	0.8 (0.5-1.4)	Median days/year=14.5 Minimum=2.5 Maximum=200
	0.99	

n=64 NHL cases; n=30,613 to 36,823 sample size, depending on the covariate adjustment set.

McDuffie et al 2001: Cross-Canada Case–control Study of Pesticides and Health

Days of use per year	**NHL RR (95% CI)**
Unexposed	1.0
>0 <=2 days	1.0 (0.63-1.57)
>2 days	**2.12 (1.20-3.73)**
p-trend	NR

n=517 NHL cases; n=2023 sample size; 184 exposed.

Eriksson et al 2008: Swedish case-control study (one of several)

Days of use per year	**NHL RR (95% CI)**
Unexposed	1.0
<=10 days/year	1.69 (0.7-4.07)
> 10 days/year	2.36 (1.04-5.37)
p-trend	NR

N = 47 exposed

8. Acknowledgements

We thank Dr Sylvaine Cordier for her valuable contributions to the early stages of this evaluation.

We thank the Agricultural Health Study, and Dr Laura Beane Freeman in particular, for providing the additional information we requested relating to various AHS publications (Alavanja et al 2014, Beane Freeman et al 2005, De Roos et al 2005).

9. References

Alavanja MC, Hofmann JN, Lynch CF, Hines CJ, Barry KH, Barker J et al. (2014). Non-Hodgkin lymphoma risk and insecticide, fungicide and fumigant use in the agricultural health study. PLoS ONE, 9(10):e109332. doi:10.1371/journal.pone.0109332 PMID:25337994

Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP et al. (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. Prostate, 71(2):168–83. doi:10.1002/pros.21232 PMID:20799287

Beane Freeman LE, Bonner MR, Blair A, Hoppin JA, Sandler DP, Lubin JH, Dosemeci M, Lynch CF, Knott C, Alavanja MC. Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon. American journal of epidemiology. 2005 Dec 1;162(11):1070-9.

Blair A, Thomas K, Coble J, Sandler DP, Hines CJ, Lynch CF et al. (2011). Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. Occup Environ Med, 68(7):537–41. doi:10.1136/oem.2010.059469 PMID:21257983

Bonner MR, Coble J, Blair A, Beane Freeman LE, Hoppin JA, Sandler DP et al. (2007). Malathion exposure and the incidence of cancer in the agricultural health study. Am J Epidemiol, 166(9):1023–34. doi:10.1093/aje/ kwm182 PMID:17720683

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM et al. (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res, 50(20):6585–91. PMID:2208120

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM et al. (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res, 52(9):2447–55. PMID:1568215

Clewell HJ, Crump KS. Quantitative estimates of risk for noncancer endpoints. Risk analysis. 2005 Apr 1;25(2):285-9.

Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, Lubin JH, Freeman LE, Blair A, Sandler DP, Alavanja MC. An updated algorithm for estimation of pesticide exposure intensity in the Agricultural Health Study. International journal of environmental research and public health. 2011 Dec 12;8(12):4608-22.

Coble J, Arbuckle T, Lee W, Alavanja M, Dosemeci M. The validation of a pesticide exposure algorithm using biological monitoring results. Journal of occupational and environmental hygiene. 2005 Mar 1;2(3):194-201.

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF et al. (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occup Environ Med, 60(9):E11 doi:10.1136/oem.60.9.e11 PMID:12937207

De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M, Sandler DP, Alavanja MC. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environmental Health Perspectives. 2005 Jan 1:49-54.

EPA-HQ-2018-002024_0004959

Dosemeci, M.; Alavanja, M.C.R.; Rowland, A.S.; Mage, D.; Zahm, S.H.; Rothman, N.; Lubin, J.H.; Hoppin, J.A.; Sandler, D.P.; Blair, A. A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. Ann. Occup. Hyg. 2002, 46, 245–260.

Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. Int J Cancer, 123(7):1657–63. doi:10.1002/ijc.23589 PMID:18623080

Eskenazi , B. , Harley , K. , Bradman , A. , Weltzien , E. , Jewell , N. P. , Barr , D. B. , Furlong , C. E. and Holland , N. T. 2004. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ. Health Perspect, 112: 1116–1124.

Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. 2008. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336:924–926.

Hardell L, Eriksson M, Nordstrom M. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leuk Lymphoma. 2002 May;43(5):1043-9.

Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
http://handbook.cochrane.org/chapter_10/10_4_3_1_recommendations_on_testing_for_funnel_plot_asymmetry.htm

Hines CJ, Deddens JA, Jaycox LB, Andrews RN, Striley CAF, Alavanja MCR (2008). Captan exposure and evaluation of a pesticide exposure algorithm among orchard pesticide applicators in the Agricultural Health Study. Ann Occup Hyg, 52(3):153–66. doi:10.1093/annhyg/men001 PMID:18326518

Hoar Zahm S, Weisenburger DD, Babbit PA, Saal R, Vaught JB, Cantor KP et al. (1990). A case control study of non-Hodgkin's lymphoma and agricultural factors in Eastern Nebraska. Epidemiology, 1:349–56. doi:10.1097/00001648-199009000-00004 PMID:2078610

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R et al. (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA, 256(9):1141–7. doi:10.1001/jama.1986.03380090081023 PMID:3801091

International Agency for Research on Cancer Volume 112: Some organophosphate insecticides and herbicides: tetrachlorvinphos, parathion, malathion, diazinon and glyphosate. IARC Working Group. Lyon; 3–10 March 2015. IARC Monogr Eval Carcinog Risk Chem Hum.

Johnson PI, Sutton P, Atchley DS, Koustas E, Lam J, Sen S, et al. 2014. The Navigation Guide—evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth.
Environ Health Perspect 122:1028–1039; doi: 10.1289/ehp.1307893.

Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, Heltshe SL, Hoppin JA, Alavanja MC, Freeman LE.
Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. Occupational and environmental medicine. 2015 Apr 23:oemed-2014.

Koutros S, Beane Freeman LE, Lubin JH, Heltshe SL, Andreotti G, Barry KH et al. (2013). Risk of total and aggressive prostate cancer and pesticide use in the Agricultural Health Study. Am J Epidemiol, 177(1):59– 74. doi:10.1093/aje/kws225 PMID:23171882

Koutros S, Silverman DT, Alavanja MC, Andreotti G, Lerro CC, Heltshe S, Lynch CF, Sandl er DP, Blair A, Beane Freeman LE. Occupational exposure to pesticides and bladder cancer risk. Int J Epidemiol. 2015 Sep 27. pii: dyv195.

Lau J, Ioannidis JP, Terrin N, Schmid CH, Olkin I. The case of the misleading funnel plot. BMJ. 2006 Sep 14;333(7568):597-600.

Lee, Cantor, Berzofsky, Zahm, Blair (2004). Non Hodgkin's Lymphoma among asthmatics exposed to pesticides. Int J Cancer:111, 298-302.

Lerro CC, Koutros S, Andreotti G, Friesen MC, Alavanja MC, Blair A, Hoppin JA, Sandler DP, Lubin JH, Ma X, Zh ang Y. Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study. Occupational and environmental medicine. 2015 Oct 1;72(10):736-44.

Lewis-Mikhael AM, Olmedo-Requena R, Martínez-Ruiz V, Bueno-Cavanillas A, Jiménez-Moleón JJ. Organochlorine pesticides and prostate cancer, Is there an association? A meta -analysis of epidemiological evidence. Cancer Causes Control. 2015 Oct;26(10):1375 -92. doi: 10.1007/s10552 -015-0643-z. Epub 2015 Aug 6.

Lewis-Mikhael AM, Bueno-Cavanillas A, Ofir Guiron T, Olmedo-Requena R, Delgado-Rodríguez M, Jiménez-Moleón JJ. Occupational exposure to pesticides and prostate cancer: a systematic review and meta-analysis. Occup Environ Med. 2016 Feb;73(2):134-44. doi: 10.1136/oemed-2014-102692. Epub 2015 Dec 7.

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA et al. (2001). Non -Hodgkin's lymphoma and specific pesticide exposures in men: cross -Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev, 10(11):1155–63. PMID:11700263

Mills PK, Yang R, Riordan D (2005). Lymphohematopoietic cancers in the United Farm Workers of America (UFW), 1988–2001. Cancer Causes Control, 16(7):823– 30. doi:10.1007/s10552-005-2703-2 PMID:16132792

Morgan RL, Thayer KA, Bero L, Bruce N, Falck -Ytter Y, Ghersi D, Guyatt G, Hooijmans C, Langendam M, Mandrioli D, Mustafa RA. GRADE: Assessing the quality of evidence in environmental and occupational health. Environment international. 2016 Jan 27.

Nachman KE, Fo x MA, Sheehan MC, Burke TA, Rodricks JV, Woodruff TJ. Leveraging epidemiology to improve risk assessment. Open Epidemiology Journal. 2011;4:3-29.

Omoike OE, Lewis RC, Meeker JD. Association between urinary biomarkers of exposure to organophosphate insecticides and serum reproductive hormones in men from NHANES 1999 –2002. Reproductive Toxicology. 2015 Jun 30;53:99-104.

Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Troussard X , Clavel J. Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case -control study. Occup Environ Med. 2009 May;66(5):291-8. doi: 10.1136/oem.2008.040972. Epub 2008 Nov 18.

Pahwa M, Harris SA, Hohenadel K, McLaughlin JR, Spinelli JJ, Pahwa P et al. (2012). Pesticide use, immunologic conditions, and risk of non-Hodgkin lymphoma in Canadian men in six provinces. Int J Cancer, 131(11):2650–9. doi:10.1002/ijc.27522 PMID:22396152

Pesatori AC, Sontag JM, Lubin JH, Consonni D, Blair A (1994). Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States). Cancer Causes Control, 5(4):310–8. doi:10.1007/BF01804981 PMID:8080942

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. Systematic review and evidence integration for literature-based environmental health science assessments. Environmental Health Perspectives (Online). 2014 Jul 1;122(7):711.

Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. Int J Environ Res Public Health, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670

Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, Carpenter J, Rücker G, Harbord RM, Schmid CH, Tetzlaff J. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011 Jul 22;343:d4002.

Thomas, K.W.; Dosemeci, M.; Coble, J.B.; Hoppin, J.A.; Sheldon, L.S.; Chapa, G; Croghan, C.W.; Jones, P.A.; Knott, C.E.; Lynch, C.F.; Sandler, D.P.; Blair, A.E.; Alavanja, M.C.R. Assessment of a pesticide exposure intensity algorithm in the Agricultural Health Study. J. Expo. Sci. Environ. Epidemiol. 2010, 20, 559–569.

Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF et al. (2001). Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control, 12(6):509–17. doi:10.1023/A:1011293208949 PMID:11519759

Woodruff TJ, Sutton P. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environ Health Perspect. 2014;122(10):1007–1014.

10. Table 68 – Results of Tier 1 Evaluation and Summary of Publications by glyphosate/Cancer Site

Multivariate risk estimates are presented

Study/ Location	Reference	Glyphosate / Non-Hodgkin Lymphoma (NHL)
Meta-analysis	Schinasi and Leon 2014	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; De Roos et al 2005a; Eriksson et al 2008; Orsi et al 2009. Ns for each meta-analysis not presented.
Agricultural Health Study	Lerro et al. 2015*	
Agricultural Health Study	Jones et al. 2015	
Agricultural Health Study	Alavanja et al. 2014	
Agricultural Health Study	Koutros et al. 2013	
Agricultural Health Study	Bonner et al. 2007	
Agricultural Health Study	Beane Freeman et al. 2005	

EPA-HQ-2018-002024_0004963

Study/ Location	Reference	Glyphosate / Non-Hodgkin Lymphoma (NHL)
Agricultural Health Study	De Roos et al. 2005	Quantitative exposure (cumulative exposure days; intensity-weighted cumulative exposure days [years of use x days/year x estimated intensity level]: in tertiles) **Risk estimates – adj. RR (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 Median days/year=2.5 Minimum=2.5 Maximum=14.5 Median days/year=7.0 Minimum=2.5 Maximum=49.5 Median days/year=14.5 Minimum=2.5 Maximum=200 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 Median days/year=2.5 Minimum=2.5 Maximum=49.5 Median days/year=7.0 Minimum=2.5 Maximum=105 Median days/year=14.5 Minimum=2.5 Maximum=200 Total N = 54,315 (49,211/36,823, depending on the analysis), with 92 incident leukaemia cases (for ever use; and 61 for analysis based on tertiles of exposure).
Agricultural Health Study	Alavanja et al 2004	
United States Midwest case–control studies	Lee et al. 2004	N.B. study population overlaps with De Roos et al 2003 below. See comment below. Qualitative - ever/never (analysis stratified by asthmatics vs not asthmatics) **Risk estimates – adj. RR (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 and 6/12 glyphosate-exposed NHL cases/controls for non-asthmatics and asthmatics, resp.

Study/ Location	Reference	Glyphosate / Non-Hodgkin Lymphoma (NHL)
United States Midwest case–control studies	De Roos et al. 2003	N.B. study population overlaps with Lee et al 2004 and total N is smaller, but as an exception this study is not excluded as it provides overall risk estimates which are comparable with other studies, whilst Lee et al 2004 only provides risk estimates stratified by asthma diagnosis. Qualitative (ever/never) **Risk estimates – adj. OR (95% CI)** Exposed **2.1 (1.1-4.0)** *from a logistic regression model; and 1.6 (0.9-2.8) from the hierarchical regression. Both adjusted for other pesticides* Total N =2583 (650 NHL cases, 1933 controls). N = 36 and 61 exposed cases and controls, resp.
United States Midwest case–control studies	Waddell et al. 2001	
United States Midwest case–control studies	Hoar Zahm et al. 1993	
United States Midwest case–control studies	Cantor et al. (1992)	Exclude – 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
United States Midwest case–control studies	Brown et al. (1990)	
Cross-Canada Case–control Study of Pesticides and Health	Pahwa et al. 2012	
Cross-Canada Case–control Study of Pesticides and Health	Hohenadel et al. 2011	

EPA-HQ-2018-002024_0004965

Study/ Location	Reference	Glyphosate / Non-Hodgkin Lymphoma (NHL)
Cross-Canada Case–control Study of Pesticides and Health	McDuffie et al. 2001	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 >0- <=2 days/year 1.0 (0.63-1.57) > 2 days/year **2.12 (1.20-3.73)** Total N = 2023. 517 cases, 1506 controls (overall). 179 cases, 456 controls (with telephone interview data i.e. detailed pesticide information) N = 51 exposed cases
Florida Pest Control Worker Study	Pesatori et al. 1994	
United Farm Workers of America	Mills et al. 2005	
Case–control study of cancer of the prostate in British Columbia	Band et al. 2011	
Sweden - N.B. some overlaps between these studies	Eriksson et al. 2008	Quantitative exposure - days of use per year (2 categories - cutpoints are given). **Risk estimates – adj. OR (95% CI)** Ever use **2.02 (1.10–3.71)** Risk estimates - **adj. OR (95% CI)** Non-farmers 1.0 <=10 days/year: 1.69 (0.7-4.07) > 10 days/year: **2.36 (1.04-5.37)** N = 29 and 18 exposed cases and controls, resp.
	Hardell et al 2002	Qualitative exposure only - ever/never use of glyphosate. A pooled analysis of Nordstrom et al 1998 and Hardell & Eriksson 1999. **Risk estimates – OR (95% CI)** Ever use 1.85 (0.55-6.2) Total N = 1656 (515 cases, 1141 controls) N = 8 exposed cases.
	Hardell & Eriksson 1999	Exclude as this study is pooled in Hardell et al 2002. Qualitative exposure only - ever/never use of glyphosate.
France	Orsi et al 2009	Qualitative - ever/never use of glyphosate (sensitivity analysis in controls - quantitative duration < vs. > median, but unusable as does not present cutpoints - **seeking information from authors**) **Risk estimates – adj. OR (95% CI)** Ever use 1.0 (0.5-2.2) N = 12 and 24 exposed cases and controls, resp.

Study/ Location	Reference	Glyphosate / Non-Hodgkin Lymphoma (NHL)
N publications after exclusions (not counting meta-analysis)		**7**
N publications eligible for quantitative risk assessment:		**3 - with different units**
Exclusions		**2**

LED, Lifetime Exposure Days; IW-LED, Intensity Weighted-Lifetime Exposure Days. We extracted the maximally adjusted risk estimates.

Reference List for Table 68

Alavanja MC, Hof mann JN, Lynch CF, Hines CJ, Barry KH, Barker J et al. (2014). Non -Hodgkin lymphoma risk and insecticide, fungicide and fumigant use in the agricultural health study. PLoS ONE, 9(10):e109332. doi:10.1371/journal.pone.0109332 PMID:25337994

Alavanja MC, Dose meci M, Samanic C, Lubin J, Lynch CF, Knott C, Barker J, Hoppin JA, Sandler DP, Coble J, Thomas K, Blair A. Pesticides and lung cancer risk in the agricultural health study cohort. Am J Epidemiol. 2004 Nov 1;160(9):876-85.

Band PR, Abanto Z, Bert J, Lang B , Fang R, Gallagher RP et al. (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. Prostate, 71(2):168 –83. doi:10.1002/pros.21232 PMID:20799287

Beane Freeman LE, Bonner MR, Blair A, Hoppin JA, Sandler DP, Lubin JH, Dosemeci M, Lynch CF, Knott C, Alavanja MC. Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon. American journal of epidemiology. 2005 Dec 1;162(11):1070-9.

Bonner MR, Coble J, Blair A, Beane Freeman LE, Ho ppin JA, Sandler DP et al. (2007). Malathion exposure and the incidence of cancer in the agricultural health study. Am J Epidemiol, 166(9):1023 – 34. doi:10.1093/aje/ kwm182 PMID:17720683

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM et al. (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer Res, 50(20):6585–91. PMID:2208120

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM et al. (1992). Pesticides and other agri cultural risk factors for non -Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer Res, 52(9):2447– 55. PMID:1568215

De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M, Sandler DP, Alavanja MC. Cancer incidence among glyphosate -exposed p esticide applicators in the Agricultural Health Study. Environmental Health Perspectives. 2005 Jan 1:49-54.

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF et al. (2003). Integrative assessment of multiple pesticides as risk facto rs for non -Hodgkin's lymphoma among men. Occup Environ Med, 60(9):E11 doi:10.1136/oem.60.9.e11 PMID:12937207

Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non -Hodgkin lymphoma including histopathological subgrou p analysis. Int J Cancer, 123(7):1657 –63. doi:10.1002/ijc.23589 PMID:18623080

Hardell L, Eriksson M. A case -control study of non -Hodgkin lymphoma and exposure to pesticides. Cancer. 1999 Mar 15;85(6):1353-60.

EPA-HQ-2018-002024_0004967

Hardell L, Eriksson M, Nordstrom M. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leuk Lymphoma. 2002 May;43(5):1043-9.

Hoar Zahm S, Weisenburger DD, Saal RC, Vaught JB, Babbitt PA, Blair A. The role of agricultural pesticide use in the development of non-Hodgkin's lymphoma in women. Arch Environ Health. 1993 Sep-Oct;48(5):353-8.

Hohenadel K, Harris SA, McLaughlin JR, Spinelli JJ, Pahwa P, Dosman JA et al. (2011). Exposure to multiple pesticides and risk of non-Hodgkin lymphoma in men from six Canadian provinces. Int J Environ Res Public Health, 8(6):2320–30. doi:10.3390/ijerph8062320 PMID:21776232

Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, Heltshe SL, Hoppin JA, Alavanja MC, Freeman LE. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. Occupational and environmental medicine. 2015 Apr 23:oemed-2014.

Koutros S, Beane Freeman LE, Lubin JH, Heltshe SL, Andreotti G, Barry KH et al. (2013a). Risk of total and aggressive prostate cancer and pesticide use in the Agricultural Health Study. Am J Epidemiol, 177(1):59– 74. doi:10.1093/aje/kws225 PMID:23171882

Lee, Cantor, Berzofsky, Zahm, Blair (2004). Non Hodgkin's Lymphoma among asthmatics exposed to pesticides. Int J Cancer:111, 298-302.

Lerro CC, Koutros S, Andreotti G, Friesen MC, Alavanja MC, Blair A, Hoppin JA, Sandler DP, Lubin JH, Ma X, Zhang Y. Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study. Occupational and environmental medicine. 2015 Oct 1;72(10):736-44.

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA et al. (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev, 10(11):1155–63. PMID:11700263

Mills PK, Yang R, Riordan D (2005). Lymphohematopoietic cancers in the United Farm Workers of America (UFW), 1988–2001. Cancer Causes Control, 16(7):823– 30. doi:10.1007/s10552-005-2703-2 PMID:16132792

Nordström M, Hardell L, Magnuson A, Hagberg H, Rask-Andersen A. Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. Br J Cancer. 1998 Jun;77(11):2048-52.

Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P, Marit G, Soubeyran P, Huguet F, Milpied N, Leporrier M, Hemon D, Troussard X, Clavel J. Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. Occup Environ Med. 2009 May;66(5):291-8. doi: 10.1136/oem.2008.040972. Epub 2008 Nov 18.

Pahwa M, Harris SA, Hohenadel K, McLaughlin JR, Spinelli JJ, Pahwa P et al. (2012a). Pesticide use, immunologic conditions, and risk of non-Hodgkin lymphoma in Canadian men in six provinces. Int J Cancer, 131(11):2650–9. doi:10.1002/ijc.27522 PMID:22396152

Pesatori AC, Sontag JM, Lubin JH, Consonni D, Blair A (1994). Cohort mortality and nested case-control study of lung cancer among structural pest control workers in Florida (United States). Cancer Causes Control, 5(4):310–8. doi:10.1007/BF01804981 PMID:8080942

Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. Int J Environ Res Public Health, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670

Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF et al. (2001). Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control, 12(6):509–17. doi:10.1023/A:1011293208949 PMID:11519759

2.7 *Studies on metabolites*

Metabolites of glyphosate

Aminomethyl phosphonic acid (AMPA) is the only identified metabolite found in the urine and faeces of orally treated rats. It was reviewed by the JMPR in 1997. The Meeting established an acceptable daily intake (ADI) of 0–0.3mg/kg bw (sum of glyphosate and AMPA) was established based on a no-observed-adverse-effect level (NOAEL) of 31mg/kg bw per day, the highest dose tested in a 26-month study of toxicity in rats with glyphosate.

Table 69. Summaries of acute toxicity studies on Amino-methyl phosphonic acid: (AMPA)

Species	Strain	Sex	Route	Purity %	Results LD50 or LC50 (mg/kg bw or mg/L)	Reference
Rat	Alpk:APfSD, Wistar	Male Female	Oral	assumed 100	>5000	Leah (1988)
Rat	Sprague-Dawley	Male Female	Oral	99.2	>5000	Cuthbert and Jackson, 1993a
Mouse	(Crj:CD-1)	Male Female	Oral	99.33	>5000	Komura (1996)
Rat	Sprague-Dawley	Male Female	Dermal	99.2	>2000	Cuthbert and Jackson, 1993b
Rat	CD®/Crl:CD®	Male Female	Dermal	98.0	>2000	Leuschner (2002a)
Guinea Pig	Dunkin-Hartley	Female	Sensitization (Magnusson-Kligman Maximization Test)	99.2	Negative	Cuthbert and Jackson, 1993c
Guinea Pig	Dunkin-Hartley	Male	Sensitization (Magnusson-Kligman Maximization Test)	98.0	Negative	Leuschner (2002b)

Acute Toxicity

In an acute oral toxicity study, 5 male and 5 female SPF Wistar -derived albino rats (Alpk:APfSD strain) were orally dosed with 5000 mg /kg bw AMPA (aminomethyl phosphonic acid: assumed purity 100%). The test material was administered as a 50% suspensi on in 0.5% aqueous polysorbate 80 at a constant dose volume of 10 mL/kg bw.

There was no mortality. Signs of toxicity included diarrhoea, chromodacryorrhea, piloerection, stains around the nose and ungroomed appearance, with recovery by Day 5. With the exception of one male, all rats gained weight days 1-8; two males and three females had weight losses days 8-15. There were no observed abnormalities at necropsy.

The oral LD_{50} of AMPA in male and female rats was > 5000 mg/kg bw (Leah, 1988).

EPA-HQ-2018-002024_0004969

In a study of acute oral toxicity , five male and five female Sprague-Dawley rats received AMPA (purity, 99.2%; dissolved in 0.5% carboxymethylcellulose) as a single dose at 5000mg/kg bw by gavage.

Clinical signs were observed 4h after dosing and included piloerection, diarrhoea, subdued behaviour, hunched appearance, and soiled anal and perigenital areas. All animals recorded normal body-weight gain throughout the experiment. No abnormalities were detected at necropsy after 14 days observation.

The acute oral LD50 of AMPA in rats is >5000mg/kg bw (Cuthbert & Jackson, 1993a).

In an acute oral toxicity study, 5 male and 5 female ICR (Crj:CD -1) mice were orally dosed with 5000 mg AMPA (purity 99.33%)/kg bw. The test material was administered as a 25% suspension in 1% CMC sodium solution at 20 mL/kg bw. There was no mortality and there were no signs of toxicity. All mice gained weight days 0-7; one male and two females had slight weight losses days 7-14. There were no observed abnormalities at necropsy.

The oral LD_{50} of AMPA in male and female mice was > 5000 mg/kg bw (Komura, 1996).

In a study of acute dermal toxicity , five male and five female Sprague -Dawley rats received AMPA (purity 99.2%) as a single dose at 2000mg/kg bw. The test substance was administered evenly onto a square dressing (5cm × 5cm) that was moistened with distilled water and then applied to the shaved back of each rat. The patch was covered with an occlusive dressing and kept in contact with the skin for 24h. At the end of the exposure period the patch was removed and the exposed skin wiped with distilled water to remove any excess test material.

There were no mortalities after a single dermal application of AMPA at 2000mg/kg bw, no clinical signs were noted and no abnormalities detected at necropsy. Thus, the acute dermal LD50 of AMPA to rats must be above this limit dose (Cuthbert & Jackson, 1993b).

In an acute dermal toxicity study, 5 male and 5 female CD ® / Crl:CD® rats received 24-hour occluded exposure to 2000 mg AMPA (purity 98.0%) in 0.5% aqueous hydroxypropylmethyl cellulose gel. The suspension was applied at a volume of 10 mL/kg. There was no mortality and there were no signs of toxicity. There was no dermal irritation. There were no observed abnormalities at necropsy.

The dermal LD_{50} of AMPA was >2000 mg/kg (Leuschner, 2002a).

The sensitisation potential of a test material, AMPA (purity 99.2%) , was investigated by means of the Magnusson -Kligman Maximisation Test in guinea pigs. A group of 20 female Dunkin - Hartley guinea pigs received AMPA by an intradermal injection (10% in w/v in carboxymethyl cellulose (CMC) and 6 days later topical application (25% w/v jn 0.5% CMC. Challenge was at a concentration of 25% w/v in CMC.

At challenge, none of the test or control g roup animals treated with AMPA at a concentration of 25% w/v in CMC showed a positive response. There is no evidence from the test results that AMPA is a sensitiser in guinea pigs (Cuthbert & Jackson, 1993c).

In a Magnusson-Kligman (Maximization Test) dermal sensitization study, a group of 10 male Dunkin-Hartley guinea pigs received injections containing 5% AMPA (98.0%) on Day 0, had their application site skin treated with sodium laurylsulfate on Day 6, and then were topically treated with 2 mL of a 50% suspension of AMPA in *aqua ad iniectabilia* on Day 7. They were challenged (along

EPA-HQ-2018-002024_0004970

with 5 negative control animals) with 2 mL of a 50% suspension of AMPA in *aqua ad iniectabilia* on Day 21. There was no resultant skin irritation in any guinea pig.

AMPA was a non-sensitizer in this assay (Leuschner, 2002b).

Short Term Studies

In a short -term toxicity study, g roups of 5 male and 5 female Sprague -Dawley rats were administered AMPA (purity 99.2%) in CMC at dose levels of 0, 10, 100, 350 and 1000 mg/kg bw per day by oral gavage for 28 days.

There was no treatment related effect on mortality, clinical signs, body weight, body weight gains, food consumption, water cons umption and macroscopic findings. T here were slight but statistically significant increases in kidney weights in males at the 350 and 1000 mg/kg bw per day when compared with values for the control group (by 7% and 8%, respectively). Histological examinations revealed a very mild reduction of serous secretion in the mandibular salivary gland of males (1/5) at the highest dose. With regard to the salivary gland findings in some of the studies with glyphosate, it is equivocal whether or not this minor finding was related to treatment.

The NOAEL is 100 mg/kg bw per day based on an increase in kidney weights seen at 350 mg/kg bw per day and above (Heath, Strutt and Iswariah, 1993).

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 dose levels 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 pretrial and on all control and high dose animals during week 12. All surviving animals were killed and necropsied at termination. Premature decedents were also necropsied. Histological examination was carried out on selected tissues from all control and h igh dose animals and all premature decedents. In addition, histological examination was carried out 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 mortali ty, clinical signs, body weight, body weight gain, food consumption, water consumption, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights, macroscopic findings and histological examination (Strutt et al., 1993).

Genotoxicity

Table 70 Summary of genotoxicity studies with AMPA

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
Reverse Mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A pKM101)	±S9 mix: 0-5000 µg/plate	>99	Negative	Callander (1988)
Reverse Mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537	±S9 mix: 0-5000 µg/plate	99.2	Negative	Jensen (1993a)

EPA-HQ-2018-002024_0004971

Reverse Mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0-5000 µg/plate	99.33	Negative	Akanuma (1996)
Unscheduled DNA Synthesis	Rat hepatocytes	0.625 – 10 mM (10 mM = 1.11 g/L)	99.9	Negative	Nesslany (2002)
Gene mutation	mouse lymphoma cells (L 5178Y)	0-5 mg/ml	99.2	Negative	Jensen (1993b)
Mouse micronucleus assay	NMRI male and female mice	5000 mg/kg bw in 0.9% Sodium chloride and 1% CMC	99.2	Negative	Jensen (1993c)

Developmental Toxicity

In a developmental toxicity study, AMPA (purity 9 9.2%) suspended in a CMC was administered to 10 copulated Sprague-Dawley female rats/dose by or al gavage at dose levels of 0, 100, 350, or 1000 mg/kg bw per day from days 6 through 1 6 of gestation (GD6-16). On day 20 of gestation, the dams were killed, pregnancy status determined, and numbers of corpora lute a, implantations, and live foetuses recorded. All live foetuses were weighed, sexed, and examined for external, visceral, and skeletal abnormalities.

There were no mortalities, and there were no clinical observations related to treatment with AMPA throu ghout the duration of the study. Body -weight gain and food consumption of the test animals were similar to those of the controls. There were no notable intergroup differences in the incidence of intrauterine deaths, or in mean fetal weights. Examination of foetuses for developmental abnormalities and variations of the viscera and skeleton (including state of ossification) showed no intergroup differences. Th e maternal and developmental toxicity NOAEL is 1000mg/kg bw per day (Hazelden, 1992).

EPA-HQ-2018-002024_0004972

N- acetyl-glyphosate and N-acetyl-AMPA

Metabolism studies in genetically modified soya beans and maize containing the glyphosate -N-acetyltransferase (GAT) gene demonstrated that new metabolites are formed that were not observed in conventional crops. The maj or metabolite in the new maize and soya bean varieties was N -acetylglyphosate (which may be degraded to glyphosate in the rat), whereas glyphosate, N -acetyl-AMPA and AMPA were found in low concentrations in the edible parts of the crops. N-acetyl glyphosate and N-acetyl-AMPA were reviewed by the JMPR in 2011. The Meeting (2011) concluded that the group ADI of 0 –1 mg/kg bw established by the 2004 JMPR for glyphosate and AMPA may also be applied to N -acetyl-glyphosate and N -acetyl-AMPA, as the available toxic ological data showed that these plant metabolites have no greater toxicity than the parent glyphosate. The 2004 JMPR decided that an ARfD for glyphosate was unnecessary. The JMPR (2011) confirmed that it is not necessary to establish an ARfD for N -acetyl-glyphosate or N-acetyl-AMPA in view of their low acute toxicity and the absence of any toxicological effects that would be likely to be elicited by a single dose.

N- acetyl-glyphosate (company code IN-MCX20)

A total of 45 male Sprague Dawley [Crl:CD(SD)IG S BR] rats each received a single oral dose of 15 mg free acid equivalent/kg bw of [^{14}C]N-acetyl glyphosate (sodium salt; purity 84.3%, radioachemical purity 99.2%) in water . Blood was collected from four animals/time point predose, and at 0.5, 1, 2, 4, 8, 12, 48 and 72 hours postdose. Excreta were collected from 5 animals at specified intervals through 168 hours postdose. Plasma, excreta and carcasses were analyzed for radioactive content. Selected samples of plasma, urine and faeces were analyzed for u nchanged parent compound and metabolites.

The mean total recovery was 95.5%, with 66.1% (61.3% within 12 hours of dosage) in urine, 26.4% (25.8% within 48 hours of dosage) in faeces, 2.79% in cage wash and wipe, and 0.23% in residual carcass. More than 90% of the total radioactivity was eliminated by 48 hours postdose. The mean maximum concentration (Cmax) in blood and plasma were 2.93 and 5.31 µg equivalents/g at 1 and 2 hours postdose, respectively. Radioactivity was eliminated from blood and plasma w ith half-life (t1/2) values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma values for area under the curve (AUC) indicates that [^{14}C]N-acetyl-glyphosate distributed preferentially into plasma.

Unchanged [^{14}C]N-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and represented less than 1% of the total radioactivity. Plasma radioactivity consisted entirely of unchanged [^{14}C N-acetyl-glyphosate (Cheng and Howard, 2004).

Acute Toxicity

The test material (purity 84.3% sodium salt, equivalent to 67.4% free acid) was suspended in water and administered to 5 male and 5 female Crl:CD ®(SD) IGS BR fasted (17 -20 hours) rats. The dose was 5000 mg free acid/kg bw, administered as a constant dose volume of 10 mL/kg bw.

One female was found dead at 6 hours after administration, and one female and one male were found dead the following day. Signs of toxicity (seen in all rats) included slight hy poactivity, irregular respiration, liquid faeces, soft faeces, light -brown perineal staining, squinted eyes, and brown nasal crust. All survivors were normal 3 days after dosing. Necropsy findings of decedents included mottled or discolored lungs, discol ored (black) liver, soft stomach, yellow fluid or gel -like clear liquid in stomach, fluid in abdominal cavity, fluid in duodenum, jejunum and ileum.

The LD_{50} in rats > 5000 mg free acid/kg bw (Vegarra, 2004).

Subacute

EPA-HQ-2018-002024_0004973

Five groups of young adult male and female Crl:CD(SD) rats (10/sex/group) were fed diets containing 0, 180, 900, 4500 or 18,000 ppm N-acetyl-glyphosate sodium salt (purity 81.8%) for 95 days (males) or 96 days (females). The mean daily intake of N-acetyl-glyphosate sodium salt in the 0, 180, 900, 4500 or 18,000 ppm groups was 0, 11.3, 55.7, 283, and 1157 mg/kg bw per day, respectively, for male rats and 0, 13.9, 67.8, 360 and 1461 mg/kg bw per day, respectively, for female rats.

No adverse test-substance-related effects on any body weight or nutritional parameters were observed. Statistically significant lower overall mean body weight gain (86% of control) was observed in 18,000 ppm males but was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean food consumption of food efficiency.

There were no test-related deaths, and no clinical, ophthalmological, or neurobehavioral observations were attributed to exposure to the test substance. There were no adverse effects on clinical pathology parameters, organ weights, gross pathology, or microscopic pathology in male or female rats. The no-observed-adverse-effect level (NOAEL) for male and female rats was 18,000 ppm, equivalent to 1157 and 1461 mg/kg bw per day in males and females, respectively (MacKenzie, 2007).

This is a supplemental report to a 90-day rat feeding study described above (MacKenzie, 2007) (with Disodium N-acetyl-N-(phosphonomethyl) glycine, purity 63% (expressed as the weight percent on a free acid basis).

Pooled urine samples for male rat groups I (control), III (180 ppm), V (900 ppm), VII (4500 ppm) and IX (18000 ppm) were collected on test day 82 and for female rats groups II (control), IV (180 ppm), VI (900 ppm), VIII (4500 ppm) and X (18000 ppm) on test day 83 for analysis of IN-MCX20 (N-acetyl glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and IN-EY252 (N-acetyl AMPA). On the same days, plasma samples from individual rats were collected for the same analyses.

For the rat urine samples, concentrations of IN-MCX20 increased with the increasing dietary levels of this test substance. Concentrations of IN-B2856 and IN-EY252 were detected above the limit of detection at higher dietary levels (900 to 18000 ppm) but at or below the limit of detection from the 180 ppm dietary group. In addition, the concentrations of these metabolites were much higher in urine samples from male rats than from corresponding female rats at 4500 and 18000 ppm. Neither IN-MCX20 nor either metabolite was detected in urine from control rats.

For the rat plasma samples, the concentrations of IN-MCX20 also increased with increasing dietary levels of this test substance. Concentrations of IN-MCX20 were < 1.0 µg/mL for males and females in the 180 ppm dietary group. Concentrations increased from a mean of ~2 µg/mL up to ~14.0 µg/mL for the other dietary groups. Little to no IN-B2856 or IN-EY252 was detected in plasma for all dietary levels. These results confirm that IN-MCX20 is metabolized in rats to small quantities of IN-B2856 and IN-EY252 (Shen, 2007).

Genotoxicity

The results of genotoxicity are summarized in Table 71.

Table 71: Results of genotoxicity studies with N-acetyl-glyphosate

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference

Reverse Mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0-5000 µg/plate	84.3%	Negative	Mecchi (2004)
Gene Mutation (*HPRT* locus)	CHO cells	±S9 mix: 0-2091 µg/ml	81.8	Negative	Glatt (2006)
Chromosomal aberration	CHO cells	±S9 mix: 0-2800 µg/ml	84.3	Negative	Murli (2004
Mouse micronucleus	Male and female Crl:CD1 (ICR) mice	Single gavage dose of 0, 500,1000 and 2000 mg/kg bw	81.8	Negative	Donner (2006)

N-Acetyl-AMPA

Acute Toxicity

The test material [(Acetylamino) methyl] phosphonic acid or Phosphonic acid [(Acetylamino) methyl], also known as N-Acetyl AMPA, purity 97%, was administered by oral gavage at 5000 mg/kg to 3 Crl (CD)SD female. The test material was suspended in deionized wa ter and administered at a constant dose volume of 20 mL/kg bw.

There was no mortality. Signs of toxicity included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia, and/or hyperreactivity. All rats were normal at 3 days aft er dosage. All rats gained weight days 0-7 and 7-14. At necropsy there were no dose-related abnormalities.

The LD_{50} of N-acetyl-AMPA in rats is > 5000 mg/kg bw (Carpenter, 2007).

Genotoxicity

Table 72: Results of genotoxicity studies with N-acetyl-glyphosate

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
Reverse Mutation	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537); *Escherichia coli* (WP2*uvr*A)	±S9 mix: 0-5000 µg/plate	76	Negative	Wagner and Klug (2007)
Gene Mutation (*HPRT* locus)	CHO cells	±S9 mix: 0-1531 µg/ml	72	Negative	Glatt (2007)
Chromosomal aberration	Human peripheral blood lymphocytes	±S9 mix: 0-1530 µg/ml	76	Negative	Gudi and Rao (2007)
Mouse micronucleus	Male and female Crl:CD1 (ICR) mice	Single gavage dose of 0, 500,1000 and 2000 mg/kg bw	72	Negative	Donner (2007)

Other formulation ingredient

Poly ethoxylated tallow amine (MON 0818; CAS 61791-26-2 (tallow); Ave POE n=15])

Ogrowsky, D. (1989) Four -Week Feeding Study of (Inert Ingredient) in Spra gue-Dawley Rats. Project Number: ML/88/273, MSL/9238, 1663. Unpublished study prepared by Monsanto Company

In a 30 -day oral toxicity study , MON 0818 (purity and lot number not reported) was administered to groups of 10 male and 10 female Sprague Dawley rats in the diet at concentrations of 0, 800, 2000, or 5000 ppm (equivalent to 0, 51.7, 122.8, and 268.7 mg/kg bw/day for males and 0, 63.2, 159.9, and 324.8 mg/kg bw/day for females).

All treated rats survived until scheduled sacrifice. Soft stools fro m three high -dose males on four occasions and from eight high -dose females on 23 occasions were observed. The body weight, body weight gain, and food consumption of high -dose male and female rats were sig¬nificantly reduced during the study; consistent wi th poor diet palatability. Food consump¬tion of mid -dose male rats was statistically decreased during the first week of treatment, as was total body weight at the end of the study, however, the final body weight was decreased by only 7% relative to con¬tr ol. No treatment-related effects were found in mid -dose female rats or in low -dose male and female rats. The absolute and relative to body organ weights of high -dose male and female rats were decreased consistent with the markedly decreased body weight. Prominent or en¬larged lymphoid aggregates in the colon of 5/10 high-dose female rats were observed at necropsy.

This 30-day oral toxicity study in the Sprague Dawley rat is unacceptable and a NOAEL and LOAEL cannot be established. Since a description o f the test material, its lot number, its purity; and its concentration, homogeneity, and stability in the diet were not provided or determined, an estimate of the dose inducing treatment-related effects to male and female rats cannot be made. In addition, very limited in-life observations and with the exception of selected organ weights and gross pathology, no post-sacrifice studies or observations were made (Ogrowsky, 1989).

Stout, L. (1990) Ninety-Day Study of (Inert Ingredient) Administered in Feed to Albino Rats. Project Number: ML/89/359, MSL/10468, 1663. Unpublished study prepared by Monsanto Company

The potential subchronic oral toxicity of the test material, MON 0818, was evaluated in Sprague-Dawley rats. The test material was administered in the diet ad libitum to three groups of 10 male and 10 female rats for 90 days. Target test diet concentrations were 500, 1500, or 4500 ppm (equivalent to 33.0, 99.3, 291.6 mg/kg bw/day in males and 39.9, 123.1, and 356.6 mg/kg bw/day in females). A similar concurrent control group of rats received basal diet only. Doses were selected based on a previous 28-day range-finding study.

Exposure to MON 0818 in the diet at the mid- and high-dose levels of 1500 and 4500 ppm resulted in statistically- and toxicologically-significant effects. Toxicity observed at 4500 ppm consists of clinical signs (soft stools, 3 incidences in 2 males and 86 incidences in all females) observed from day 16 through day 92 of the study, decreased mean body weights throughout the study (ranging from 12 - 20% and 8 -18% in males and females, respectively), and decreased mean total body weight gains in males (31%) and females (35%). Food consumption was also significantly reduced throughout most of the study (13 weeks for males and 10 weeks f or females), particularly during the first week of the study (32% decrease in males and 27% decrease in females). Since a food efficiency assessment was not conducted, it is not possible to determine if the decreases in body weights, body weight gains, and food consumption were due, in part, to the unpalatability of the diet. Statistically -significant changes in hematological parameters observed in females may be a result of the inflammation observed in the intestines. Statistically -significant changes in c linical chemistry parameters and organ weights observed in high -dose males and females are likely a result of decreased food consumption/nutrient absorption and body weight.

EPA-HQ-2018-002024_0004976

At both the 1500 and 4500 ppm dose levels, microscopic examination conducted at necropsy revealed lesions, including: (1) hypertrophy and/or vacuolation of histiocytes in the lamina propria of the ileum in all high-dose males and females, and 4 of 10 mid-dose males and 4 of 10 mid-dose females; (2) hypertrophy and/or vacuolation of histiocytes in the lamina propria of the jejunum in 4 of 10 high-dose males, 7 of 10 high-dose females, and 1 mid-dose female; and (3) sinus histiocytosis in 9 of 10 high-dose males, 6 of 10 high-dose females, and 2 of 10 mid-dose males and females; and (4) accumulation of macrophage aggregates in the cortex and medullary cords of the mesenteric lymph node in 8 of 10 high-dose males, 7 of 10 high-dose females, and 2 of 10 mid-dose females. These inflammatory changes are likely the cause of the soft stools observed during the study and are considered treatment-related.

No statistically-significant treatment related effects on body weight, body weight gain, food consumption, hematological/clinical chemistry parameters, and organ weights were observed at the low-dose level of 500 ppm. In addition, no gross abnormalities or histopathological findings related to treatment were observed at this dose level.

Based on review of the study, the no-observable-adverse-effect-level (NOAEL) for MON 0818 is 500 ppm (33.0 mg/kg bw/day in males and 39.9 mg/kg bw/day in females). The lowest-observable-adverse-effect-level (LOAEL) is 1500 ppm (99.3 mg/kg/day in males and 123.1 mg/kg bw/day in females), based on irritation in the intestines and colon (hypertrophy and vacuolation of histiocytes in the lamina propria of the jejunum and ileum, and histiocytosis and accumulation of macrophage aggregates in the mesenteric lymph node (Stout, 1990).

Knapp, J. (2007) A Reproduction/Developmental Toxicity Screening Study of MON 0818 in Rats. WIL Research Laboratories, LLC (Ashland, Ohio). Study No. WIL-50282, January 4, 2007. Unpublished.

In a screening study, the potential reproductive toxicity and developmental (prenatal and postnatal) toxicity of the test article, MON 0818 (69-73% a.i.; Lot# GLP-0309-14324-I), was evaluated in CD (Sprague-Dawley) rats through two successive generations. The study was designed to evaluate the effects of MON 0818 on male and female reproduction within the scope of a screening study. The study was extended to a two-generation study when a decrease in live litter size was observed at the high-dose level. In the study, MON 0818 was administered orally via the diet to three groups of 20 male and 20 female CD rats. Target test diet concentrations were 100, 300 or 1000 ppm. A similar concurrent control group of rats received basal diet only. At approximately 10 weeks of age, the P animals were dosed via diet for at least 70 days prior to mating and continuing to sacrifice (males) or LD 21 (females). All P adults were sacrificed following selection of the F1 generation on PND 21.

Selection of parents for the F1 generation was made from the weaned F1 litters. Between PND 21 or 22 and 70, the weanling F1 animals (3/sex/litter, if possible) were administered the test diet on a mg/kg basis (so not to overexpose the rapidly growing F1 animals) at target concentrations of 0, 6, 18, or 61 mg/kg/day for the F1 males and 0, 7, 22, or 74 mg/kg/day for the F1 females. Beginning on PND 70, the F1 animals selected for breeding from the control and high-dose groups only (2/sex/litter) were administered the test diet at a constant concentration (0 or 1000 ppm) for a minimum of 80 to 88 days prior to mating. The selected F1 males continued to receive the test diet throughout mating and continuing until sacrifice (after the F2 pups reached LD 4). The selected F1 females continued to receive the test diet throughout mating, gestation and lactation and until the day of sacrifice (after the F2 pups reached LD 4).

Mortality and clinical signs, body weights, body weight gains, food consumption, reproductive function, fertility and mating performance, absolute and relative organ weights, macroscopic abnormalities at necropsy, and histopathological findings were recorded for all parental/adult animals. In addition, blood samples for testosterone and/or thyroid hormone concentration determinations were collected from one F1 male and one F1 female per litter at the scheduled necropsy. Sperm evaluation (motility and morphology) was also performed on all F1 male animals at termination. Litter size,

EPA-HQ-2018-002024_0004977

viability, clinical signs, body weights, body weight gains, developmental (sexual and physical) parameters, and macroscopic abnormalities at necropsy were recorded for the F1 and F2 pups.

Survival and clinical conditions, mean body weights and food consumption (pre -mating, gestation, and lactation), reproductive performance, mean organ weights, and macroscopic and microscopic morphology of the P and F1 parental generations were unaffected by adm inistration of MON 0818 at all dose levels. Treatment -related effects were also not seen in estrous cyclicity, spermatogenic endpoints and testosterone and thyroid hormone levels of the F1 generation or in the clinical signs, mean body weights, and developmental landmarks of the F1 and F2 pups, as well as the litter viability and postnatal survival of the F2 litters.

Potential treatment -related effects were observed in litter loss, increased mean number of unaccounted-for implantation sites, and decreased mean number of pups born, live litter size and postnatal survival from birth to LD 4 in the high -dose P females and F1 litters. These effects were limited to a small number of litters, not always statistically-significant, and were not reproduced in the F2 litters. However, the increased (statistically -significant) mean number of unaccounted -for implantation sites exceeded the maximum mean value in the laboratory historical control data. While not statistically-significant, the corresponding reduced number of pups born and live litter size, as well as the reduced postnatal survival, were at or below the limits observed in the laboratory historical control data.

Therefore, the lowest -observed-adverse-effect level (LOAEL) for parental reproductive toxicity (P) and offspring developmental/neonatal toxicity (F1) is 1000 ppm (56.1 and 52.8 mg product/kg/day (equivalent to 41 and 38.5 mg/kg/day) for the P and F1 males, respectively, and 66.6 and 64.9 mg product/kg/day (equivalent to 48.6 and 47 mg/kg/day) for P a nd F1 females, respectively), based on litter loss, increase mean number of unaccounted -for implantation sites and decreased mean number of pups born, live litter size and postnatal survival from birth to LD 4. The no-observed-adverse-effect level (NOAEL) is 300 ppm (16.6 and 14.9 mg product /kg/day (equivalent to 12 and 11 mg/kg/day) for the P and F1 males, respectively, and 19.5 and 18.9 mg product/kg/day (equivalent to 14 and 13.7 mg/kg/day) for the P and F1 females, respectively). The NOAEL for parental (P and F1) systemic toxicity is 1000 ppm. A LOAEL for parental systemic toxicity was not determined(Knapp, 2007).

Knapp, J.F. (2008) A Combined 28 -Day Repeated Dose Oral (Dietary) Toxicity Study with the Reproduction/Developmental Toxicity Screening T est of MON 8109 and MON 0818 in Rats. WIL Research Laboratories, LLC., Ashland OH Study number WIL-50337, April 3, 2008. Unpublished.

Nord, P.J. (2008) A Combined 28 -Day Repeated Dose Oral (Dietary) Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of MON 8109 and MON 0818 in Rats - Sub-Report on Analysis of Dietary Formulations. Mosanto Company St. Louis, MO. Study number WI - 2007-013, January 17, 2008. MRID 47405101. Confidential Attachment. Unpublished.

In a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, MON 8109 (Coco amine ethoxylates, CAS No. 61791-31-9, (coco); Ave POE n=2]) ; purity 100%) or MON 0818 (purity 100%) was administered to 12 Crl:CD(SD) rats/sex/dose in the diet at dose levels of 0, 30, 100, 300, or 2000 ppm MON 8109 or 1000 ppm MON 0818. Males received the test or basal diets for a total of 71-72 days, and the females received the test or basal diets for a total of 69 -72 days. Functional observational battery (FO B) and locomotor activity data were recorded for 6 males/group near the end of diet administration and for 6 females/group on lactation day 4. Parental animals were sacrificed approximately 2.5 weeks after lactation day 4, and offspring were sacrificed on lactation day 4.

No mortality related to the test substance occurred. One female in the 1000 ppm MON 0818 group was found dead with dystocia on lactation day 1 and another was euthanized in extremis on gestation day 30 and found to have a ruptured uterus. Increased incidences of red material around the nose, reddened nose, and reddened mouth were test substance -related findings in males and females treated with 2000 ppm MON 8109. Mean body weight losses were noted at 2000 ppm MON 8109 in

EPA-HQ-2018-002024_0004978

male and females during the first week of test diet administration. Lower mean body weight and/or body weight gain with corresponding reduction in food consumption were usually observed in the animals from this group throughout the study. Absolute and relative organ weight values that were statistically different from the corresponding control were not treatment -related due to the significantly lower body weight of the 2000 ppm MON 8109 treated animals. The females from this group had a lower number of implantation sites and lower live litter size. Offspring of these females had lower postnatal survival on PND0, PND0-1, PND1-4, and birth to PND4 compared to the control group. No effect of treatment was observed in male and female mating and fertility, male copulation and female conception indices, gestation length, functional observational battery, locomotor activity, hematology, or serum chemistry. No test substance-related findings were noted in the 30, 100, or 300 ppm MON 8109 or 1000 ppm MON 0818 group males, females, or offspring.

The parental systemic LOAEL is 2000 ppm MON 8109 (126 mg/kg bw/day in males, 147, and 181 mg/kg bw/day in females through gestation and lactation, respectively), based on clinical findings, decreased mean body weight and body weight gain, and food consumption. The parental systemic NOAEL is 300 ppm MON 8109 (19 mg/kg bw/day in males, 22, and 34 mg/kg bw/day in females through gestation lactation, respectively).

The reproductive/developmental LOAEL is 2000 ppm MON 8109 based on decreased postnatal survival, lower live litter size on postnatal day 0, lower number of pups born, and lower number of implantation sites. The reproductive NOAEL is 300 ppm MON 8109.

A parental LOAEL for MON 0818 was not demonstrated in this study. The parental NOAEL is 1000 ppm MON 0818 (66 mg/kg bw/day in males, and 86 mg/kg bw/day in females).

The reproductive/developmental LOAEL for MON 0818 was not demonstrated in this study. The reproductive NOAEL is 1000 ppm MON 0818 (Knapp, 2008 and Nord 2008)).

Holson, J. (2006) A Developmental Toxicity Study of (Inert Ingredient) in Rats. Project Number: WI/89/388, WIL/50097, 1663. Unpublished study prepared by Monsanto Company and WIL Research Laboratories, Inc.

In a developmental toxicity study (MRID 46902005), MON 0818 (100% a.i., Lot No. PIT-8907-7571) was administered in Mazola® Corn Oil to 25 Charles River Crl:CDBr female rats/dose by gavage at dose levels of 0 (corn oil only), 15, 100 or 300 mg/kg bw/day from days 6 through 15 of gestation. On day 20 of gestation, all surviving females were sacrificed for a scheduled Cesarean section. Developmental parameters observed and noted included: number of viable fetuses, early and late resorptions, total implantations, total corpora lutea, sex and weight of fetuses and external, visceral and skeletal examinations of all fetuses.

Six of the twenty five high-dose females died during GD 6-15. Clinical signs were also observed in the high-dose females and included: rales (12/25), labored respiration (3/25), yellow uro- (15/25) or anogenital (14/25) matting and mucoid feces (22/25) compared to none of the control animals. Few to no clinical signs were observed in the mid-dose and low-dose females. High-dose females weighed significantly ($p<0.01$) less than the controls from study day 9 until sacrifice at study day 20. High dose females also gained 59% less weight compared to controls during treatment (days 6-16). Body weight was similar to controls in the low- and mid-dose groups. Gravid uterine weight was not affected by treatment in any of the groups. High-dose females ate statistically ($p < 0.01$) less food compared to the control rats with the most significant decrease (55% less than controls) on days 6-9 before gradually improving to become comparable to controls by day 16. Overall for days 6-16, the high-dose group ate 29% less than the controls. Food consumption for the low-dose and mid-dose females was comparable to that of controls throughout the study, except for days 6-9 when the mid-dose group had a statistically significant ($p<0.05$) decrease. There were no treatment-related effects observed on liver weight or gross pathology at necropsy in any of the treated dams.

The maternal lowest -observed-adverse-effect level (LOAEL) for MON 0818 in rats is 300 mg/kg bw/day, based on increased mortality, clinical signs, and decreased body weight, body weight gain, and food consumption. The maternal no -observed-adverse-effect level (NOAEL) for MON 0818 is 100 mg/kg bw/day.

No treatment-related differences were o bserved in the mean number of corpora lutea, implantations, live fetuses or resorptions. Mean fetal weight was not affected by maternal treatment with the test article. The mean number of malformations on external examination of the fetuses from the high-dose dams appeared to be high but most were observed in a single one fetus and a dose response was not observed. On visceral examination, in the high -dose group, one fetus was missing a urinary bladder, one fetus had stenosis of the right carotid artery and two fetuses had situs inversus. One control fetus also had situs inversus. These were not considered treatment -related as there was not a dose response for the situs inversus and the others were within the historical control data range. Vertebral anomalie s with or with/out rib anomalies were observed in one fetus in the high -dose group but this was within the range of historical control data. No malformations were observed in the low - or mid-dose groups. Several skeletal variations in the sternebrae and ri bs were identified but they were observed in both the control and treated groups at similar incidences and are not considered treatment-related.

The developmental lowest -observed-adverse-effect level (LOAEL) for MON 0818 in rats could not be determined as no effects were associated with treatment. The developmental no-observed-adverse-effect level (NOAEL) for MON 0818 is 300 mg/kg bw/day (Holson, 2006).

Stegeman, S.D. and Li, A.P. (1990). Ames/Salmonella Mutagenicity Assay of MON 0818. Monsanto Co. Envi ronmental Health Laboratory, St. Louis, MO. Project No. ML -89-461; Study No. 89178, dated November 12, 1990; Registrant Submission dated, July 31, 2006. Unpublished.

In independent trials of the reverse gene mutation assay in bacteria, strains TA1535, TA 1537, TA98 and TA100 of *Salmonella typhimurium* were exposed to MON 0818 (Purity not available). In the first trial, all tester strains were exposed to 0.001, 0.003, 0.01, 0.03 or 0.1 mg/plate with S9 activation and 0.0003, 0.001, 0.003, 0.01 or 0.03 mg/pla te without S9 activation. A repeat assay was performed on TA1535 and TA1537 (± S9) using the same concentrations tested in trial 1. Because cytotoxicity was not observed with all tester strains, a second cytotoxicity assay was conducted and the test arti cle concentrations were adjusted for the subsequent mutagenicity trials (trials 3 and 4). Concentrations of MON 0818 ranging from 0.01 to 1.0 mg/plate +S9 and 0.003 to 0.3 mg/plate –S9 were tested in strain TA98; 0.001 to 0.10 mg/plate ±S9 in TA100; 0.001 to 0.1 mg/plate –S9 in TA1535; 0.003 to 0.3 mg/plate +S9 and 0.001 to 0.1 mg/plate –S9 in TA1537. The S9 -fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

All tester strains were evaluated at the concentrations listed above in the presence and absence of S9 activation. No evidence of mutagenicity was observed in Trial 1. A statistically significant ($p<0.01$), increase in the number of revertant colonies was observed at 0.03 mg/plate (-S9) in TA98 and 0.0003 mg/plate in TA153 5 (-S9); however, the increases were <2 -fold and were not concentration - dependent. All strains were retested in trials 3 and 4 at concentrations described above. Cytotoxicity was seen at ≥0.3 mg/plate +S9 and ≥0.1 mg/plate –S9 in TA98, ≥0.03 mg/plate ±S9 in TA100, at 0.1 mg/plate –S9 in TA1535 and at ≥0.1 mg/plate ±S9 TA1537. Although slight increases in the number of revertants were seen at non -cytotoxic concentrations of 0.01 and 0.1 mg/plate +S9 in TA98, the increases were < 2 -fold greater than the s olvent controls and did not satisfy the criteria for a positive response. No concentration -dependent increase in the number of revertant colonies was observed in any of tester strains ±S9.
Overall, no evidence of mutagenicity was observed at non -cytotoxic concentrations in the presence or absence of S9 activation

EPA-HQ-2018-002024_0004980

MON 0818 was tested up to cytotoxic concentrations in all strains, but failed to induce a mutagenic response in this test system. The positive controls induced the expected mutagenic responses i n the appropriate strain. **There was no evidence of induced mutant colonies over background** (Stgeman and Li, 1990).

Stegeman, S.D. and Kier, L.D. (1998). Mouse Micronucleus Screening Assay of MON 0818. Monsanto Co. Environmental Health Laboratory (EHL), St.Louis, MO; Project No.: ML -89-463, EHL-89182, R.D.No. 1663, dated March 26, 1998. Unpublished

In a bone marrow micronucleus assay , adult male and female Crl:CD -1® (ICR) mice (5/dose/sex) were treated once via an intraperitoneal injection (i.p.) with 0 or 100 mg/kg MON 0818, which was estimated to be ~61% of the LD50 (Batch/Lot No. PIT -8907-757-I; Purity: 100%), prepared in corn oil. Bone marrow cells were harvested at 24 and 48 hours following dosing and scored for micronucleated polychromatic erythrocytes (MPCEs). Cyclophosphamide (60 mg/kg) served as the positive control.

No deaths, signs of overt clinical toxicity or cytotoxicity to the target organ (bone marrow) were observed at the selected dose. Although no toxicity was seen at 100 mg/kg, t he selected level was considered acceptable in accordance with the high dose recommended by the U.S. EPA Gene -Tox Program (i.e., when a dose that is not less than 50% of the LD50 is used to define the maximum tolerated dose) for the micronucleus assay (Ma vournin, et al., 1990). Administration of 60 mg/kg Cyclophosphamide caused a significant ($p<0.01$) induction of MPCE in both sexes. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any harvest time up to the maximum tolerated dose (Stegeman and Kier, 1990).

3. Observations in humans

Occupational Exposure

Occupational exposure to glyphosate can occur via dermal and inhalation routes of exposure. In vito and in vivo percutaneous absorptio n studies suggest very limited dermal penetration of glyphosate formulation. Exposure through inhalation is considered minimal route of exposure due to the low vapor pressure of glyphosate.

Both passive dosimetry and biomonitoring have been used as techni ques to assess exposure. Biomonitoring results represent systemic (internal) exposure, whereas the results obtained from passive dosimetry quantify external deposition. There is general agreement that biological measurements as obtained through biomonitori ng provide the most relevant information for safety assessments (Frankinlin et al., 1986 nd Chester et.al., 1986).

The biomonitoring study of the Farm Family Exposure Study (FFES) was supported by seven agricultural companies. In this study, e ligible farm families from Minnesota and South Carolina were randomly selected from a roster of licensed private pesticide applicators. Eligibility required that the family include a farmer, spouse, and at least one child between the ages of 4 and 17 years, that the family live on the farm, that the farmer planned to apply one of the target pesticides [glyphosate, chlorpyrifos, 2,4-dichlorophenoxy acetic acid (2,4-D)] to at least 10 acres (4.1 hectares) of land within 1 mile (1.6 kilometers) of the house. For each fam ily member, geometric means were calculated for 24-hour composite urinary samples, with a 1 ppb (part per billion) limit of detection, the day before, the day of, and for 3 days after the application. For the farmers, the peak geometric mean concentrations were 3 ppb for glyphosate, 64 ppb for 2,4 -D, and 19 ppb for the primary chlorpyrifos metabolite. For the spouses and children, the percentage with detectable values varied by chemical, although the average values for each chemical did not vary during the study period. The applicators had the highest urine pesticide concentrations, children had much lower values, and spouses had the lowest values. Exposure to family members was largely, though not exclusively, determined by the

EPA-HQ-2018-002024_0004981

degree of direct contact with the application process. The exposure profile varied for the three chemicals for each family member (Mendel et al. 2005).

As part of the Farm Family Exposure Study, we evaluated urinary glyphosate concentrations for 48 farmers, their spouses, and their 79 children (4-18 years of age). We evaluated 24-hr composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Sixty percent of farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean (GM) concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. Farmers who did not use rubber gloves had higher GM urinary concentrations than did other farmers (10 ppb vs. 2.0 ppb). For spouses, 4% had detectable levels in their urine on the day of application. Their maximum value was 3 ppb. For children, 12% had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb . All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application. None of the systemic doses estimated in this study approached the U.S. Environmental Protection A gency reference dose for glyphosate of 2 mg/kg/day. Nonetheless, it is advisable to minimize exposure to pesticides, and this study did identify specific practices that could be modified to reduce the potential for exposure (Acquavella et al. 2004).

Some earlier biomonitoring studies were performed on silvicultural workers who sprayed a glyphosate formulation in a variety of forestry and tree farming activities. In one study, the United States Department of Agriculture's Forest Service, in collaboration wit h Monsanto Company and the University of Arkansas, sponsored a study to investigate exposure of workers to glyphosate at two forestry nurseries (Phipps Nursery in Oregon and Ashe Nursery in Massachusetts) where glyphosate was used for weed control (Lavy et al, 1992). Urine samples were collected from the weeders and scouts prior to working with glyphosate and for an eight -month period thereafter. Continuous total urine sampling was conducted for the first 12 consecutive weeks of the study, after which a 24 -hour sample was collected each Wednesday for the next fivee months.

Of the 355 daily urine samples analysed, none were found to contain quantifiable levels of glyphosate. The limit of quantification was 10 ppb (Lavy et al. 1992).

In a se parate collaborative study conducted by the USDA Forestry Service, Georgia Tech Research Institute, and Monsanto (Cowell et al., 1990) the exposure of applicators to glyphosate during a hand -held directed spray foliar application at three sites maintained by the USDA For estry Service. Urinary samples were collected for 5 days after exposure. Of the 96 urine samples analysed, 5 were found to contain quantifiable levels of glyphosate. The highest glyphosate measure was 14 ppb and the highest estimated internal dose was 0.0006 mg/kg body weight (Cowell et al. 1990).

Two other studies have been conducted to measure exposure of forestry workers to glyphosate during normal silvicultural applications one in Finland (Jauhiainen et al., 1991) and the other in Canada (Center de T oxicologie du Quebec, 1988). In the Finnish study, workers sprayed glyphosate each day for 5 consequitive days in August 1988. Urine samples were collected from each workers at the end of the day. In addition, each worker received a medical examination on the first day and last day, which included haematology, clinical chemistry, ECG, pulmonary function tests, an interview for a health questionnaire, and a general clinical examination (including blood pressure, pulse rate and pressure craft of hands). All urine samples had less than detectable concentrations of glyphosate. There were no statistically significant differences in the findings of the medical examinations conducted before and after exposure (Jauhiainen et al. 1991).

The Canadian study of the ex posure of forestry workers to glyphosate following normal silviculture uses of glyphosate was conducted over two growing seasons (in 1986) and involved 45 workers conducting various operations. Glyphosate was not detected in the majority of urine samples. For the two flagmen and the operator, glyphosate concentrations in all urine samples were less than 0.03 ppm (the limit of quantitation). In contrast, 14 of 33 urine samples from the mixer and two urine samples for the foreman contained glyphosate conce ntrations greater than 0.03 ppm. Maximum glyphosate concentrations in the foreman's and mixer's urine were 0.043 and 0.055 ppm, respectively. In the follow up study in 1987, glyphosate concentrations in urine of exposed workers were very low.

EPA-HQ-2018-002024_0004982

In the majo rity of samples, glyphosate was not detectable. In those samples, which did contain detectable levels of glyphosate, concentrations were less than 0.1 ppm in all cases, and typically less than 0.035 ppm (Center de Toxicologie du Quebec, 1988).

Pesticide Poisoning Reporting:

It has been stated that glyphosate is a leading cause of pesticide poisoning in California. These claims are based upon a counting of telephone calls to the California Environmental Protection Agency's Pesticide Poisoning Information System (PISP). PISP was created in 1982 as a clearinghouse for telephone calls of pesticide -related illness. Review of the California data indicates that the number of reported cases simply reflects greater use of the product relative to other herbicides and shows that glyphosate has relatively low toxicity among pesticides used in California (Goldstein et al., 2002). An analysis of the database spanning the time frame of 1982 through 1997 shows that there were 815 calls involving glyphosate herbicide prod ucts. Of those 815 calls, 399 were eye irritation only cases, 250 were skin irritation only cases, 7 were respiratory only cases and 32 were mixed cases (eye, skin and respiratory). Only 20 of the 815 calls reported systemic symptoms following use of only a glyphosate product.

Acquavella et al. (1999) evaluated ocular effects from reported human exposures to Roundup herbicides based on 1513 calls to an American Association of Poison Control Centers (AAPCC) certified regional poison center during the years 1993 through 1997. The preponderance of reported exposures were judged by poison center specialists to result in either no injury (21%) or transient minor symptoms (70%). There was some temporary injury in 2% of cases; one injury took more than 2 weeks to resolve. In no instance did exposure result in permanent change to the structure or function of the eye (Acquavella et al. 1999).

Various studies reported in the literature describe the effects observed after accidental and intentional ingestion of conce ntrated formulations of glyphosate. Large amounts of glyphosate -based herbicides are occasionally deliberately ingested to attempt suicide and may result in serious gastrointestinal, cardiovascular, pulmonary and renal effects and possibly death (Talbot et al., 1990 Tominack et al, 1990 and Lee et al., 2000). Aggressive supportive care is recommended (Tominack et al., 1989).

In a published study by Chang et al. (1999), reported glyphosate surfactant herbicide poisoning cases from ingestion during the per iod from January 1994 through June 1998 in Taiwan, China. Only 50 patients out of 132 reported cases met the criteria established by the authors.Ingested Esophageal injury was seen in 68% of the patients, gastric injury in 72%, and duodenal injury in 16%. The upper gastrointestinal tract injuries caused by glyphosate surfactant were minor in comparison with those by other strong acids (Chang et al., 1999). In a published report by Pushny, Avnon, and Care (1998) reported that the pneuomonitis as result of R oundup exposure. However, Goldstein et al. (1999), suggested that o ccupational pneumonitis has never been reported in connection with Roundup. Although there are reports of the aspiration of Roundup concentrate resulting in lung injury, almost all reported pulmonary effects have occurred following suicidal ingestion of the undiluted Roundup concentrate and appear to be the result of cardiovascular toxicity producing nonvasogenic pulmonary edema (Goldstein et al. 1999).

The nature of the clinical symptoms ob served in cases of suicide suggests that hypovolemic shock was the cause of death (Sawada et al., 1988; Tominack et al., 1989). Because similar responses have been observed in cases involving ingestion of other surface -active agents, it has been suggested that the acute toxicity of concentrated glyphosate formulations is likely due to the surfactant. Accidental exposure results in, at most, only mild effects; no deaths have been reported (Goldstein et al., 2002).

Barobosa et al. (2001) published a single case report of a 54 -year old man who accidentally sprayed himself with a glyphosate -based formulation in his garden (manufacturer and formulation details unknown). According to the authors within 6 hours of the incident the man developed conjunctival hype remia and a generalized rash. One month later he presented with Parkinsonian

EPA-HQ-2018-002024_0004983

symptoms in all four extremities and one year later developed a resting tremor of one hand and complained of memory deficits.

In published study by Bradberry, Proudfoot and Vale (2004), reported that a ccidental ingestion of glyphosate formulations is generally associated with only mild, transient gastrointestinal features. Most reported cases have followed the deliberate ingestion of the concentrated formulation of Roundu. The authors also suggested reasonable correlation with amount ingested and the likelihood of serious systemic sequelae or death (Bradberry, Proudfoot and Vale, 2004).

Comments

Biochemical aspects

Toxicological data

.

Biochemical and toxicological data on metabolites and/or degradates

Human data

Toxicological evaluation

References

References

Akanuma, M. (1996) AMPA reverse mutation test. The Institute of Environmental Toxicology, Tokyo, Japan. Laboratory Project ID IET 96-0076. Sponsor Sankyo Co., Ltd., Tokyo, Japan. Unpublished study.

Allen, S.L. (1996) Glyphosate acid: Comparison of salivary gland effects in three strains of rat. Unpublished report No. CTL/P/5160, study No. PR 1029, dated 18 August 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Atkinson, C., Perry, C.J., Hudson, P. & Snodgrass, E. (1989) Glyphosate: 4 week dietary toxicity study in rats. Inveresk Research International report No.: 5626. Cheminova Report No.: 49 GLY

Atkinson, C., Martin, T., Hudson, P. & Robb, D. (1993a) Glyphosate: 104 week dietary carcinogenicity study in mice. Unpublished report No. 7793, IRI project No. 438618, dated 12 April 1991, fr om Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

EPA-HQ-2018-002024_0004984

Atkinson, C., Strutt, A.V., Henderson, W., Finch, J. & Hudson, P. (1993b) Glyphosate: 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks.). Unpub - lished report No. 7867, IRI project No. 438623, dated 7 April 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Baldrick P and Reeve L. Carcinogenicity Evaluation: Comparison of tumour data from dual control groups in CD -1 mouse. Toxicologic Pathology 2007; 35: 562-569.

Bhide, M.B. (1988): Carcinogenicity and chronic toxicity study of glyphosate (technical) in mice. Excel Industries Ltd.

Bhide, R.M. (1997), Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat, Indian Institute of Toxicology, Pune India, Study Nol.: 1231, Sankyo Co., Ltd., Japan, Unpublished study.

Bhide, M.B., Patil, U. M.; (198 9) Rabbit Teratology Study with Glyphosate Technical. Indian Institute of Toxicology, Sanpada, New Bombay – 400. Data owner: Excel, Study no.: IIT Project No. 1086, Unpublished study.

Botham, P.A. (1996) First revision to glyphosate acid: 90 day feeding st udy in rats. Unpublished report No. CTL/P/1599, study No. PR 0663, dated 7 November 1996, from Zeneca Agrochemicals, Central Toxicol - ogy Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Brammer, A. (1996) Glyphosate acid: 1 year dietary toxicity study in dogs. Unpublished report No. CTL/P/5079, study No. PD 1006, dated 24 September 1996, from Zeneca Agrochemicals, Central Toxi - cology Laboratory, Alderley Park, Macclesfield, Ch eshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Brammer, A. (2001) Glyphosate acid: two year dietary toxicity and oncogenicity study in rats. Unpublished report No. CTL/PR1111, study No. PR1111, dated 15 March 2001, fr om Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Brooker, A.J., John, D.M., Anderson, A. & Dawe, I.S. (1991a) The effect of glyphosa te on pregnancy of the rat (incorporates preliminary investigation). Unpublished report No. CHV 43 & 41/90716, dated 14 October 1991, from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Brooker, A.J., Brennan, C., John, D.M., Anderson, A. & Dawe, I.S. (1991b) The effect of glyphosate on preg - nancy of the rabbit (incorporates preliminary investigations). Unpublished report No. CHV 45 & 39 & 40/901303, dated 14 October 1991, from Huntingdon Research Centre Ltd., Huntingdon, England. Sub - mitted to WHO by Cheminova A/S, Lemvig, Denmark.

Brooker, A.J., Myers, D.P., Parker, C.A., Offer, J.M., Singh, H., Anderson, A. & Dawe, I.S. (1992) The effect of dietary administration of glyphosate on reproductive fu nction of two generations in the rat. Unpublished report No. CHV 47/911129, dated 14 May 1992, from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Callander, R.D. (1988) Aminomethyl phosphonic acid : An evaluation of the mutagenic potential using S. typhimurium and E. coli. Unpublished report No. CTL/P/2206, study No. YV2280 and YV2281, dated 21 September 1988, from ICI Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Carpenter C (2007) IN -EY252: acute oral t oxicity study in rats —up-and-down procedure. Unpu blished report No. DuPont-22229. DuPont Haskell Laboratory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

EPA-HQ-2018-002024_0004985

Chan, P.O. & Mahler, J.F. (1992) NTP technical report on toxicity studies of glyphosate (CAS No. 1071 -83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. National Toxicolog y Program, Research Triangle Park, NC, USA. NTP Toxicity Report Series No. 16, NIH Publication 92 -3135, July 1992. Sub- mitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Chandra M and Frith CH. Spontaneous renal lesions in CD -1 and B6C3F1 mice . Exp Toxic Pathol 1994; 46:189 - 198.

Cheng T, Howard S (2004) Mass balance, metabolism, and pharmacokinetics of [14C] *N*-acetyl-glyphosate following administration of a single oral dose to rats. Unpublished report No. Covance 7535 -100, amended report. Covance Laboratories, Inc., Vienna, VA, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Coles, R.J. and Doleman, N. (1996) Glyphosate technical: Oral gavage teratology study in the rabbit. Safepharm Laboratories Limited, Shardl ow Business Park, Shardlow, Dernyshire, DE72 2GD, UK. Data owner: Nufarm, SPL project no.: 434/020, Unpublished study.

Coles, LJ, Thomas, ON, Bartlett, AJ and Brooks, PN (1996) Technical glyphosate: Ninety day sub -chronic oral (dietary) toxicity study in t he rat. Safepham Laboratories Limited, Derby, UK. SPL project number: 434/016. Sponsor by Mastra Industries Sdn. Bhd., Port Klang, Malaysia and Maruzen Kako Co., Ltd., Tokyo, Japan. Unpublished study.

Colvin, L.B. & Miller, J.A. (1973a) CP 67573 Residue an d metabolism. Part 8: the gross metabolism of N - phosphonomethylglycine-14C (CP 67573-14C) in the laboratory rat following a single dose. Unpublished report No. 297, study No. 9 -23-760.06-7863, dated 15 June 1973, from Monsanto Commercial Products Co., Agricultural Division, Research Department. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Colvin LB and Miller JA (1973b) CP 67573 Residue and Metabolism. Part 13: The Dynamics of Accumulation and Depletion of Orally Ingested N-Phosphonomethylglycine-14C, Monsanto report MSL-309. NON GLP STUDY

Colvin, L.B. & Miller, J.A. (1973c) CP 67573 Residue and metabolism. Part 9: the metabolism of N - phosphonmethylglycine-14C (CP 67573 -14C) in the rabbit. Unpublished report No. 298, study No. 9 -23-760.06-7863, dated 20 June 1973, from Monsanto Commercial Products Co., Agricultural Division, Research Department. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Cuthbert, J.A. & Jackson, D. (1993a) AMPA: Acute oral toxicity (limit) test in rats. Unpublished report No 8763, IRI project No. 552409, dated 28 January 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1993b) AMPA: acute dermal toxici ty (limit) test in rats. Unpublished report No 8764, IRI project No. 552409, dated 28 January 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1993c) AMPA: Magn usson-Kligman maximisation test in guinea pigs. Unpub - lished report No 8765, IRI project No. 552409, dated 28 January 1993, from Inveresk Research Interna - tional, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Davies, D.J. (1996a) Glyphosate acid: excretion and tissue retention of a single oral dose (10 mg/kg) in the rat. Unpublished report No. CTL/P/4940, dated 26 April 1996, from Zeneca Agrochemicals, Central Tox - icology Laboratory, Alderley Park, Macclesfield, Cheshire, England . Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Davies, D.J. (1996b) Glyphosate acid: excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat. Unpublished report No. CTL/P/4942, dated 19 June 1996, from Zeneca Ag rochemicals, Central Tox - icology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

EPA-HQ-2018-002024_0004986

Davies, D.J. (1996c) Glyphosate acid: excretion and tissue retention of a single oral dose (1 0 mg/kg) in the rat following repeat dosing. Unpublished report No. CTL/P/4944, dated 22 May 1996, from Zeneca Agro - chemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Davies, D.J. (1996d) Glyphosate acid: whole body autoradiography in the rat (10 mg/kg). Unpublished report No. CTL/P/4943, dated 10 June 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Davies, DJ (2003) Glyphosate SL (360g/L) formulation (A12798Q) In Vitro absorption through Human Epidermis CTL/JV1732/Regulatory/Report. Syngenta CTL laboratory, UK CTL Study No.: JV1732, Document no. CTL/JV1732/REG/REPT, Unpublished.

Dhinsa, N.K., Watson, P., Brooks, P.N. (2007) Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Dernyshire, DE72 2GD, UK. Data owner: Nufarm, SPL project no.: 2060/0013, (amended 2008-04-08 and 2008-08-08), Unpublished study.

Donner EM (2006) IN -MCX20: mouse bone marrow micronucleus test. Unpublished report No. DuPont -20154, DuPont Haskell Laboratory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Donner EM (2007) IN -EY252: mouse bone marrow micronucleus test. Unpublished report No. DuPont -22226. DuPont Haskell Laboratory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Com - pany, Wilmington, DE, USA.

Enomoto, K. (1997), HR -001: 24-Month Oral Chronic Toxicity and Oncoge nicity Study in Rats, The Institute of Environmental Toxicology, Kodaira -shi, Tokyo, Japan, Arysta Life Sciences, Study No.:IET 94 -0150. Also referred to as Arysta Life Sciences, 1997, Unpublished study.

Gaou, I (2007) 13-Week toxicity study by oral route (capsule) in beagle dogs. CIT, Evreux, France. Laboratory study number 29646 TTC. Submitted by Nufarm Asi Sdn Bhd, Selangor, Malaysia. Unpublished study.

Glatt CM (2006) IN -MCX20: in vitro mammalian cell gene mutation test (CHO/HGPRT). Unpublished report N o. DuPont-20155. DuPont Haskell Laboratory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Glatt CM (2007) IN -EY252: in vitro mammalian cell gene mutation test (CHO/HGPRT). Unpublished report No. DuPont-22224. DuPont Haskell Laboratory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Goburdhun, R. (1991) Glyphosate: 52 week oral toxicity study in dogs. Unpublished report No 7502, IRI project No. 642675, dated 12 April 1991, from Inveresk Research International, Tranent, Scotland. Sub - mitted to WHO by Cheminova A/S, Lemvig, Denmark.

Goburdhun, R. & Oshodi, R.O. (1989) Glyphosate: oral maximum tolerated dose study in dogs. Unpublished report No 5660, IRI project No. 640683, dated 22 June 1989, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Gudi R, Rao M (2007) IN -EY252: in vitro mammalian chromosome aberration test in human peripheral blood lymphocytes. Unpublished report No. DuPont -22225. BioReliance, Rockville, MD, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Hadfield, N. 2012a Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [14C] -glyphosate, Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK. Study No.: JV2182, Report No.: JV2182-REG,Date: 2012-04-18, Unpublished.

EPA-HQ-2018-002024_0004987

Hadfield, N. 2012b Glyphosate: Glyphosate 360IPA salt (CA 2273): In Vitro Penetration through Human Epidermis using 14C-glyphosate. Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK. Study No.: JV2147, Report No.: JV2147-REG, Unpublished study.

Haag, V (2008) 52 -Week toxicity study by oral route (capsule) in beagle dogs. CIT, Evreux, France, Laboratory study number 29647 TTC, Sponsor Nufarm Asia Sdn Bhd, Selangor, Malaysia. Unpublished study.

Haas, M.C. (2010) An 8 -Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats, WIL -50361 GTF, Unpublished study.

Haas, M.C. (2012) Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice. WIL Research Laboratories, LLC, Ashland, OH 44805 -8946, USA, ImmunoTox®, Inc., Virginia Bio Technology Research Park, Richmond, VA 23219, USA, Project No.: WI -10-460 (Study No.: WIL -50393), Data owner: Monsanto, Unpublished study.

Hatakenaka, N. (1995) HR -001: Teratogenicity Study in Rats. The Institute of Environmental Toxicology, T okyo, Japan. Data owner: Arysta LifeScience, Study No.: IET 94-0152, Unpublished study.

Hazelden, K.P. (1992) AMPA: teratogenicity study in rats. Unpublished report No 7891, IRI project No. 490421, dated 29 December 1992, from Inveresk Research Internation al, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Heath, J., Strutt, A. & Iswariah, V. (1993) AMPA: 4 week dose range finding study in rats with administra - tion by gavage. Unpublished report No. 7803, IRI project No. 450860, dated 16 March 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Hodge, M.C.E. (1996) First revision to glyphosate acid: 90 day oral toxicity study in dogs. Unpublished report No. CTL/P/1802, study No. PD 0674, dated 14 November 1996, from Zeneca Agrochemicals, Central Tox - icology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Hojo, H. (1995). HR -001: A Teratogenicit y Study in Rabbits. The Institute of Environmental Toxicology, Kodaira - shi, Tokyo, Japan. Data owner: Arysta Life Sciences, Study no.: IET 94-0153, Unpublished study.

Horner, S.A. (1996a) Glyphosate acid: acute neurotoxicity study in rats. Unpublished repo rt No. CTL/P/4866, study No. AR5968, dated 11 March 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Horner, S.A. (1996b) Gl yphosate acid: subchronic neurotoxicity study in rats. Unpublished report No. CTL/P/4867, study No. PR1009, dated 11 March 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by S yngenta Crop Pro- tection AG, Basel, Switzerland.

Howe, R.K., Chott, R.C. & McClanahan, R.H. (1988) The metabolism of glyphosate in Sprague-Dawley rats. Part II. Identification, characterisation and quantitation of glyphosate and its metabolites after intr avenous and oral administration. Unpublished report No. MSL -7206, study No. 206300, dated February 1988, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, and Monsanto Life Sciences Research Center, Chesterfield, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Jensen, J.C. (1993a) Mutagenicity test: Ames Salmonella test with AMPA, batch 286 -JRJ-73-4. Unpublished report Lab. No. 13269, dated 18 February 1993, from Scantox A/S, Lille Skensved, Denmark. Submit ted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1993b) Mutagenicity test: in vitro mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y). Test compound: AMPA, batch 286 -JRJ-73-4. Unpublished report Lab. No. 13270, dated 18

EPA-HQ-2018-002024_0004988

February 1993, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1993c) Mutagenicity test: micronucleus test with AMPA, batch 286 -JRJ-73-4. Unpublished report Lab. No. 13268, dated 18 February 1993, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Johnson, AJ (1982) Glyphosate: 21-Day dermal toxicity study with rabbits. International Research and Development Corporation. Sponsor Monsanto, St. Louis, MO., Monsanto number IR-81-195. Unpublished study.

Johnson, A.J. (1996) Glyphosate acid: acute delayed neurotoxicity study in the domestic hen. Unpublished report No. CTL/C/3122, project No. ISN 361/960244, dated 23 August 1996, from Huntingdon Life Scienc es Ltd., Huntingdon, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Kinoshita, M (1995) HR -001: 13 -Week subchronic oral toxicity study in rats. The institute of environmental toxicology, Tokyo, Japan. Laboratory Project ID IE T-94-0138. Sponsor by Sankyo Co., Ltd., Tokyo, Japan. Unpublished study.

Knezevich, A. and Hogan, G. (1983) A Chronic Feeding Study of Glyphosate (Roundup Technical) in Mice: Project No. 77-2061: Monsanto Report BDN-77- 420.

Knowles, S.L. and Mookherjee, C .R. (1996) [14C] -Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat. Corning Hazleton Europe, Harrogate, North Yorkshire, UK, Data owner: Nufarm, Report No.: 1413/2-1011, Date: 1996-10-23, not published.

Komura, H. (1996) AMPA: Acute oral toxicity study in mice. The Institute of Environmental Toxicology, Tokyo, Japan. Laboratory project ID Study No. IET 96 -0075, Sponsor Sankyo Co., Ltd, Tokyo, Japan. Unpublished study.

Kumar, D.P.S. (2001) Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice Toxicology Department Rallis Research Centre, Rallis India Limited, Peenya II Phase, Bangalore, 560 058 India. Data owner: Feinchemie Schwebda GmbH, Study No.: Toxi: 1559.CARCI-M, Unpublished study.

Lankas, G.R. (1981) A Lifetime Feeding Study of Glyphosate (ROUNDUP Technical) in Rats Bio/dynamics Inc., Division of Biology and Safety Evaluation, East Millstone, New Jersey, Data owner: Monsanto Study/Project No.: 77-2062 (BDN 77-416). Unpublished study.

Leah, A.M. (1988) Aminomethyl phosphonic acid: acute oral toxicity to the rat. Unpublished report No. CTL/P/2266, study No. AR4690, dated 26 August 1988, from ICI Agrochemicals, Central ToxicologyLaboratory, Alderley Park, Macclesfield, Cheshire, England. S ubmitted to WHO by Syngenta Crop Pro- tection AG, Basel, Switzerland.

Leuschner, PJ (2002a) Acute toxicity of AMPA (aminomethyl phosphonic acid) in CD rats by dermal administration-Limit test. LPT laboratory of Pharmacology, Hamburg, Germany. LPT Report No . 16168/02. Sponsor Agan Chemical Manufacturers Ltd. Ashdod, Israel.

Leuschner, PJ (2002b) Examination of AMPA (aminomethyl phosphonic acid) in the skin sensitization test in guinea pigs according to Magnusson and Kligman (Maximisation test). LPT laborator y of Pharmacology, Hamburg, Germany. LPT Report No. 16169/02. Sponsor Agan Chemical Manufacturers Ltd. Ashdod, Israel.

MacKenzie SA (2007) IN -MCX20: subchronic toxicity 90 -day feeding study in rats. Unpublished report No. DuPont-19008. DuPont Haskell Laboratory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Macpherson, D. (1996) Glyphosate acid: biotransformation in the rat. Unpublished report No. CTL/P/5058, dated 28 June 1996, from Zeneca Agrochemicals , Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

EPA-HQ-2018-002024_0004989

Maibach, H.I. (1983) (a) Elimination of 14C -glyphosate in Rhesus monkeys following a single parentera l dose, (b) Percutaneous absorption of 14C -glyphosate in Roundup formulation in Rhesus monkeys following a single topical dose. Unpublished report No. MA -81-349, dated 1 April 1983, from University of California, School of Medicine; San Francisco, Californ ia, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

McEwen, A.B. (1995) HR -001: Metabolism in the rat. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, UK, Data owner: Arysta Lifescience SAS, Report No.: SNY 332/951256, D ate:1995-08-16, not published, ASB2012-11379

Mecchi MS (2004) Salmonella –Escherichia coli/mammalian -microsome reverse mutation assay with a confirma - tory assay with N -acetyl-glyphosate. Unpublished report No. Covance 7535 -101. Covance Laboratories, Inc., Vienna, VA, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA

Milburn, G.M. (1996) Glyphosate acid: one year dietary toxicity study in rats. Unpublished report No.CTL/P/5143, study No. PR 1012, dated 2 October 1996, from Zen eca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Pro - tection AG, Basel, Switzerland.

Mizuyama, K. (1987) Irritating effect of glyphosate, surfactant and roundup on stomach and small intestine in dogs. University of Tsukuba, clinic medicine, Japan. Unpublished study.

Moxon, M.E. (1996a) Glyphosate acid: developmental toxicity study in the rat. Unpublished report No.CTL/P/4819, study No. RR0690, dated 27 March 1996, CTL/P/4819 /amendment-001, dated 20 Novem - ber 2002, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland, Unpublished study.

Moxon, M.E. (1996b) Glyphosate acid: developmental toxicity study in the rabbit. Unpublished report No. CTL/P/5009, study No. RB0709, dated 2 July 1996, from Zeneca Agrochemicals, Central Toxicology Lab - oratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WH O by Syngenta Crop Protection AG, Basel, Switzerland.

Moxon, M.E. (2000) Glyphosate acid: multigeneration reproduction toxicity study in rats. Unpublished report No. CTL/P/6332, study No. RR0784, dated 16 June 2000, from Zeneca Agrochemicals, Central Toxic ol- ogy Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Murli H (2004) Chromosomal aberrations in Chinese hamster ovary (CHO) cells. Unpublished report No. Covance 7535-102. Covance Laboratories, Inc., Vienna, VA, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Nakashima, N. (1997) HR -001: 12 -Month Oral Chronic Toxicity Study in Dogs. The Institute of Environmental Toxicology, Tokyo, Japan. Study No.: IET 94-0157. Data owner: Arysta LifeScience, Unpublished study.

Nesslany, F. (2002) Measurement of unscheduled DNA synthesis (UDS) in rat hepatocyte using an *in vitro* procedure with AMPA (aminomethylphosphonic acid). Institut Pasteur DElille, Gene tic toxicology Laboratory, Lille Cedex. Report No. IPL -R 020625/AMPA (aminomethylphosphonic acid)/Calliope SAS. Sponsor Mrs. Florence Leconte., Unpublished study.

Parker, RM (1993) 90 day range finding study of glyphosate in rats. TSI Redfield Laboratories , 100 East Boone Street, Redfield, AR. Laboratory project no. 011 -0001. Submitted/Sponsor by Alkaloida Co. Ltd., Hungry. Unpublished study.

Pavkov, KL and Turnier, JC (1983) Two-year chronic toxicity and oncogenicity dietary study with SC-0224 in mice. Stauffer Laboratory Farmington, CT, study no. T -11813, unpublished study. Submitted by Syngenta, Basle, Switzerland.

EPA-HQ-2018-002024_0004990

Pavkov, KL and Wyand, S (1987) Two -year chronic toxicity and oncogenicity dietary study with SC -0224 in rats. Stauffer Laboratory Farmington, CT, study no. T -11082, unpublished study. Submitted by Syngenta, Basle, Switzerland.

Perry, C.J., Atkinson, C., Strutt, A., Henderson, W. & Hudson, P. (1991a) Glyphosate: 13 week dietary toxi - city study in rats. Unpublished report No. 7136, IRI project No . 437876, dated 7 March 1991, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Perry, C.J., Atkinson, C., Strutt, A., Hudson, P. & Jones, M. (1991b) Glyphosate: 13 week dietary toxicity study in mice. Unpublished report No 7024, IRI project No. 437918, dated 7 March 1991, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Pinto, P.J. (1996) Glyphosate Acid: 21 Day Dermal Toxicity Study In Rats. Central Toxicology Labaoratory, Alderley Park, Macclesfield, Cheshire, UK, Report No.: CTL/P/4985, Data owner: Syngenta, Unpublished study.

Powles, P. (1992a) 14C -glyphosate: absorption and distribution in the rat —preliminary study. Unpublished re port No. 6365 -676/1, dated 10 June 1992, from Hazleton UK, Harrogate, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Powles, P. (1992b) 14C -glyphosate: absorption, distribution, metabolism, and excretion in the rat. Unpub - lished report No. 7 006-676/2, dated 30 June 1992, from Hazleton UK, Harrogate, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Prakash, PJ (1999) Subchronic (90 day) oral toxicity study with hlyphosate technical in beagle dogs. Test compound: Glyphosate technical. Rallis Research center, Rallis India Limited, Banglore, India. Study no. 1816. Sponsor M/s Feinchemie Schwebda GmbH, Koln, Germany, unpublished study.

Reyna MS and Ruecker FA (1985) Twelve -month Study of Glyphosate administered by Gelatin Capsule to Bea gle Dogs, Monsanto report ML-83-137.

Reyna, M.S. & Thake, D.C. (1989) Range finding study of glyphosate administered in feed to Sprague -Dawley rats. Unpublished report No. ML -88-272, study No. 88181, dated April 1989, from Monsanto Environ - mental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Reyna MS (1990) Glyphosate: Two Generation Reproduction Feeding Study with Glyphosate in Sprague -Dawley Rats. Monsanto Company Environmental Health L aboratory, Missouri, US, Monsanto Unpublished Report No. ML-88106, Unpublished Study.

Ridley, W.P. (1983) A study of the plasma and bone marrow levels of glyphosate following intraperitoneal administration in the rat. Unpublished report, study No. 830109, project No. ML -83-218, dated 24 October 1988, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Ridley, W.P. & Mirly, K. (1988) The metabolism of glyphosate in Sprague -Dawley rats. Part I. Excretion and tissue distribution of glyphosate and its metabolites following intravenous and oral administration. Unpub - lished report, study No. 86139, project No. ML -86-438, dated March 1988, from Monsanto Environmen - tal Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Shen ZA (2007) IN -MCX20: subchronic toxicity 90 -day feeding study in rats. Unpublished report No. Du - Pont-19008, Supplement No. 1. DuPont Haskell Laborat ory, Newark, DE, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Schroeder RE & Hogan GK (1981) Glyphosate: A Three Generation Reproduction Study in Rats with Glyphosate. Bio/Dynamics, Inc., New Jersey, US. Bio/Dynamics Unpublished Report No. BDN-77-417, Unpublished study.

EPA-HQ-2018-002024_0004991

Stout, L.D. & Johnson, C.W. (1987) 90 -day study of glyphosate administered in feed to Sprague/Dawley rats. Unpublished report No. ML -86-351, study No. EHL 86128, dated 30 November 1987, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Stout, L.D. & Ruecker, F.A. (1990) Chronic study of glyphosate administered in feed to albino rats. Unpub - lished report No. MSL -10495, job/project No. ML -87-148/EHL 87122, dated 22 October 1990, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Strutt, A.V., Atkinson, C., Hudson, P. & Snodgrass, E . (1993) AMPA: 13 week toxicity study in rats with administration by gavage. Unpublished report No. 7866, IRI project No. 450876, dated 16 April 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Sugimoto, K. (1997), HR -001: 18 -Month Oral Oncogenicity Study in Mice, The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan, Laboratory Project I IET 94 -0151, Sankyo Co., Ltd., 7-12, Ginza 2-chome, Chou-ku, Tokyo 104, Japan, not published. Also referred to as Arysta Life Sciences, 1997.

Suresh, T.P. (1993a) Two Generation Reproduction Study in Wistar Rats. Toxicology Department, Rallis India Ltd., Rallis Agrochemical Research Station, Bangalore, India. Data owner: Feinchemie Schwebda GmbH, Study No.: TOXI 885-RP-G2, Unpublished study.

Suresh, T.P. (1993b) Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990), Rallis India Limited, Rallis Agrochemical Research Station, Beng alore, India. Data owner: Feinchemie Schwebda GmbH, Study No.: TOXI: 884-TER-RB, amended 1994-06-18, Unpublished study.

Suresh, T.P. (1996) Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats, Rallis Research Centre , Rallis India Ltd., Bangalore, India, Data owner: Feinchemie Schwebda GmbH, Study No.: 886.C.C-R, Unpublished study.

Takahashi, M or K?. (1997) HR -001: A two -generation reproduction study in rats. The Institute of Environmental Toxicology, Tokyo, Japan, Data owner: Arysta Life Sciences, Study No.: IET 96-0031, Unpublished study.

Takahashi, M (1999a). Oral Feeding Carcinogenicity Study in Mice with AK -01, Nippon Experimental Medical Research Institute Co., Ltd., Technical PROJECT No. H -95056, 3303 -58, Oaza Odo, Agatsuma -macho, Agatsuma-gun, Gunma, Japan.

Takahasi, M (1999b) A COMBINED CHRONIC TOXICITY/CARCINOGENICITY STUDY OF AK -01 BULK SUBSTANCE BY DIETARY ADMINISTRATION IN RATS, Nippon Experimental Medical Research Institute Co., Ltd, PROJECT No. H -95053, Oaza Odo, Agatsuma -machi, Agatsuma -gun, Gunma, Japan. Unpublished study.

Tasker, E.J., Rodwell, D.E., Jessup, D.C. (1980a) Glyphosate: Teratology Study in Rats With Technical Glyphosate. International Research and Development Corp., Michigan, US, Monsant o Unpublished Report No. IR -79-016, Unpublished study.

Tasker, E.J., Rodwell, D.E., Jessup, D.C. (1980b) Technical Glyphosate: Teratology study in rabbits International Research and Development Corporation, Michigan, USA. Monsanto Report No.: IR-79-016, Unpublished study.

Teramoto, I (1998). A 12 -month chronic toxicity study of AK -01 bulk substance by oral gavage administration in dogs. Nippon Experimental Medical Research Institute Co., Ltd., Project No. H-95059, Nippon, Japan.

Tierney W.J and Rinehart WE (1979) A Three Month Feeding Study of Glyphosate (Roundup® Technical) in Mice. Monsanto report BDN-77-419.

EPA-HQ-2018-002024_0004992

Tornai, A. (1994) Glyphosate technical - Repeated dose twenty -eight day toxicity study in rabbits, Institute of Toxicology, Keszthely, Testing facil ity code GLY -94-410/N, Sponsor Alkaloida Co. Ltd., Tiszavasvari, Sponsor's report no. MUF 214/94. Unpublished study.

Vegarra MM (2004) Acute oral toxicity study in rats with N -acetyl-glyphosate, sodium salt (acute toxic class method). Unpublished report No . Covance 7535 -103. Covance Laboratories, Inc., Vienna, VA, USA. Sub - mitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Vereczkey, L. and Csanyi, E. (1992/ revised version): 18-month carcinogenicity study of glyphosate in mice.

Wagner VO, Klug ML (2007) IN-EY252: bacterial reverse mutation assay. Unpublished report No. Du - Pont-22227. BioReliance, Rockville, MD, USA. Submitted to WHO by E.I. du Pont de Nemours and Company, Wilmington, DE, USA.

Ward, RJ (2010a) 450g/L Glyphosate SL f ormulation (MON 79545) In Vitro absorption of glyphosate through Human Epidermis JV2083-REG. Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK. Study No.: JV2083, Report No.: JV2083 -REG, Unpublished.

Ward, RJ (2010b) 480 g/L Glyphosate SL formulation (MON 79351) In Vitro absorption of glyphosate through Human Epidermis JV2085-REG. Dermal Technology Laboratory Ltd., Med IC4, Keele University Science and Business Park, Keele, Staffordshir e, ST5 5NL, UK. Study No.: JV2085, Report No.: JV2085 -REG, Unpublished.

Wood, E. (1996) Glyphosate Technical: Pharmacology Screening Study in the Rat. Safepharm Laboratories Ltd., Derby, UK. Data owner: Nufarm, Study No.: 434/021, Unpublished study.

Wood, E., Dunster, J., Watson, P., Brooks, P. (2009a), Glyphosate technical: Dietary Carcinogenicity Study in the Mouse, Harlan Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK SPL Project No.: 2060-0011. Unpublished study.

Wood, E., Dunster, J., Watson, P., Brooks, P (2009b) Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat, Harlan Laboratories Ltd., Shardlow, Derbyshire, UK Study No.: 2060 - 0012, Unpublished study.

Yoshida, A (1996) HR -001: 13 Week oral subchronic toxicity study in dogs. The Institute of Environmental Toxicology, Tokyo, Japan, Laboratory Project ID IET 94 -0158, Sponsor Sankyo Co. Ltd., Tokyo, Japan. Unpublished study.

EPA-HQ-2018-002024_0004993